Unravelling the Past and Present: Marine Phytoplankton Population Dynamics in the face of the Anthropocene

Doctoral thesis for obtaining the

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Schmidt, Alexandra

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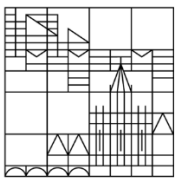

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Referee: Prof. Dr. Laura Epp

Referee: Prof. Dr. Eric Capo

Referee: Prof. Dr. Peter Kroth

"The genome is a book that wrote itself, continually adding, deleting and amending over four billion years."

Matt Ridley

(*Genome: The Autobiography of a Species in 23 Chapters*)

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Summary of thesis

This research investigates the resilience and adaptability of the Baltic Sea's phytoplankton populations over millennia, with a particular focus on the diatom *Skeletonema marinoi*. It demonstrates how genomic monitoring can provide a comprehensive picture of ecosystem health in response to environmental changes. By means of a series of multi-layered studies, the study establishes a new framework for ecosystem assessment, combining marker-based genetics with advanced genetic techniques, including analyses of sedimentary ancient DNA (sedaDNA) and resurrected individuals. This approach permits the reconstruction of phytoplankton population dynamics, here *S. marinoi*, across both recent decades and millennia, thereby providing insight into shifts that have occurred due to natural, climatic and anthropogenic influences.

In Chapter 1, a molecular index is developed to track the dominance relationship between a diatom species (*S. marinoi*) and a dinoflagellate species (*Apocalathium malmogiense*) over time. This index provides valuable historical data that extends beyond traditional monitoring programs, allowing us to detect responses to changes. In Chapter 2, we examine the genetic structure of *S. marinoi* populations through hybridization capture of sedaDNA over the last ~8,000 years. Our findings reveal historical resilience, with profound changes in genetic structure occurring over the last 1,000 to 2,000 years. This demonstrates the species' capacity for resilience despite historical disturbances and underscores the potential of genomic data to reflect long-term ecosystem stability. In Chapter 3, the revival of diatom *S. marinoi* resting cells from sediments over 6,800 years old demonstrates that these populations retain functional traits even after millennia of dormancy, though phenotypic convergence can be observed, whereby similar phenotypes exist despite genetic differences. This allows for the real-time study of evolutionary adaptations. Finally, in Chapter 4, the genomes of these resurrected strains are compared to modern locally distributed Baltic Sea strains of *S. marinoi*, revealing patterns in genetic structure. Results suggest that while there are marginal spatial impacts, there are significant temporal ones. This indicates the existence of a single large Baltic Sea population with high genetic diversity.

The findings reveal that genomic monitoring, which encompasses both genetic and physiological information, represents an instrument for evaluating ecosystem health. Importantly, this research suggests that maintaining genetic diversity in phytoplankton could be essential for resilience against rapid environmental shifts, a conclusion with considerable implications for marine conservation. The Baltic Sea serves as a case study in this research, offering a model for monitoring marginal seas and coastal regions worldwide, where biodiversity is under similar pressures from climate change,

pollution, and eutrophication. In light of these findings, it is evident that genomic monitoring has the potential to profoundly impact marine conservation. It can facilitate more responsive and adaptive management strategies, thereby enhancing the capacity to protect ecosystems in the face of the Anthropocene.

Zusammenfassung

Die vorliegende Arbeit untersucht die Resilienz und Anpassungsfähigkeit von Phytoplanktonpopulationen über Jahrtausende in der Ostsee. Der Fokus liegt auf der Kieselalge (Diatomee) *Skeletonema marinoi* als Modelorganismus. Die Ergebnisse (basierend auf Sedimentarchiven) zeigen das Potenzial des genomischen Monitorings zur Bewertung der ökologischen Resilienz und Adaptivität in Reaktion auf Umweltveränderungen. Diese Arbeit könnte einen neuen Ausgangswert/"Baseline" für marine/aquatische Ökosysteme entwickeln, der auf einer Kombination von markerbasierter Genetik und fortschrittlichen genetischen Techniken basiert. Dazu zählen die Analyse alter sedimentärer DNA (sedaDNA) und wiederbelebter Individuen. Dieser Ansatz erlaubt es die Populationsdynamik des Phytoplanktons für die vergangenen Jahrzehnte und Jahrtausende zu rekonstruieren, im Besonderen/mit Fokus auf für *S. marinoi*. Somit lassen sich Veränderungen ermitteln, die durch natürliche, klimatische und anthropogene Einflüsse entstanden sind.

In Kapitel 1 wird ein molekularer Index entwickelt, der das Dominanzverhältnis zwischen einer Kieselalgenart (*S. marinoi*) und einer Dinoflagellatenart (*Apocalathium malmogiense*) im Laufe der Zeit verfolgt. Dieser Index liefert wertvolle historische Daten, die über die traditionellen Monitoring-Programme hinausgehen, und ermöglicht es uns, Reaktionen auf Veränderungen zu erkennen. In Kapitel 2 erfolgt eine Untersuchung der genetischen Struktur von *S. marinoi*-Populationen über einen Zeitraum von 8.000 Jahren unter Verwendung der DNA-Hybridisierung zur Anreicherung von sedaDNA. Unsere Ergebnisse demonstrieren eine historische Resilienz, die durch wesentliche Veränderungen der genetischen Struktur in den vergangenen 1.000 bis 2.000 Jahren gekennzeichnet ist. Dies veranschaulicht die Fähigkeit der Art, sich trotz historischer Störungen zu erholen, und unterstreicht das Potenzial genomischer Daten, die Langzeitstabilität von Ökosystemen widerzuspiegeln. Dies unterstreicht das Potenzial genomischer Daten, die die Langzeitstabilität von Ökosystemen widerspiegeln. In Kapitel 3 zeigt die Wiederbelebung von Kieselalgen-Überdauerungsstadien aus über 6.800 Jahre alten Sedimenten, dass diese Populationen funktionelle Merkmale selbst nach Jahrtausenden der Dormanz beibehalten, obwohl phänotypische Konvergenz beobachtet werden kann, wobei ähnliche Phänotypen trotz genetischer Unterschiede existieren. Dies ermöglicht die Untersuchung evolutionärer Anpassungen in Echtzeit. In Kapitel 4 werden schließlich diese alten und moderne *S. marinoi*-Stämme genomisch verglichen, um Muster in der genetischen Struktur zu untersuchen. Die Ergebnisse deuten darauf hin, dass es zwar geringe räumliche, aber signifikante zeitliche Auswirkungen gibt. Dies legt die Schlussfolgerung nahe, dass eine einzige, große Ostseepopulation mit hoher genetischer Diversität existiert.

Die Ergebnisse zeigen, dass 1) das genomische Monitoring ein geeignetes Instrument zur Bewertung des Ökosystemzustands ist. 2) die genetische Diversität im Phytoplankton für die Resilienz gegenüber Umweltveränderungen entscheidend ist und somit große Bedeutung für den Meeresschutz hat. Die Ostsee dient dabei als Fallstudie für das Monitoring von Randmeeren und Küstengebieten weltweit, welche Klimawandel, Verschmutzung und Eutrophierung (frühzeitig) ausgesetzt sind. Dabei birgt das genomische Monitoring das Potenzial, den Meeresschutz essenziell zu beeinflussen und Managementstrategien zu entwickeln, die den Schutz der Ökosysteme angesichts des Anthropozäns verbessern.

General Introduction

Phytoplankton

Phytoplankton, the microscopic, photoautotrophic organisms at the base of aquatic food webs, are essential to both aquatic ecosystems and global climate regulation. Serving as sensitive indicators of environmental changes, they reflect variations in temperature, nutrient availability, and light, making them invaluable for assessing ecosystem health and anthropogenic impacts, such as climate change (Buchwald et al., 2024). As primary producers, they support a diverse range of aquatic life from zooplankton to large predators. Their productivity and diversity directly influence the stability and productivity of aquatic ecosystems (Huisman and Weissing, 1999). However, their sensitivity to climate change introduces vulnerability, as changes in sea surface temperature, salinity, and nutrient dynamics can profoundly alter community composition and productivity (Hays et al., 2005), with cascading effects on higher trophic levels (bottom-up effects) and overall ecosystem functioning (Krumhardt et al., 2022).

A remarkable aspect of phytoplankton is the "paradox of the plankton", described by Hutchinson (1961) and expanded by Huisman and Weissing (1999). Traditional ecological theories predict that in a stable environment with limited resources, competitive exclusion should lead to the dominance of a single or a few species. However, in marine ecosystems, a considerable number of phytoplankton species coexist despite the apparent scarcity of essential resources like nitrogen, phosphorus, silica and light (Behrenfeld et al., 2021). This paradox highlights the incredible diversity within phytoplankton communities, a diversity that is key to ecosystem stability and the maintenance of biodiversity in marine environments. Understanding the genetic diversity within and between phytoplankton populations offers valuable insights into the adaptive mechanisms that enable them to occupy various ecological niches and respond to environmental fluctuations (Litchman et al., 2012).

In both temperate and polar regions, profound seasonal variations are observed in phytoplankton populations, with spring blooms occurring with particular frequency and intensity (Hjerne et al., 2019; Waga et al., 2021). These spring blooms occur when increasing light availability and nutrient levels, often due to winter mixing, create optimal conditions for rapid phytoplankton growth (Browning et al., 2020). Spring blooms are of particular importance as they provide a substantial food source for higher trophic levels, including zooplankton and fish larvae, thereby supporting the entire food web (Chiswell et al., 2015). The timing and magnitude of these blooms can substantially impact the reproductive success and survival of various species (Dong et al., 2021).

Beyond their role in food webs, phytoplankton are crucial to global carbon cycling. Phytoplankton serve as critical bioindicators of ecosystem health due to their sensitivity to changes in environmental conditions such as nutrient availability, salinity, and temperature (Smol and Stoermer, 2010). Ecosystem health refers to the stability, resilience, and functionality of ecosystems, ensuring that ecosystems can continue to provide essential services and maintain biodiversity over time (Rapport, 1989). Shifts in phytoplankton community composition or biomass often precede observable changes at higher trophic levels, making them an early warning system for ecological change (Wasmund et al., 2016). For example, diatoms and dinoflagellates reflect nutrient cycling and eutrophic conditions, providing valuable insights into the state and trends of marine ecosystems. This sensitivity, coupled with their fundamental ecological role, underscores the importance of understanding phytoplankton responses to environmental variability and anthropogenic impacts. Through photosynthesis, they convert carbon dioxide (CO_2) into organic matter, contributing to carbon sequestration in oceanic sediments and reducing atmospheric CO_2 levels (Falkowski et al., 1998). Their ability to absorb light through various pigments enhances their photosynthetic efficiency, further influencing growth rates and ecosystem dynamics (Kirk, 1994).

However, anthropogenic activities, particularly the introduction of nutrients from agricultural runoff and urban wastewater, frequently disrupt these intricate ecological dynamics. Eutrophication, the excessive enrichment of water bodies with nutrients, can lead to rapid phytoplankton growth and subsequent oxygen depletion (Rabalais et al., 2014). While phytoplankton produce oxygen through photosynthesis, their decomposition following blooms can create hypoxic or anoxic conditions, particularly in bottom waters, leading to “dead zones” that severely impact marine life and ecosystem functioning (Diaz and Rosenberg, 2008).

Within the diverse phytoplankton community, the groups diatoms and dinoflagellates play key roles. Diatoms are integral to biogeochemical cycles, particularly silica cycling and carbon fixation, and their silica cell walls contribute to sediment formation and nutrient cycling (Tréguer et al., 2018). The sinking of diatom frustules to the seafloor is a common phenomenon following the death of these organisms. This process provides an important benthic food source and contributes to the sedimentary silica pools found in marine environments (Ehrlich and Witkowski, 2015). Diatoms exhibit unique reproductive strategies that further enhance their adaptability in fluctuating environments (Zhou et al., 2021). Predominantly diploid during their vegetative phase, diatoms reproduce asexually through clonal mitotic division, gradually decreasing in cell size with each generation (Bulankova et al., 2022). While primarily reproducing asexually through clonal mitotic division, diatoms also undergo periodic sexual reproduction triggered by critical size thresholds. This sexual phase results in the formation of larger

initial cells called auxospores, which reset the cell size cycle and introduce genetic diversity into the population (Bilcke et al., 2022; Johansson et al., 2019). These dual reproductive modes – rapid clonal expansion and genetically diversifying sexual reproduction – enable diatoms to maintain resilience under varying conditions. Dinoflagellates, with their diverse metabolic pathways and adaptability, can form harmful algal blooms, impacting nutrient cycling and ecosystem health (Mena et al., 2024). In contrast to diatoms, dinoflagellates mainly undergo demineralization in the pelagic zone, where their organic matter is dissolved and recycled within the water column (Skeffington et al., 2022). Both groups serve as bioindicators of environmental change due to their sensitivity to nutrient levels and water quality fluctuations (Smol and Stoermer, 2010). Diatoms and dinoflagellates include species that are capable of forming resting stages, spores and cysts respectively, which enable them to survive unfavourable conditions, including those that occur during the warm season (Hinners et al., 2017; Stenow et al., 2020). Diatoms and dinoflagellates contribute to biogeochemical cycles and are useful for monitoring the environment. Diatoms, through their sedimentation and silica deposition, and dinoflagellates, through their pelagic dissolution and nutrient recycling, both play vital roles in maintaining ecosystem balance and responding to environmental changes.

The Baltic Sea

The Baltic Sea presents an ideal study system for investigating the ecological roles and responses of phytoplankton to environmental changes and anthropogenic impacts. The Anthropocene, defined by the unprecedented impact of human activities on Earth's systems (Crutzen and Stoermer, 2000), has significantly shaped the Baltic Sea ecosystem, likely starting from the mid-20th century. Accelerated industrialization, urbanization, and agricultural expansion over the last two centuries have introduced anthropogenic stressors such as eutrophication, pollution, and habitat degradation, fundamentally altering the natural dynamics of the region (Reckermann et al., 2022). The interplay of these anthropogenic pressures with natural climatic variability creates a unique setting to study ecosystem robustness and adaptability in the face of compounded stressors. Phytoplankton, as bioindicators, are of particular importance in monitoring these changes, offering insights into the ecosystem's trajectory in this human-dominated era. In the Baltic Sea, where environmental conditions such as salinity and nutrient dynamics are particularly variable, phytoplankton serve as vital indicators of ecosystem health, providing insights into both natural and human-driven changes. Despite being relatively young, the Baltic Sea has a dynamic history that offers valuable insights into the interplay between natural environmental changes and phytoplankton dynamics (Wasmund and Uhlig, 2003). The dual influence

of natural and anthropogenic factors highlights the Baltic Sea's value as a model system for investigating the resilience and adaptability of phytoplankton populations, such as the diatom *Skeletonema marinoi*, in response to changing environmental conditions.

The Baltic Sea has undergone significant transformations over the course of millennia, evolving from a glacial freshwater lake to the brackish marine environment observed today (Fig. 1). This evolutionary process commenced following the conclusion of the last glacial period, with the region undergoing a series of notable transitions. The melting ice masses and the uplift of landmasses led to these changes in salinity. The initial phase of the Baltic Ice Lake (14,600-12,300 Before Present cal years Before Present (BP=1950)) was characterized by the presence of a large freshwater body, formed by the accumulation of ice and land, with water levels reaching up to 25 meters above sea level at its zenith (Andrén et al., 2011). As the ice sheet receded, the Yoldia Sea (12,300-11,500 cal yr BP) emerged, exhibiting brackish conditions resulting from the influx of marine water. Subsequently, the Ancylus Lake (11,500-10,000 cal yr BP) underwent a transition back to freshwater conditions due to the severing of its connection to the ocean as a result of land uplift. The subsequent Littorina Sea (9,500-4,000 cal yr BP) exhibited a resurgence of marine conditions, with salinity levels exceeding those of the present-day Baltic Sea (Andrén et al., 2011). The modern Baltic Sea, which has existed since 4,000 cal yr BP, is distinguished by its brackish water conditions, which are the consequence of its restricted exchange with the North Sea (Weckström et al., 2017).

Climatic events have profoundly impacted the Baltic Sea and the broader Northern European region throughout the Holocene, which commenced at 11,700 cal yr BP and persists to the present day (Borzenkova et al., 2015) (Fig. 1). The Holocene Thermal Maximum (HTM, 10,000-6,000 cal yr BP) brought temperatures 2.5-3°C warmer than the preindustrial period, particularly in summer, due to favourable orbital configurations (Andrén et al., 2011). In contrast, the Late Antique Little Ice Age (LALIA, 1,414-1,290 cal yr BP) was distinguished by notable cooling, potentially initiated by volcanic eruptions, which may have influenced the Baltic Sea's ecology and precipitated societal changes (Büntgen et al., 2016). The Medieval Climate Anomaly (MCA), which occurred between 1,000 and 700 cal yr BP, was a period of relatively warm temperatures in the Northern Hemisphere. This climatic event is believed to have had an impact on Baltic Sea productivity and ecosystem dynamics (Zillén et al., 2008). The subsequent period, the Little Ice Age (LIA, 550-100 cal yr BP), was marked by cooler temperatures in the Baltic region, which influenced sea ice extent, water column mixing, and potentially altered phytoplankton communities (Niedźwiedź et al., 2015). The Modern Warm Period (MWP), spanning from ~100 cal yr BP to the present, has been distinguished by anthropogenic

warming, which has resulted in a reduction in sea ice, alterations in stratification, and shifts in phytoplankton composition (Zillén et al., 2008).

Human activity has had a progressively detrimental impact on the Baltic Sea, particularly in recent centuries (Fig. 1). The most profound impacts on the Baltic Sea ecosystem have occurred during the period of the modern Baltic Sea, particularly since the nineteenth century. Earlier, during the Bronze Age (approximately 4,000-3,500 cal yr BP), the region witnessed a surge in interaction and trade, accompanied by the advent of fortified settlements and the dissemination of metalworking technology (Hald et al., 2024). The Pre-Roman Iron Age (approximately 3,500-1950 cal yr BP) saw further advancements in agriculture and settlement patterns, with local iron production expanding in the region (Hald et al., 2024). The Roman Iron Age (approximately 1,950–1,200 cal yr BP) was characterised by an increased trade with the Roman Empire and the advent of early urban settlements supported by agricultural development (Hald et al., 2024). The Viking Age (approximately 1,200-800 cal yr BP) saw the Baltic region become a significant area for Viking activity, with extensive trade and cultural exchange (Hald et al., 2024). The Hanseatic League, a powerful commercial and defensive alliance of merchant guilds and market towns, exerted control over trade in the Baltic and North Seas from the 13th to the 17th centuries (800-200 cal yr BP), thereby further influencing the economic and cultural landscape of the region (Dollinger, 1970). The processes of industrialization and urbanization that have occurred in recent centuries have resulted in increased nutrient loading, pollution, and habitat destruction, which have collectively led to a significant alteration of the ecosystem (Ojaveer et al., 2010; Reckermann et al., 2022). Since the 1940s, accelerated industrialization and exploitation of natural resources have resulted in the deterioration and degradation of the Baltic Sea Large Marine Ecosystem (BSLME) (Reckermann et al., 2022).

Phytoplankton, which also form the base of the Baltic Sea food web, are of pivotal importance to the region's ecosystem (Canuti and Penna, 2024). The spring bloom, which is typified by accelerated phytoplankton growth and elevated primary production, is markedly influenced by climate change and anthropogenic factors, including nutrient inputs. The Dia/Dino-index, which assesses the spring bloom (biomass) of diatoms (Dia) and dinoflagellates (Dino), serves to reflect the overall health and dynamic changes within the Baltic Sea ecosystem (Wasmund et al., 2017). However, conventional monitoring projects, such as microscopic assessments, lack historical data, as they only began in the 1980s (HELCOM, 2021), a period when significant environmental damage had already occurred (Mack et al., 2020; Storie et al., 2021). In order to ascertain whether the responses of phytoplankton to current stressors are more pronounced than those to past natural events, it is imperative to investigate the

paleoenvironment and expand time series data, which will provide a more comprehensive understanding of the ecosystem's changes over time.

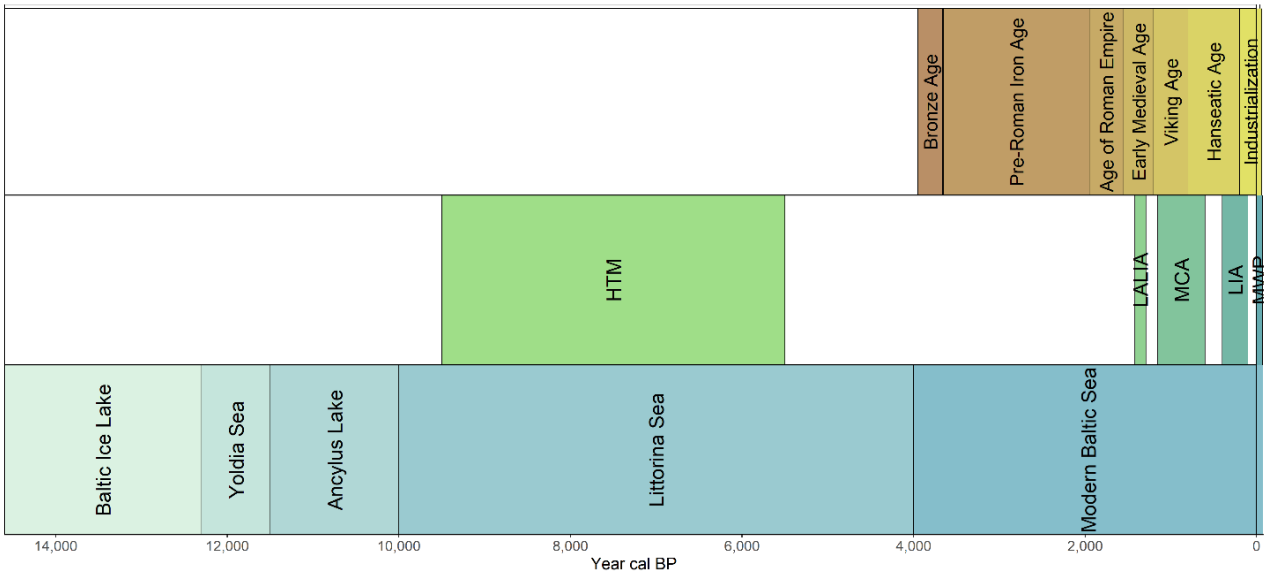

Fig. 1: Phases of the Baltic Sea's development from its origin around 14,600 years cal yr BP to the modern day, including major climate events and human periods. Climate events: HTM: Holocene Thermal Maximum, LALIA: Late Antique Little Ice Age, MCA: Medieval Climate Anomaly, LIA: Little Ice Age, MWP: Modern Warm Period.

Anthropocene

As previously discussed, the dynamics of phytoplankton in the Baltic Sea are becoming increasingly influenced by human activities, which is complicating our understanding of their natural responses to environmental changes. The Holocene, which began around 11,700 cal yr BP, provides a crucial baseline for understanding these natural dynamics. In order to elucidate these effects, it is necessary to consider the Anthropocene, a proposed epoch that highlights the significant impact of human activity on Earth's systems (Lewis and Maslin, 2015).

The concept of the Anthropocene, first introduced by ecologist Eugene F. Stoermer and subsequently popularized by atmospheric chemist Paul Crutzen in the early 2000s, suggests that human influence on the Earth's systems has become so significant that it warrants the designation of a new geological epoch, succeeding the Holocene (Crutzen and Stoermer, 2000). The defining markers of this epoch are increased atmospheric CO_2; radionuclides resulting from nuclear testing; and the pervasive presence of microplastics (Steffen et al., 2011). The Anthropocene Working Group of the International Commission on Stratigraphy actively debated the formal recognition of this epoch, with discussions

centring on its starting date and the stratigraphic evidence supporting it (Zalasiewicz et al., 2008), but a formal suggestion to establish a new chronostratigraphic was refuted (IUGS-ICS, 2024).

Nonetheless, the implications of the Anthropocene are numerous and pervade numerous academic disciplines. In the field of environmental science, it underscores the imperative to confront the consequences of human activities on ecosystems and biogeochemical cycles (Vitousek et al., 1997). For geologists, it presents a challenge to traditional views on the nature of Earth system changes and the timescales involved (Autin and Holbrook, 2012). In the fields of anthropology and philosophy, it calls for a reassessment of human-environment interactions and ethical responsibilities (Ellis, 2011; McNeill and Engelke, 2014). From an economic perspective, the necessity for sustainable development practices in the context of ongoing environmental transformations is self-evident (Steffen et al., 2011).

In order to address the impact of the Anthropocene on the Baltic Sea, a variety of strategies are being employed, including the implementation of international agreements and the establishment of local conservation initiatives. The Helsinki Convention's (HELCOM) objective is to protect the Baltic marine environment from all sources of pollution through intergovernmental cooperation (Korpinen et al., 2010). Similarly, the EU Marine Strategy Framework Directive (European Commission, 2008) requires EU member states to achieve Good Environmental Status in their marine waters. The Baltic Sea Action Plan establishes particular objectives for the reduction of nutrient inputs and the restoration of the ecological status of the Baltic Sea (HELCOM, 2007). These measures are of critical importance for addressing the issues of eutrophication, pollution, and climate change, thereby facilitating the restoration and conservation of the Baltic Sea ecosystem.

Sediment Archives

The current dynamics of phytoplankton are substantially shaped by anthropogenic influences, including the aforementioned nutrient loading and climate change, which introduce artificial stressors into aquatic ecosystems. These anthropogenic impacts complicate the investigation of how phytoplankton would naturally respond to environmental changes in the absence of such pressures. While these dynamics are relevant across all aquatic systems, marine environments, such as the Baltic Sea, are of particular interest (Canuti and Penna, 2024). To gain a deeper understanding of the natural responses of these organisms to environmental stressors, it is essential to examine paleoenvironmental data, including sediment archives.

Sediment archives form when organic and inorganic material transported by water, wind, or biological processes settles on lake or sea beds. The structure and composition of these archives depend on factors like sedimentation rates, water chemistry, and seasonal variations (Wakeham and Canuel, 2006). In aquatic systems, sedimentation is influenced by biological productivity, riverine input, and particle settling, which can vary seasonally and geographically (Ellegaard et al., 2020). For instance, more phytoplankton in nutrient-rich ecosystems can make sediments more organic (Holman et al., 2023). Sediment characteristics differ between oxic and anoxic environments. Anoxic conditions preserve organic material, such as biomarkers and sedimentary ancient DNA (sedaDNA), by reducing microbial activity (Wakeham and Canuel, 2006). Floods, storms, and volcanic eruptions can introduce material layers that serve as chronological markers, enabling precise dating (Ellegaard et al., 2020). In regions like the Baltic Sea, laminated sediments with distinct seasonal layers allow high-resolution analysis (Holman et al., 2023; Zillén et al., 2008). Over time, these layers compact, creating a stable archive of past environmental and biological conditions (Wakeham and Canuel, 2006).

Sediment archives serve as important repositories of environmental and biological data, safeguarding a comprehensive record of past ecosystems and climatic conditions (Meyers, 2003). These archives encompass a wide array of proxies (Fig. 2), including microfossils (foraminifera, diatoms, radiolarians, dinoflagellates, ostracods, molluscs), dormant resting stages (cysts, spores, seeds), biomarkers (lipidic remains, pigments, DNA and RNA fragments, amino acids), and elemental compositions (trace metals, stable isotopes, major elements, organic carbon and nitrogen), which collectively offer valuable insights into long-term ecological changes and environmental dynamics (Smol, 2009). The diversity and richness of these proxies render sediment archives indispensable for the reconstruction of historical environmental conditions and the provision of an understanding of past ecological landscapes.

Accurate interpretation of sediment archives relies on robust age-depth models. As Appleby (2001) observes, these models are of great significance in establishing correlations between sedimentary records and historical events, as well as climatic changes. Principal techniques include carbon-14 (^{14}C) dating and lead-210 (Pb210) dating. C14 dating entails the measurement of the remaining C14 isotope in organic materials, with the objective of estimating the age of the material in question. C14 is formed in the atmosphere and subsequently absorbed by living organisms, where it undergoes a known decay process following death (half-life of 5,730 years) (Lal and Krishnaswami, 1998). This method is effective for dating up to 50,000 years; however, contamination and variations in atmospheric C14 levels may impact its precision. The use of Pb210 dating is primarily confined to the analysis of sediments deposited in the recent past, specifically within the last 100 to 150 years. Pb210, a decay product of radon-222, is deposited onto the Earth's surface and incorporated into sediments. Given a half-life of

22.3 years, the concentration of Pb210 in sedimentary layers can be used to establish an age (Foucher et al., 2021). This method provides high-resolution age models, but it is restricted by a constant supply of Pb210. Furthermore, it is susceptible to the effects of bioturbation. The combination of these techniques provides a comprehensive age-depth model. C14 dating is employed for sediments of greater age, whereas Pb210 dating and the stratigraphic examination of traces from historical occurrences, including nuclear weapon testing and volcanic eruptions, are utilized for more recent layers. This approach provides a detailed chronology, which is essential for the interpretation of sedimentary records and the understanding of historical and climatic changes.

Biomarkers and microfossils serve as important indicators within sediment archives, offering insights into past environmental and biological conditions (Florenzano et al., 2023; Schouten et al., 2013; Smol and Stoermer, 2010). Biomarkers, including lipid remnants, pigments, and DNA fragments, are molecular fossils that provide insights into the organisms that previously inhabited an ecosystem and their metabolic processes (Summons et al., 2022). These compounds are of particular value for the reconstruction of the history of microbial life and environmental changes over geological timescales. Microfossils, including foraminifera, diatoms, radiolarians, and dinoflagellates, serve as proxies for past climatic and oceanographic conditions (Samant, 2024). The morphological characteristics and abundance of these organisms in sediment layers can be used to identify historical shifts in climate, productivity, and water chemistry. Together, biomarkers and microfossils provide insights into the complex interactions within past ecosystems, thus contributing to the high-resolution reconstruction of environmental changes.

The resting stages of phytoplankton, including cysts, spores, and akinetes, also play a pivotal role in sediment archives, retaining viability for millennia (Ellegaard and Ribeiro, 2018). These dormant forms serve as a "seed bank" within the sedimentary environment, with the potential to germinate when conditions become favourable again. The isolation and resurrection of these resting stages have provided invaluable insights into the evolution and adaptation of phytoplankton (Härnström et al., 2011). This aspect of sediment archives contributes an additional dimension to our understanding of past aquatic ecosystems, highlighting the resilience and adaptability of these communities over time.

The capacity to trace historical population dynamics through sediment archives provides a means of integrating biological insights with ecological understanding, thereby facilitating a comparative analysis of past and present ecosystem states. The sediment archives of the Baltic Sea, when considered in combination with advanced molecular tools such as sedaDNA and resurrection ecology, offer an unparalleled opportunity to reconstruct historical baselines and trace population dynamics over

millennia. By comparing these historical records with modern data, it becomes possible to assess the extent to which recent anthropogenic pressures deviate from natural variability. This approach not only informs us about past resilience mechanisms but also provides critical insights for predicting the future adaptability of phytoplankton populations under ongoing climate change and human impact scenarios. In consideration of the dynamic history and susceptibility to anthropogenic pressures of the Baltic Sea, this comparative framework provides a valuable tool for analysing the patterns of change in its ecosystem.

In recent years, the field of paleo aquatic research has undergone a substantial transformation as a result of the introduction of sedaDNA analysis (Crump, 2021). This technique has yielded insights into the evolutionary history and long-term ecological changes of aquatic communities that were previously undocumented, particularly with regard to phytoplankton (Lammers et al., 2021). SedaDNA is formed when genetic material from organisms, that inhabit or are associated with water bodies, is deposited and preserved in sedimentary deposits (Nguyen et al., 2023). Its preservation is especially robust in anoxic conditions, which prevent microbial degradation and enhance the longevity of genetic material (Pedersen et al., 2015). This knowledge has expanded the ability to explore the ancient biodiversity of aquatic ecosystems, offering a molecular perspective on past ecological dynamics. This advancement in sedaDNA analysis has expanded the ability to explore the ancient biodiversity of aquatic ecosystems, offering a molecular perspective on past ecological dynamics.

The Baltic Sea serves as a striking example of the impact of the Anthropocene, displaying notable alterations to its ecosystem as a result of eutrophication, pollution, and climate change. By examining long-term data and employing techniques such as analyses of laminated sediments, a better understanding of the pre-human environment and the extent of anthropogenic impact can be gained. This provides insights into the broader narrative of the Anthropocene and its implications for marine ecosystems.

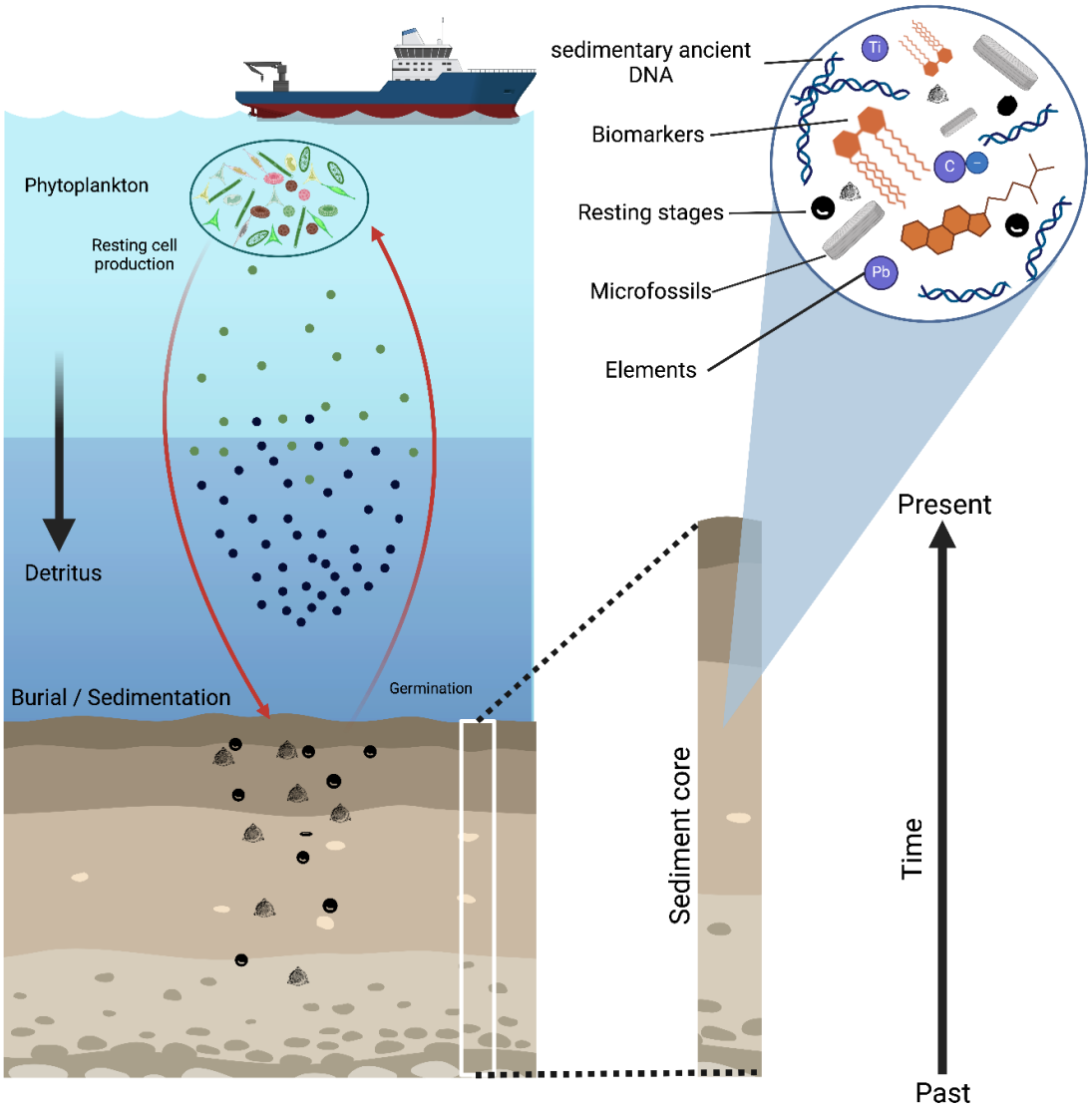

Fig. 2: Overview of various environmental and biological data types retrievable from sediment archives. Left: showing the deposition of organismal remains in sediment. Red arrows depict the production of resting stages, their deposition into the sediment, and the germination of these stages from the sediment. The black arrow indicates the sinking of dead organic matter (detritus) to the seafloor. The sediment contains a mix of preserved organismal remains, including both resting stages and detritus. Right: Close-up view of the sediment core, highlighting different data types that can be extracted (Hochfeld et al., 2024, in review).

Sedimentary Ancient DNA

Environmental DNA (eDNA) has become an important tool in the field of ecosystem research, offering non-invasive techniques for assessing biodiversity through the analysis of genetic material exuded by organisms into the surrounding environment. This approach allows for the efficient monitoring of

species without the need for direct observation (Capo et al., 2021). An interesting part of eDNA, sedaDNA, captures the genetic record of past organisms preserved in aquatic sediments. This provides insights into historical biodiversity and ecosystem changes over time (Capo et al., 2021).

SedaDNA is formed when genetic material from organisms is deposited into sediments through a range of processes, including excretion, decay, and the shedding of cells. This DNA may become embedded in the sediment matrix and preserved for thousands to millions of years (Armbrecht et al., 2019). The preservation of sedaDNA is influenced by a number of environmental factors, including temperature, oxygen levels, and sedimentation rates (Mejbel et al., 2022). These factors can affect the degradation and stability of the DNA (Torti et al., 2015). As a consequence of the degradation of the DNA, the fragments that are recovered are frequently of a short length, which presents a challenge for the subsequent analysis (Pedersen et al., 2016).

SedaDNA is valuable for reconstructing long-term ecological and evolutionary changes since ancient DNA can remain intact over extensive periods. Researchers can examine biodiversity shifts over time, helping to understand modern ecological processes. Metabarcoding, for instance, detects various taxa in a single sample, advancing biodiversity assessments (Pawlowski et al., 2020). It has revealed community shifts linked to historical climate changes and human impacts (Nguyen et al., 2023). Additionally, shotgun metagenomics offers a broader view of past communities and their functional potential beyond targeted sequencing (Lammers et al., 2019).

Monitoring long-term changes in aquatic biodiversity is a major sedaDNA application (Li et al., 2023). Metabarcoding has shown community composition shifts associated with climate changes and human impacts (Nguyen et al., 2023), while shotgun metagenomics offers deeper insights into past communities and their ecological roles (Özdoğan et al., 2024). Combining sedaDNA with paleoenvironmental proxies has enhanced understanding of past climate change's effects on ecosystems. SedaDNA research has expanded knowledge of deep-sea ecosystems, revealing new microbial diversity and their roles in global biogeochemical cycles (Orsi et al., 2017). It has also reconstructed ancient food webs and trophic interactions, providing insights into past ecosystem functions (Özdoğan et al., 2024).

Despite advances in biodiversity and community analysis, sedaDNA applications for studying population-level changes remain less explored. Hybridization capture can help bridge this gap by recovering specific sequences from sedaDNA without extensive sequencing, supporting targeted analyses of particular taxa and genes (Murchie et al., 2021). Analysing population structure and dynamics reveals how community shifts reflect at the population level, tracing responses to historical

climate changes and human impacts. For example, hybridization capture has been applied to study ancient larch populations, shedding light on past forest dynamics and climate responses (Schulte et al., 2021). Similarly, sedaDNA reconstruction of Ice Age algal populations offers insights into adaptations to ancient environments (Lammers et al., 2021). Such studies showcase sedaDNA's potential to reconstruct ancient population dynamics and responses to climate shifts.

The main challenges in sedaDNA research include DNA degradation, contamination, and preservation biases across taxa (Armbrecht, 2022). DNA degradation varies significantly with environmental conditions. For example, the cold and stable conditions characteristic of Arctic permafrost facilitate the preservation of DNA to a greater extent than the warm and humid conditions prevalent in tropical environments, which accelerate the breakdown of DNA (Özdoğan et al., 2024; Pedersen et al., 2016). The contamination of ancient samples with modern DNA is a significant issue, particularly in densely populated areas where human activity can introduce modern DNA, thereby complicating the analysis of such samples (Özdoğan et al., 2024). These challenges highlight the need for continued improvements in methodologies and technologies to accurately reconstruct past environments and biodiversity from sedaDNA.

The integration of sedaDNA with advanced molecular techniques enables the conduct of population genomic analyses of key phytoplankton species, particularly diatoms, in the Baltic Sea. This approach enables the tracing of historical population structures and the investigation of how these fundamental organisms have responded to past environmental changes, thereby providing a baseline for comparison with the Anthropocene epoch. The distinctive and evolving history of the Baltic Sea, in conjunction with the substantial anthropogenic influences of the Anthropocene, provides a compelling setting for examining the natural versus human-induced responses of phytoplankton populations. By reconstructing past population dynamics and comparing them with modern data, insights can be gained into the resilience and adaptability of phytoplankton communities.

Dormant Resting Stages

Resting stages represent a critical survival strategy for a multitude of aquatic organisms, enabling them to persist through periods of unfavourable environmental conditions. For phytoplankton such as dinoflagellates and diatoms, these organisms produce cysts or spores (Ellegaard and Ribeiro, 2018), while zooplankton, like *Daphnia*, produce resting eggs called ephippia (Conde-Porcuna et al., 2014). This capacity for dormancy ensures the population's persistence across fluctuating environmental

conditions. The resting stages in sediments serve as seed banks, contributing to biodiversity and facilitating adaptation to shifting environmental conditions. This reservoir of genetic diversity can enhance populations' ability to respond to environmental changes, thereby ensuring the survival of species in fluctuating ecosystems (Brendonck and De Meester, 2003). This genetic repository is of great importance for our understanding of evolutionary dynamics and modern biodiversity.

Several environmental factors influence the survival and viability of dormant stages in sediments, affecting their longevity and potential for revival. Sedimentation rates and environmental conditions like temperature and oxygen levels are key factors; faster sedimentation can enhance the preservation of resting stages (Casabianca et al., 2020). For example, studies have demonstrated that dinoflagellate cysts can be revived after more than a century in sediments (Hinners et al., 2017; Lundholm et al., 2011), and *Daphnia* ephippia have successfully hatched after remaining dormant for up to 700 years old (Frisch et al., 2014). Additionally, diatom resting spores have been successfully revived after an estimated 6,600 years of dormancy, demonstrating the remarkable longevity of these dormant stages (Sanyal et al., 2022). However, rising temperatures and decreased oxygen levels due to climate change threaten this resilience, potentially impacting the longevity and viability of dormant stages (Ellegaard et al., 2016; Hinners et al., 2017). Human impacts like pollution and eutrophication further complicate the survival of these resting stages, especially in regions with altered sediment composition, such as the Baltic Sea (Hinners et al., 2017; Lundholm et al., 2011).

The study of dormant stages also contributes to the field of resurrection ecology, providing insights into the ways in which species adapt across generations. Sediment cores act as natural archives of past populations, allowing scientists to isolate and resurrect species from resting cells alongside DNA analysis (Härnström et al., 2011). This approach has provided important insights, including comparative studies on different taxa. For example, diatoms such as *S. marinoi* frequently demonstrate superior viability in older sediments when compared to certain dinoflagellates (Härnström et al., 2011; Lundholm et al., 2011). While variability exists due to factors such as sediment composition and species type (Ellegaard et al., 2016), resurrection ecology has revealed distinctive patterns of dormancy and viability across species.

Sediments offer great potential for studying evolutionary processes due to their preservation of dormant stages and genetic material over extended periods (Ellegaard et al., 2020; Holman et al., 2023). Dormant stages serve as a 'genetic repository', allowing whole-genome sequencing and phenotypic analyses of both historical and ancient populations. This enables researchers to track genetic shifts in response to past environmental pressures, providing insights into adaptation and

resilience that are valuable for understanding current and future climate responses (Ellegaard et al., 2020). The fragmented nature of sedaDNA makes dormant stages particularly valuable for reconstructing complete genomes, which are essential for tracking historical genetic changes and evolutionary dynamics.

In addition to genetic data, examining the physiological traits of resurrected species can provide further insights into their adaptability and resilience. Experimental analysis of growth rates, photosynthetic efficiency, and stress resistance to temperature, for example, provides insights into the survival strategies of ancient populations (Gechev et al., 2021; Sharma et al., 2020). By integrating physiological data with genomic analyses, researchers can obtain a more comprehensive understanding of population dynamics and adaptability at both genomic and physiological levels.

The analysis of dormant resting stages in phytoplankton, such as those of the diatom *S. marinoi*, adds a valuable dimension to understanding phytoplankton dynamics over time. These resting stages permit researchers to access intact genetic material from specific historical populations, thereby providing a more detailed representation of how phytoplankton populations have adapted to past environmental conditions. In contrast to fragmented sedaDNA, these dormant stages permit whole-genome analysis and phenotypic studies, thereby providing deeper insights into historical resilience and adaptability. By examining resting stages in conjunction with modern data, researchers can track evolutionary responses within phytoplankton communities, thereby providing a more nuanced baseline for understanding natural and human-induced shifts in marine ecosystems.

Contribution of the Thesis

The overarching aim of this research is to explore the resilience and adaptability of phytoplankton populations of the important spring bloom species *Skeletonema marinoi* in the Baltic Sea, particularly in the context of the Anthropocene. By integrating various methodologies, including population size reconstruction, genomic analysis, and resurrection ecology, this research seeks to provide a comprehensive understanding of how these fundamental organisms respond to both natural and anthropogenic environmental changes. The study is structured into four main chapters, each addressing a specific aspect of phytoplankton dynamics and their implications for ecosystem health.

This multi-layered approach reflects a progression from basic population monitoring to more intricate analyses that reveal both genetic diversity and stability in response to environmental changes. The thesis follows a structured approach, with the first two chapters focusing on sedaDNA to analyse species abundance based on marker analysis and genomic analyses of organelle genomes. The subsequent chapters shift to samples from resurrected resting stages, examining dormancy and phenotypic traits, and culminating in whole-genome sequencing investigations. This progression allows for a focused exploration of resilience while also scaling outward temporally and regionally, offering a comprehensive view of ecosystem dynamics. Each chapter contributes new insights that enhance our understanding of ecosystem robustness. This structure provides a foundation for evaluating ecosystem health while demonstrating the adaptability of genomic tools in identifying resilience mechanisms in marine systems, with implications for conservation and management.

Chapter Perspectives

1. **Reconstructing Population Size and Ecosystem Status:** The first chapter focuses on reconstructing changes in the population size of two key phytoplankton species, the diatom *S. marinoi* and the dinoflagellate *Apocalathium malmogiense*. By developing an index that reflects ecosystem status and changes, this chapter provides a foundational understanding of how population dynamics can inform on the overall health of the Baltic Sea ecosystem. These findings are essential for establishing historical baselines that guide the detection of early ecosystem shifts and inform future conservation priorities.

2. **Genomic Architecture and Intraspecific Diversity:** The second chapter investigates whether the changes observed in the population size of *S. marinoi*, as discussed in the first chapter, are

reflected in the diversity of their mitochondrial and chloroplast genomes, and whether changes in the genetic composition are linked to natural and/or anthropogenic environmental changes. By analysing the genomic architecture at two distinct locations over multiple millennia, this chapter sheds light on the genetic mechanisms underlying population dynamics and their potential adaptive significance on long timescales.

3. **Resurrection Ecology and Genetic Distinctiveness:** In the third chapter, the focus shifts to resurrection ecology, where cultures resurrected from millennial-old *S. marinoi* resting stages are compared to current populations. This chapter investigates whether the observed genetic differences are also reflected in phenotypic and physiological traits, thereby providing insights into the evolutionary processes that have shaped these populations over millennia.

4. **Temporal and Spatial Genomic Changes:** The final chapter employs a full genomic approach to examine the changes in millennial-old resurrected strains of *S. marinoi*. This chapter compares ancient genomes with modern genomes to investigate the impact of spatial and temporal factors on the genomic composition and the underlying drivers of genetic diversity. By linking spatial homogeneity with temporal genetic turnover, this chapter demonstrates how connectivity can buffer populations against localized pressures, while highlighting the influence of long-term environmental shifts on current genetic structures.

Central Question

This research addresses the central question of whether the phytoplankton populations of the Baltic Sea can achieve a state of equilibrium or if they are undergoing continuous shifts. The question thus arises as to whether we are witnessing a response phase preceding the re-establishment of resilience, or whether the current shift is indicative of a more profound and lasting change. By addressing this question, the research aims to provide critical insights into the future trajectory of the Baltic Sea ecosystem and inform conservation and management strategies to mitigate the impacts of climate change and human activities.

This study makes a valuable contribution to our understanding of phytoplankton dynamics in the Baltic Sea, with a particular focus on organisms involved in the Baltic Sea spring bloom. The spring bloom is of particular relevance as it represents a period of rapid phytoplankton growth, which is crucial for the food web and overall ecosystem productivity. By examining the resilience and adaptability of these spring bloom organisms, this research offers a broader perspective on the resilience and adaptability

of aquatic ecosystems in the context of ongoing environmental change. It also establishes a framework for integrating molecular and genomic data into conservation strategies, providing tools for identifying turning points and thresholds that can inform timely interventions.

Chapter 1 – Decoding the Baltic Sea's Past and Present: A simple Molecular Index for Ecosystem Assessment

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Alexandra Schmidt[1,2*], Juliane Romahn[3,4,5], Elinor Andrén[6] , Anke Kremp[7], Jérôme Kaiser[7], Helge W. Arz[7], Olaf Dellwig[7], Miklós Bálint[3,4,5], Laura S. Epp[1*]

1. Department of Biology, University of Konstanz, Constance, Germany
2. International Max Planck Research School Quantitative Behaviour Ecology & Evolution, Constance, Germany
3. Senckenberg Biodiversity and Climate Research Centre, Frankfurt am Main, Germany
4. Loewe Center for Translational Biodiversity Genomics (LOEWE-TBG), Frankfurt am Main, Germany
5. Institute for Insect Biotechnology, Justus Liebig University, Giessen, Germany
6. Department of Natural Science, Technology and Environmental Studies, Södertörn University, Stockholm, Sweden
7. Leibniz Institute for Baltic Sea Research Warnemünde, Rostock, Germany

* Correspondence

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Abstract

Marginal sea ecosystems, such as the Baltic Sea, are severely affected by anthropogenic pressures, such as climate warming, pollution, and eutrophication, which increased in the course of the past century. Biodiversity monitoring data and assessment of environmental status in such systems have typically been carried out only for the past few decades, if at all, and knowledge on pre-impact stability and good ecological status is limited. An extension of monitoring time series can potentially be achieved through analyses of palaeoecological records, e.g. for phytoplankton, which form the base of the food web and are highly susceptible to environmental changes. Within the phytoplankton community, dinoflagellates and diatoms play a significant role as primary producers, and their relative dominance in the spring bloom, calculated as Dia/Dino index, is used as an indicator for the environmental status of the Baltic Sea. To extend time series on the dominance patterns and include non-fossilized dinoflagellates, we here establish a simple droplet digital PCR (ddPCR) reaction on ancient DNA from sediment cores that decodes phytoplankton dynamics. We focus on two common spring bloom species, the diatom *Skeletonema marinoi* and the dinoflagellate *Apocalathium malmogiense*, for which we evaluate a DNA based dominance index. It performs very well in comparison to DNA metabarcoding and modern monitoring and can elucidate past species dominance across the past century and across millennia in different basins of the Baltic. For the past century, we see a dominance shift already starting before the mid-20th century in two of the Baltic Sea basins, thus substantially predating current monitoring programs. Shifts are only partly coeval among the cores and the index shows different degrees of stability. This pattern is confirmed across millennia, where a long-term stable relationship between the diatom and the dinoflagellate is observed in the Eastern Gotland Basin, while data from the Gulf of Finland bear testimony to a much more unstable relationship. This confirms that good ecological status based on the dominance pattern of diatoms and dinoflagellates must be established locally and exemplifies how sediment core DNA can be employed to extend monitoring data.

1. Introduction

Marine ecosystems, currently providing a main source of livelihood for three billion people worldwide (“Ocean economy and developing countries - OECD,” 2023), are under extreme pressure due to a variety of stressors (Pandion et al., 2022), such as current temperature rise (Cheng et al., 2022), biodiversity loss through overexploitation (Ward et al., 2022; Worm et al., 2006), pollution (Datta, 2023; Yu and Singh, 2023) and increased nutrient loads (Howarth et al., 2021; Malone and Newton, 2020). In

particular, coastal marine systems and their inhabitants are increasingly affected by climate change and anthropogenic pressures, such as elevated nutrient loads (Breitburg et al., 2018). Over the past century, significant changes have been observed in these ecosystems, which are largely ongoing. However, systematic monitoring of biological communities typically only began a few decades ago. This has resulted in a lack of data on communities prior to the onset of these alteration, i.e. on pre-impact reference states, on the exact timing and potential historical causes of the anthropogenic community changes (Costello et al., 2017; Obst et al., 2020; Van De Putte et al., 2021) and on the long-term stability of the ecosystem prior to the current rise in anthropogenic pressure.

The few existing biodiversity records that extend beyond the monitoring time series, such as historical plankton counts (Wasmund, 2017), cannot fully address these issues. They do not extend beyond the realm of historical memory, are not continuous in time and exist only for a few locations. The latter aspect limits the possibility to investigate dynamics and establish pre-impact reference states and good ecological status (GES) on local scales, i.e. for separate basins in marine systems. This shortcoming can potentially be addressed through the use of palaeoecological archives, from which the history of ecosystems and their constituent species can be studied through the fossil record (Nguyen et al., 2023; Wingard et al., 2017).

Palaeoecological archives, such as sediment cores, can deliver data on past aquatic assemblages from a multitude of sites and thus provide relevant local time series. They can elucidate the range of natural variation and the speed and trajectory of change, and thus provide a long-term perspective to evaluate present day observations (Saunders and Taffs, 2009). However, the direct incorporation of palaeoecological datasets and the subsequent extension of monitoring data into the past is complicated by several factors.

Current monitoring datasets rely on discrete samples or observations at specific timepoints, accounting for seasonal patterns. In contrast, palaeoecological archives, like sediment core slices, integrate information from longer periods. Living organisms incurrent ecosystems can generally be identified to species level visually, while palaeoecological archives are based on remains, often not identifiable to species level. These remains can be pollen or microfossils with hard parts or shells (McQuoid et al., 2002), or also cysts and spores of soft-bodied plankton (Rengefors et al., 1998; Sundqvist et al., 2018). They can become damaged or degraded over time. Additionally, many aquatic organisms are soft-bodied in all life stages and are thus absent from the visual fossil record.

These problems can be diminished or overcome by using not visible, but molecular remains, in particular ancient DNA preserved in sediments (sedimentary ancient DNA or sedaDNA). This delivers a

record of aquatic communities with a high degree of spatial fidelity (Wang et al., 2023), making it suitable for investigations of local aquatic ecosystem history. It is increasingly employed on marine sediment cores to investigate past biodiversity changes across centuries (Siano et al., 2021), millennia (Zimmermann et al., 2023) and reaching up to a million years (Armbrecht et al., 2022). This highly complex source of biodiversity information is typically investigated for changes in past communities, using either DNA metabarcoding or shotgun sequencing to obtain large inventories of taxa. While it provides a good overview of biodiversity, the well-established DNA metabarcoding approach (Taberlet et al., 2012) must target relatively conserved fragments of the genome to prime PCR amplifications of complete higher level phylogenetic groups. This often results in a lower than optimal taxonomic resolution (Ficetola et al., 2010). Certain primers can lead to under-amplification of some organisms, such as diatoms (Armbrecht et al., 2021). Despite this, correlations between sedaDNA metabarcoding and traditional biomonitoring have been found within specific groups (Thorpe et al., 2024). However, these correlations are not universal across all groups, emphasizing the need for careful interpretation of sedaDNA data. Moving away from these conserved markers to more variable genomic loci, however, sedaDNA offers the potential for high taxonomic resolution, discriminating between sister species and reaching population level (Epp et al., 2018; Lammers et al., 2021). It also includes taxa that are not well-preserved in the fossil record (Nguyen et al., 2023), such as a number of phytoplankton taxa lacking rigid structures.

Among these are dinoflagellates, which, together with diatoms, represent a dominant group of phytoplankton world-wide (Bi et al., 2021; Kang et al., 2021; Zhang et al., 2019). In temperate waters, these groups are often typical components of the spring bloom. Due to differences in nutritional value, biochemical composition, and phenology of diatoms and dinoflagellates, their relative dominance and the timing of their temporal succession has consequences for the complete ecosystem (Wasmund et al., 2017). Current global changes have induced shifts in their relative dominance and successions (Bi et al., 2021; Zhang et al., 2019).

For example in the Baltic Sea, where the two groups occur simultaneously in the spring bloom, a shift in dominance from diatoms to dinoflagellates has lately been observed, with far reaching ecological consequences (Spilling et al., 2018). In particular, it influences the function of the food web: the increased deposition of pelagic diatoms to the sea floor provides an abundant food supply for zoobenthos, while the pelagic dinoflagellates provide a food source for zooplankton or encyst. A shift from diatoms to dinoflagellates signifies a nutrient pathway change favouring pelagic organisms and indicates eutrophication due to silicate limitation (Conley et al., 2008; Wasmund et al., 2017). Thus, a

regime shift towards dinoflagellate dominance is considered an indicator for eutrophication (Wasmund et al., 2017).

The recent shift towards a dinoflagellate dominance is attributed to both eutrophication, mainly caused by anthropogenic discharge of nitrogen and phosphorus, and to climate warming (Klais et al., 2011). The degree of this change in proportions of the two taxa has been utilized to develop a simple index for the environmental status of the Baltic Sea, used in the assessments of the Marine Strategy Framework Directive (MSFD) (Wasmund et al., 2017), named the Dia/Dino-index. It is currently calculated based on microscopically produced phytoplankton biomass data collected in regular biomonitoring surveys of the HELCOM monitoring and assessment program (Wasmund et al., 2017), using seasonal spring-bloom averages for diatom and dinoflagellate biomass.

These regular biomonitoring surveys in the Baltic Sea were initiated in the 1980s due to the tremendous anthropogenic pressures on its unique marine and coastal environment (Storie et al., 2021). Within this timeframe, the Dia/Dino-index identified a regime shift at the end of the 1980s in the Baltic Proper, which has been attributed to climate warming (Wasmund et al., 2017). However, at the start of the biomonitoring, changes in trophic state had already exerted stress on the complete Baltic ecosystem and its communities (Mack et al., 2020; Storie et al., 2021). Eutrophication predates the 1980s, based on available nutrient data extending back to 1900 (HELCOM, 2021; Wasmund, 2017). Thus, the ecosystem's responses were putatively already underway, rendering it impossible to accurately determine the onset of shifts in response to multiple impacts of the past century, or to define values for the index that would characterize a potential pre-impact reference state, or GES. The initial shift, and the conditions preceding it, can only be investigated by turning to historical data or paleoenvironmental archives.

Historical records of plankton counts, although few and temporally patchy, confirm a clear dominance of diatoms over dinoflagellates at the beginning of the 20th century, with higher index values than in later years (Wasmund, 2017). Across this timescale, there seems to be a clear trend related to human impact, and no fluctuation of the index. However, records are not continuous, missing large parts of the past century. In addition, the existing data - historic and recent - also show a clear heterogeneity in the index values, prompting the development of different values for the GES for different basins (Wasmund, 2017). As historic data are not available for all basins, the question of pre-impact stability cannot be conclusively answered for the complete Baltic Sea, also considering the relatively short period covered in relation to the history of the ecosystem. Analysing the diatom/dinoflagellate ratio in the spring bloom on longer, millennial time scales, where they were governed by natural environmental

changes, can elucidate the relevance of recent, anthropogenic impact. In light of these dynamics, the Baltic Sea represents an optimal system for conducting a proof of principle investigation for a novel type of molecular index, which is based on quantitative data of two pivotal species within these taxa. The documented diatom-dinoflagellate dynamic in this region enables us to illustrate how such a molecular index can elucidate environmental changes in past periods, for which no biomonitoring data exists, but which experienced pronounced eutrophication.

In this study, we develop and test a simple sedaDNA approach relying on species-specific PCR reactions and evaluate its use to infer the stability across the 20th century and across the millennial history of the Baltic Sea. To overcome the inherent problem of temporal resolution in sediment core samples and extract seasonally resolved information, our approach exclusively targets key members of the spring-bloom in the diatoms and the dinoflagellates, i.e., the diatom *Skeletonema marinoi* (Sarno et al., 2005) and the dinoflagellate *Apocalathium malmogiense* (Craveiro et al., 2017). Both provide stable records through the Baltic history and can therefore be used as models, representing the two major taxonomic groups at large. We evaluate quantitative results obtained by digital droplet PCR (ddPCR). This method provides precise, sensitive, and robust quantification of nucleic acids without the need for external standards, while being able to perform massive sample partitioning, leading to reliable and sensitive measurements (NRCM and Bio-Rad, 2020) of (species-)specific DNA fragments. We calculate an index from these results, which is equivalent to the existing Dia/Dino index, and compare resulting values to data obtained from metabarcoding of sedaDNA spanning the past decades and to Baltic Sea biomonitoring data reaching back to the beginning of the 1980s. We evaluate 1) how this approach compares to the other employed methods, and investigate 2) the initial timing of the recent, anthropogenically induced dominance shift from diatoms to dinoflagellates in the spring bloom and 3) the degree of stability of the pre-impact reference state across millennia in relation to natural environmental changes in two open Baltic Sea locations. By purely focusing on species that are relevant for the index, we introduce a cost-effective and efficient molecular tool, designed to reconstruct the historical dynamics of these key taxa with the potential to be used also in current monitoring applications on modern environmental DNA (eDNA).

2. Material & Methods

2.1. Study Area

The Baltic Sea is a brackish, shallow, and semi-enclosed sea with limited water exchange to the North Sea and lower biodiversity than fully marine systems (Snoeijs-Leijonmalm et al., 2017) and is especially at risk due to a multitude of reasons. It is particularly vulnerable due to its low biodiversity and its unique position as a sea enclosed by land, densely populated human settlements, and high industrial activity. It suffers from decreasing oxygen levels caused by a number of intertwined processes. Higher oxygen levels are, for one, induced by the higher water temperatures, which diminish the amount of dissolved oxygen and lead to an increase in oxygen-deficient areas (Ito et al., 2017). In addition, eutrophication, caused among other factors by the input of agricultural waste, such as fertilizers, exacerbates the formation of anoxic areas (Carstensen et al., 2014a). Discharge of nutrients, such as nitrogen and phosphorus, furthermore promotes cyanobacteria and algal blooms. These primary producers consume nutrients and fix carbon, but they also lead to an increase of decomposing biomatter in the benthic zone. Bacterial decomposition again consumes oxygen (Conley et al., 2009). Taken together, increasing hypoxia is a major challenge facing the Baltic Sea. The Baltic Marine Environment Protection Commission (HELCOM), the governing body for the Baltic Sea, is actively working to reverse the impacts of anthropogenic pressures, including efforts to reduce nutrient levels (HELCOM, 2021).

Since the latest glaciation, the Baltic Sea has undergone profound changes (Snoeijs-Leijonmalm and Andrén, 2017). Following the retreat of the Scandinavian ice sheet, the Baltic Sea underwent a number of distinct phases. The initial glacial retreat left a giant meltwater lake, the Baltic Ice Lake (12050-9750 yr BCE, years Before Common Era) in the Baltic basin, which finally drained as a connection to the ocean opened over middle Sweden and became the Yoldia Sea (9750-8750 yr BCE). Further meltwater and isostatic rebound of the Scandinavian land masses led to the closure of the oceanic connection, and the formation of the freshwater Ancylus Lake (8750-7850 yr BCE). After a transitional, almost freshwater phase, the Initial Littorina Sea (7850-5550 yr BCE), the salinity increased, related to a rising sea level, and the brackish Littorina Sea phase started around 5550 BCE (Andrén et al., 2011). Between 5550-2050 yr BCE, during the Holocene Thermal Maximum, the temperature in the Baltic Sea region was 1-2 °C above modern values (Seppä et al., 2009) and the Baltic Sea salinity reached a maximum between 4050-2050 yr BCE (Gustafsson and Westman, 2002). Both temperature and salinity decreased between 2050 and 50 yr BCE (Seppä et al., 2009; Snoeijs-Leijonmalm and Andrén, 2017). The Baltic Sea

temperature increased again during the Medieval Climate Anomaly (950-1250 yr CE; years Common Era; Mann et al., 2009) and the Modern Warm Period (since 1850 yr CE) (Kabel et al., 2012).

2.2. Study Organisms

The cold-water diatom *Skeletonema marinoi* and the cold-adapted dinoflagellate *Apocalathium malmogiense* are particularly abundant species of the Baltic Sea spring bloom (Hällfors, 2004) and characterize the phytoplankton communities. Both form resting stages, spores and cysts, respectively, to overcome unfavourable conditions such as during the warm-season (Hinners et al., 2017; Stenow et al., 2020). For both species, continuous decadal and centennial records of living resting stages have been established from Baltic coastal sediments as well as deposits of the deep basins (Härnström et al., 2011; Kremp et al., 2018).

2.3. Sampling & Data Acquisition

Our study is based on two main sources of data: biomonitoring data and data extracted from sediment archives. By combining and comparing these two datasets, we aim to establish a comprehensive time series of key phytoplankton species and extend existing monitoring approaches. This approach allows us to understand not only the current state of these communities but also their historical changes and potential future trends.

Five sediment cores were retrieved (Fig. 3) in April 2021, during expedition EMB262 in April 2021 onboard the research vessel Elisabeth Mann Borgese from three different locations (Fig. 3): 1) Eastern Gotland Basin (EGB, 57°17.004'N, 020°07.244'E, Water Depth: 241 m water depth, cores EMB262/6-28MUC and EMB262/6-30GC); 2) Gulf of Finland (GOF, 59°34.443’N, 023°36.461’E, Water Depth: 81 m bsl, cores EMB262/12-2MUC and EMB262/12-3GC). 3) Landsort Deep (LD, 58°38.391’N, 018°15.997’E, Water Depth: 436m bsl, core EMB262/13-8MUC) (see supplementary Table S1). Short cores (ca. 50 cm) were retrieved at all locations using a multicorer (MUC) preserving the water-sediment interface undisturbed. Long cores (ca. 500 cm) were retrieved from the EGB and GOF using a gravity corer (GC). The EGB long core sampling began at a depth of 32 cm, corresponding to the 1980s. Thus, it misses the final decades, which are only captured in the short core. The cores were sampled onboard using sterile syringes according to Epp et al. (2019) and immediately frozen for storage.

In addition to the sediment cores, phytoplankton monitoring data, specifically for diatoms and dinoflagellates, were obtained from the ICES database (ICES Data Portal, Dome Phytoplankton. (2022), October 18th) and filtered based on a 5% proximity to sediment core locations. Data focusing on the spring bloom period (March to May) were verified and taxonomy obtained from WoRMS using the "worrms" package. Incomplete years were excluded, size classes were merged and data were averaged monthly between stations. Annual biomass proxies from biomonitoring data were calculated for the genera *Skeletonema*, *Apocalathium*, *Scrippsiella* and *Peridinium*, with the latter two treated as *Apocalathium* due to systematic changes. The average count data per month and genus were summed over the three months as an annual biomass proxy.

Information on the history and paleoenvironmental proxies of the Baltic Sea is derived from the corresponding core results and published records. The annual average air temperature, which represents the climate of the Baltic Sea, is based on a core from Lake Flarken in southern Sweden (Seppä et al., 2005). The Mn/Ti ratio from our EGB core is employed as an oxygen level proxy (Supplementary Data, Core dating).

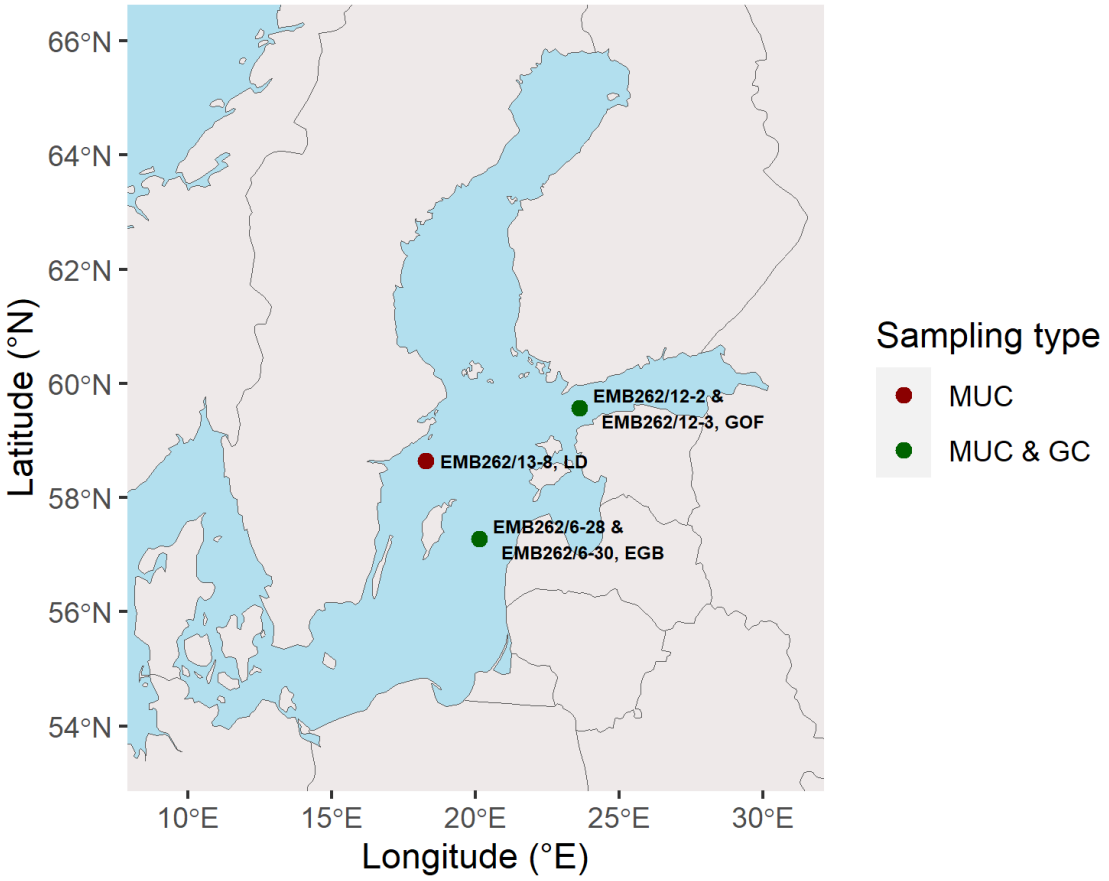

Fig. 3. Location of the coring sites in the central Baltic Sea. Gulf of Finland (GOF; cores EMB262/12-2MUC and EMB262/12-3GC), Landsort Deep (LD; core EMB262/13-8MUC) and Eastern Gotland Basin (EGB; cores EMB262/6-28MUC and EMB262/6-30GC)

2.4. Core Dating

2.4.1. Short cores (MUCs)

The short sediment cores from the EGB (EMB262/6-28MUC) and LD (EMB262/13-8MUC) were dated by transferring published age models of short sediment cores from the same or nearby locations using Hg content and Mn counts (Fig. S1; Häusler et al., 2018; Kaiser et al., 2023). Concerning the GOF short core (EMB262/12-2MUC), an event stratigraphy approach has been applied, which is a current method used to date recent sediments of the Baltic Sea (Dellwig et al., 2018; Häusler et al., 2018; Kaiser et al., 2023; Moros et al., 2017). Three parameters were considered to build the GOF short core stratigraphy: the activity of ^{241}Am and ^{137}Cs radionuclides, and the Hg content. Altogether, five stratigraphical events, or time markers, were used to date the sediment (Figure S1). The uncertainties of the time markers were estimated as in Kaiser et al. (2023). The early increase in Hg content reflects the early increase in

coal combustion around 1870 CE related to the beginning of the Second Industrial Revolution. A sharpening in Hg increase starting around 1950 CE is related to a further increase in coal combustion after the Second World War. The early increase and peak in ^{241}Am activity reflect the beginning and maximum in the global fallout related to the atmospheric nuclear bomb tests in 1953 and 1963 CE, respectively. The synchronous increase in ^{241}Am and ^{137}Cs has been attributed to the Chernobyl accident in 1986 CE. The year of the core recovery (2021 CE) was attributed to the core top layer. Linear sedimentation rates were assumed between the time markers.

2.4.2. Long cores (GCs)

Organic carbon records from post-Littorina-transgression sediments in the central Baltic Sea exhibit patterns ideal for detailed inter-core correlations (Moros et al., 2020; Warden et al., 2017). The chronology of core EMB262/6-30GC from the Eastern Gotland basin is based on such a correlation using the relative content of S and the Br/K ratio (XRF scanner elemental data), which reflect changes in the bulk organic carbon content of the sediments (Ziegler et al., 2008). The data were visually tuned with XRF scanner and organic carbon data of dated sediment cores from the central Baltic Sea (Figure S2). For the mid-Holocene part (~5000-3000 BCE), the chronology of sediment core P435/2-1 (Warden et al., 2017), retrieved from the same location as EMB262/6-30 in the EGB, was used as a reference. For the late Holocene part (the last ~3500 years), sediment cores M86-1a/36-4GC (Häusler et al., 2018) and MSM62-60GC (Moros et al., 2020) from the western Gotland Basin were used as reference chronologies. Due to the terrigenous nature of the sediments, a precise age assignment of pre-Littorina-Stage is difficult for Baltic Sea sediments (Andrén et al., 2000). However, the oldest sediments of the core likely belong to the late Ancylus Lake phase (< 6500 BCE) of the Baltic Sea, which is the unit preceding the Littorina-Stage in Baltic Sea sediments (Snoeijs-Leijonmalm and Andrén, 2017).

For sediment core EMB262/12-3GC from the Gulf of Finland, an independent Bayesian age model using BACON 2.5.5 (Blaauw and Christen, 2011) was established based on seven radiocarbon dates of the bulk organic matter in the sediment (Figure S3, Table S2). With average sedimentation rates of ~100 cm/1000 years, the core dates back to ~2300 BCE.

2.5. Laboratory Work & Data Treatment

2.5.1. DNA Extraction

DNA was extracted from all sediment samples (n = 226, see supplementary Table S3 & S4) using the PowerSoil Pro Kit from Qiagen (Hilden Germany) and a starting amount of ~0.5 g, resulting in 226 DNA extracts and 22 extraction blanks. From 15 random horizons more than one extract was generated for additional PCR pre-tests. The extraction followed the manufacturer's protocol with an additional overnight incubation at 56°C and 20µL of proteinase K (20mg/ml) to optimize lysis ensuring complete digestion of proteins and removing contaminants that could interfere with the subsequent extraction process (Seeber et al., 2024). The washing steps were performed using a Qiagen Vacuum Pump (Hilden, Germany) following the manufacturer's instructions. Afterwards, we centrifuged for 3 minutes instead of the recommended 2 minutes before conducting the elution. The spin columns were incubated twice with 75 µl of elution buffer for 5 minutes and eluted into the same tube.

2.5.2. Metabarcoding

Data on overall changes in phytoplankton communities was obtained across all sediment cores using DNA metabarcoding, targeting all eukaryotic organisms with the Euka02 (V7), or Allshorts, primer pair (forward: 5'-TTTGTCTGSTTAATTSCG-3', reverse: 5'-CACAGACCTGTTATTGC-3') (Guardiola et al., 2015). The PCR setup was automated using the workstation Biomek i7 (Beckman Coulter GmbH) and included 2 PCR positive controls, 4 PCR negative controls, and 8 multiplexing controls, including the extraction controls for 4 PCR replicates. Extracts were 1:2 diluted and amplified using the AmpliTaq Gold ™ Mastermix (Thermo Fisher). The PCR amplification started with an initial denaturation step at 94°C for 5 min, followed by 40 cycles of 94°C for 30s, 45°C for 30s, 68°C for 45s and a final elongation step at 72 °C for 10 min. Unique tag combinations were used to separate replicates and samples (Taberlet et al., 2018). All PCR products were equally pooled and 1200 µL of each pool was purified using the MinElute PCR Purification Kit (Qiagen) according to manufacturer's instructions. PCR-free library preparation and paired-end sequencing (2 × 150 bp, sequencing instrument: NovaSeq6000 on a shared run) were performed at Fasteris SA (Geneva, Switzerland).

Raw metabarcoding paired-end reads were processed using ObiTools3 (v.3.01b13). Read pairs were merged, and sequences with an average alignment score less than 0.8 were discarded. Afterwards reads were demultiplexed, dereplicated and pre-filtered based on count and length. Reads with low counts (<10 counts) were removed and only those within a specified length range (80-250 bp) were

retained. Reads were assigned using mothur (v.1.40.4) (Schloss et al., 2009) against the pr2 database (v. 4.14.0) (Vaulot et al., 2022) with a consensus confidence threshold of 80%.

The data were further filtered in R (v 4.1.3). ASVs were defined as rare based on frequency plots, and ASVs with low read counts (<90 reads) or present in few replicates (<10 replicates) were discarded. Additionally, filtering according to ObiTools3 (Flück et al., 2022) was performed to remove ASVs with higher "internal" counts than head or singleton counts. The maximum read count of each ASV in the negative controls was subtracted from the dataset. Read counts were normalized based on wet sample weight. ASVs belonging to dinoflagellates or diatoms were summed for each replicate (Table S5). This normalization was performed to ensure consistency in the comparison of data across samples, although we point out that the ratio of the two taxa remains unaffected by this normalization.

2.5.3. Droplet digital PCR (ddPCR) on the target species

A duplex droplet digital PCR was performed to quantitatively investigate the DNA copies of the two key target species. With this duplex reaction the copies of both species could be simultaneously determined and thus directly compared. To this end, two species-specific primers were designed to target the study organisms *S. marinoi* and *A. malmogiense*. ITS1, 5.8S rRNA and ITS2 sequences from GenBank (Benson et al., 2015) were downloaded and aligned separately for both species using the MUSCLE alignment function in Geneious Prime (v. 21.2.2). Primer pairs were manually selected and compared by eye to the respective species alignment and outgroups (*Homo sapiens*, *Nannochloropsis limnetica*) based on criteria such as short fragment length and specificity to the target species. The primers were then tested and optimized in four steps: 1) primer specificity and taxonomic resolution was determined by running *in silico* PCR with the program ecoPCR (OBITools ecoPCR, version 1.0.0, Ficetola et al., 2010); 2) the optimal annealing temperature *in vitro* was determined by gradient PCR on DNA extracts of tissue and upper sediment layers; 3) specificity of the reactions was tested *in vitro* by running PCRs on DNA of both target species (tissue from stock cell cultures and resurrected cultures) and non-target environmental samples from Lake Constance Site S23 (Wang et al., 2023); and 4) PCR with sedaDNA. From these tests, an optimal primer pair for *A. malmogiense* (forward: 5'-GATACCCTTGTGCAGAAACTC-3', reverse: 5'-ATTAGACAAGAAGCAAGAAGTAG-3') and *S. marinoi* was selected (forward: 5'-CTTGTGAGTTGCCGAGGC-3', reverse: 5'-TCCATAGATGAGGTACATTCAT-3').

The ddPCRs were run in a probe-based assay, for which ddPCR probes specific to the amplicon were designed using Geneious Prime (v. 22.1.1) according to the Bio-Rad Laboratories (Hercules CA, USA) application guide. The *A. malmogiense* probe was 19 base pairs in length (GCAGGATGGGTGCTTGTCA),

while the *S. marinoi* probe was 20 base pairs long (TCTCCAGCGAATTGGGCTAC). Both probes were double quenched in a duplex ddPCR reaction, with *A. malmogiense* marked by FAM (blue) and *S. marinoi* by HEX (green) fluorescent dyes.

Each 22 µL ddPCR reaction contained the following five components: 1) 11 µL ddPCR supermix for probes (Bio-Rad); 2) 6.8 µL DEPC treated H_2O; 3) 1.1 µL 20x Target-Primers/Probes FAM (900nM/250nM); 4) 1.1 v 20x Target-Primers/Probes HEX (900nM/250nM); 5) 2 µL DNA sample. The ddPCR was performed according to the Bio-Rad manufacturer's protocol. 21 µL of reaction mix was transferred to the Bio-Rad QX200 droplet generator, avoiding bubble formation by only pushing the pipette to the first pressure point. The resulting droplets were transferred to a 96 well PCR plate for amplification on a Bio-Rad C1000 Touch™ thermal cycler using the following program: 10 min at 95°C, 40 cycles of 30s at 94°C, 30s at 50°C and 30s at 60°C, followed by a final enzyme deactivation at 98°C for 10 min. The Bio-Rad QX200 droplet reader and the corresponding QuantaSoft™ Analysis Pro software (v. 1.0.596) were used to analyse the plate and determine the number of positive droplets, measured in copies per µL. Three replicates of the 226 DNA extracts and 22 extraction blanks were run, with each run consisting of 56 PCR reactions, two master mixes, and two NTCs.

2.6. Analyses

In the present study, four datasets were prepared for comparative analyses, each serving a specific purpose. 1) ddPCR copies: This dataset, derived from sedaDNA, targets two species that are abundant in the spring bloom. It provides a direct quantification of these species. 2) Metabarcoding read numbers: This dataset, also derived from sedaDNA, captures the entire respective phylum, providing a broader taxonomic context. Currently, metabarcoding data are the most common type of data generated from sedaDNA. 3) Biomonitoring (phylum level): This dataset provides the relative abundance of the two phyla from biomonitoring counts, thereby enabling the reconstruction of the Dia/Dino-index for the location of the sediment cores. 4) Biomonitoring (species level): This dataset provides the relative abundance of the two species from biomonitoring counts, thereby facilitating an evaluation of their potential as proxies.

To facilitate comparison of the datasets, both the overall relative abundance and 5-year intervals were considered. The latter accounts for the accumulation of molecular compounds in the sediment samples over several years, thereby providing a more accurate representation of long-term trends.

2.6.1. Consistency check

To ensure that it is valid to use the four datasets quantitatively, we implemented a consistency check. This check is based on the five-year intervals of all four datasets described above. We restricted our analyses of correlation between datasets to data derived from the short cores due to their temporal resolution, particularly in the years when biomonitoring was initiated (~1980s). Importantly, only the years where data was available across all four datasets were included in the comparison. This approach ensured a comprehensive comparison.

The ddPCR concentrations, measured in copies per µL, of the three replicates per sample were correlated for consistency, with each dye copy number (FAM and HEX) representing a separate reaction (supplementary Fig S4). Afterwards, the average DNA copy number of *S. marinoi* and *A. malmogiense* per sample was determined by ddPCR. The results were normalized based on the wet weight of the sediment used in the DNA extraction process (Brasell et al., 2022). This normalization was performed to ensure consistency in the comparison of data across samples. However, it should be noted that the ratio of the two species remains unaffected by this normalization.

Dinoflagellate and diatom community dissimilarities were assessed across all PCR replicates using nonmetric multidimensional scaling (NMDS) via the metaDMS function (seed number 12345) from the R-package vegan (Dixon, 2003). Ordination plots were generated for each short core location.

2.6.2. Correlations among datasets

All four datasets were pairwise correlated against each other, calculating Pearson's correlations. Rows that did not show measurements in all datasets (presence of NAs) and outliers were removed according to the interquartile range method, calculating the first (0.25) and third (0.75) quartile of the input vector and the interquartile range (IQR) of the input vector. Then the IQR was multiplied by 1.5 to get H, which determines the threshold for which data points are considered outliers. Any variable that is less than the first quartile minus H, or greater than the third quartile plus H, was treated as outlier and was removed. No outliers were found using the interquartile range method, so no data points were removed. This pairwise correlation was done to evaluate if the two target species are appropriate proxies for our proposed molecular index.

For the analyses and visualizations, the following R packages were utilized: corrplot v0.92 (Taiyun, 2023), scales v1.2.1 (Wickham et al., 2022), ggpubr v0.6.0 (Kassambara, 2023), ggimage v0.3.3 (Yu, 2023), gridExtra v2.3 (Auguie and Antonov, 2017) and cowplot v1.1.1 (Wilke, 2020). Additionally, the

biomonitoring Dia/Dino-index was correlated against the biomonitoring ske/apo-index (extracted from the biomonitoring data), which is constructed from the target species *S. marinoi* (ske) and *A. malmogiense* (apo) representing the Dia/Dino-index. This was done to evaluate if the ske/apo-index represents the Dia/Dino-index. Prior to performing further analysis with the molecular ddPCR ske/apo-index, we controlled for any correlation between ddPCR copies of *A. malmogiense* and *S. marinoi*. This was done to ensure that any observed trends in the copy numbers of each species were independent of the overall DNA copies fluctuations in the sediment cores.

2.6.3. Ecological indices derived from the data

The ratio between ddPCR copies of *Skeletonema marinoi* and *Apocalathium malmogiense*, defined as the ske/apo-index, for each horizon, 5-year interval and the biomonitoring data was calculated using the following formula:

$$\text{ske/apo-index} = \frac{Sm\ conc.}{Sm\ conc.\ + Am\ conc.}$$

The Dia/Dino-index of the biomonitoring data is based on Wasmund (2017). It was calculated with the following formula (Wasmund et al., 2017):

$$\text{Dia/Dino-index} = \frac{diatom\ relative\ abundance}{diatom\ relative\ abundance + dinoflagellate\ relative\ abundance}$$

The relative abundance of a group or species at a sampling site was determined by calculating its proportion of the overall biomass at the site. The ddPCR copies, read numbers and abundances were formatted and combined with sample metadata using the R packages “tidyverse”, “readxl” and “writexl” (Ooms et al., 2023; Wickham et al., 2023, 2019).

2.6.4. Changes over time

The R package “ggplot2” v0.4.3 (Wickham, 2016) was used to visualize the relative abundance of the two species or their phyla over time at the three locations using all four datasets. The ske/apo-index, the Dia/Dino index of the biomonitoring data, and ddPCR data were visualized over time. A linear trend

analysis was performed by calculating the correlation between the ddPCR copies and time for both species, utilizing all data points from the three short cores. This was done using Pearson's correlation.

3. Results

3.1. Data Consistency

The ddPCR copy number measurement of the replicates resulted in consistent copies per sample, justifying the use of average copy numbers. Full data of replicate copy numbers are available in the supplements (See Supplementary Tab. S5). The dinoflagellate and diatom communities of the metabarcoding replicates (EMBL Project PRJEB74483) resulted in similar composition except for a few single replicates (see Supplementary Figure S5, Table S6). After bioinformatic processing and cleaning, the metabarcoding dataset contained 6,590 ASVs and 30,775,892 reads. Thereof 120 AVSs and 2,164,127 reads corresponded to diatoms and 852 ASVs and 8,311,983 reads to dinoflagellates. Biomonitoring data has only been available since the 1980s, and the relative abundance of taxa in the datasets varies dynamically (Figure S6). We found no statistically significant correlation between *A. malmogiense* and *S. marinoi* (R=-0.004, p=0.96) (See Fig. S7).

3.2. Correlation Analyses

Figure 4 presents a correlation analysis of four datasets for *A. malmogiense* and *S. marinoi* or their respective phyla, which are employed as a Dia/Dino-index control. It is noteworthy that *A. malmogiense* exhibits a robust correlation between ddPCR copies and genus-level biomonitoring data (R = 0.891), although the correlation is less pronounced at the phylum level (R = 0.414) and with metabarcoding read count (R = 0.262). For *S. marinoi*, correlations are stronger at the phylum level (R = 0.501) and with metabarcoding read count (R = 0.492), but weaker at the genus level (R = 0.363). Despite these variations, there are notable correlations between ddPCR copies and biomonitoring data for both species.

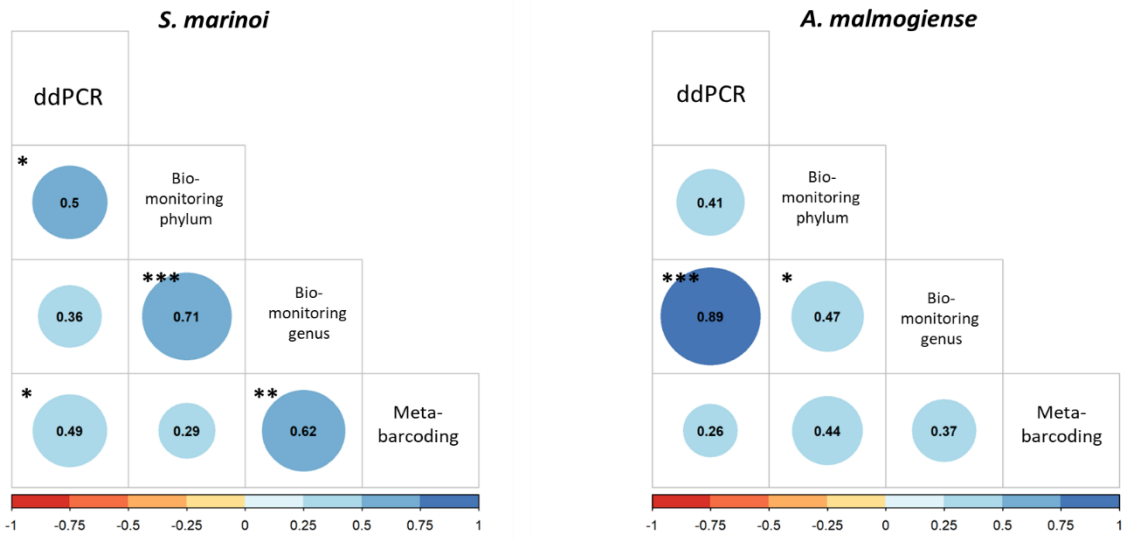

Fig. 4: Pearson's correlation between the four different datasets for *A. malmogiense* and *S. marinoi*: 1) ddPCR copies , 2) Biomonitoring phylum, 3) Biomonitoring species, 4) Metabarcoding phylum. Correlation coefficients from -1 (red) to 1 (blue) and their significance. *: p<0.05, **: p<0.01, ***: p<0.001. Detailed information in supplementary Table S7.

3.3. Trends Over the past Decades and Centuries

A linear trend analysis of ddPCR copies over time reveals a significant positive correlation between the sediment sample age (Year) and the ddPCR copies of *A. malmogiense*, indicating a substantial increase in its copies in recent years (R=0.21, p<0.05). Conversely, *S. marinoi* shows a weaker correlation (R=0.09) that is not statistically significant (p=0.366). These findings support the interpretation of a recent shift in dominance from *S. marinoi* to *A. malmogiense*, a trend further corroborated by the regression analysis (Fig. 5A).

This shift is further supported by the trends in the ske/apo-index, which is based on the ddPCR data and calculated at 5-year intervals, and the Dia/Dino-index, which is based on the biomonitoring data of the area of the respective core location (Diatom and Dinoflagellate complex of the spring bloom) (Fig. 5B). Both indices show drops in recent years. Notably, however, the biomonitoring based Dia/Dino-index starts at values below 1 during the timespan of monitoring, while the ske/apo-index reaches values of 1 in the early years of the core. This record suggests that the initial shift in dominance - and hence drop in ske/apo-index - occurs earlier than the timespan covered by the biomonitoring data. In the EGB, a first drop can be observed in the ddPCR data around 1980, which then advances into the late 1990s. The Dia/Dino-index shows that the index was around 0.6 in the 1980s, and decreased to

0.12 in recent years. In the GOF, the ske/apo-index and Dia/Dino-index show contradicting dynamics. While the ddPCR results show a first decrease in the 1930s and a second in the 1960s-1970s, the biomonitoring results show an increase since the first data point in the 1980s, though never reaching 1. In the LD, a first decrease in the ske/apo-index can be observed in the 1930s as well, followed by a second decrease in the 1980s and a third decrease around the 2000s. The Dia/Dino-index also shows an ongoing decrease since the 1980s, becoming more rapid in the 2010s.

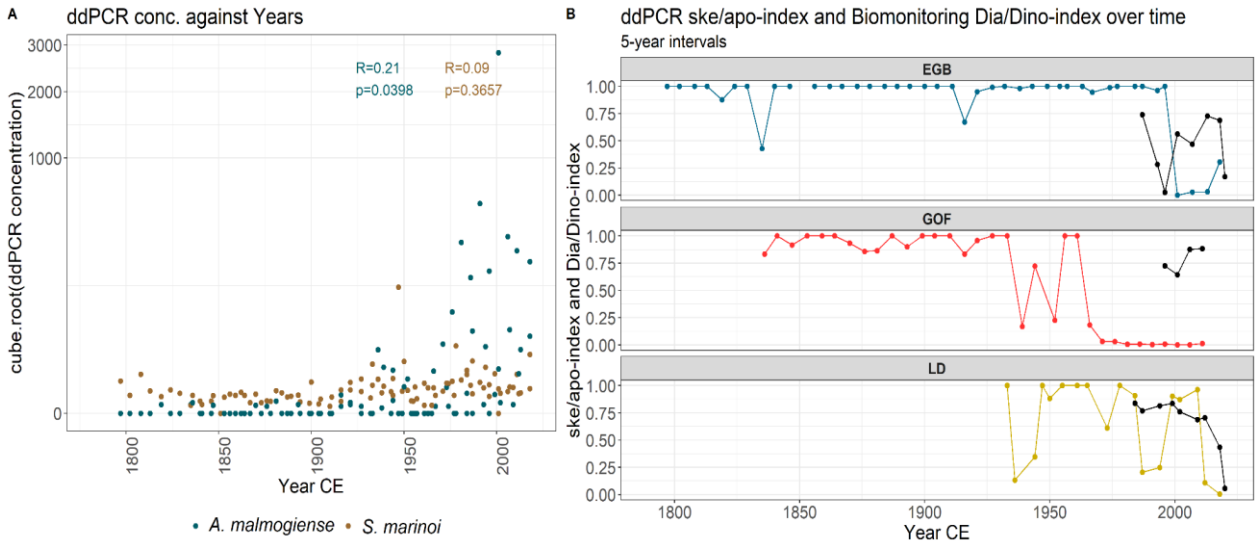

Fig. 5: A) Pearson's correlation between time and ddPCR concentration (measured in copies/µL, cube root transformed) for *A. malmogiense* (blue) and *S. marinoi* (brown). B) ddPCR ske/apo-index over time in five year intervals at all the locations (1798-2018) (coloured) and biomonitoring Dia/Dino-index over time in five year intervals at all three locations (1984-2021) (black).

Overall, the two biomonitoring indices display a weak but significant positive correlation (R=0.39, p=0.012). This suggests that the molecular ske/apo-index could serve as a useful tool in certain contexts, as it shows a similar trend to the Dia/Dino-index. However, the relationship does not hold consistently across all regions or time intervals, particularly in the GOF where no clear relation is observed.

3.4. Trends over Millennia

To evaluate long-term variability and stability of the target species ratio, we extended our investigation into the past. This revealed different patterns in EGB and the GOF. As depicted in Figure 6, in the EGB the species ratio was generally stable, with *S. marinoi* being more abundant once it occurred. However, there were two drops in the index, indicating a dominant shift to *A. malmogiense*. In contrast, the GOF

shows lower copy numbers of *S. marinoi,* and *A. malmogiense* occurred more frequently than in the EGB. As a result the index in the GOF was more dynamic with multiple dominance shifts.

These patterns were compared visually with the historical development of the Baltic Sea, focusing on detailed changes in oxygen and atmospheric temperature, and considering overall changes in salinity in the different stages of the Baltic. The air temperature, indicated by a blue line, showed an overall upward trend despite intermittent fluctuations. The dynamics of XRF Mn/Ti proportion at the EGB, represented by a black line, and the main anoxic phases in the central Baltic Sea, indicated by red bars, were also considered in this comparison.

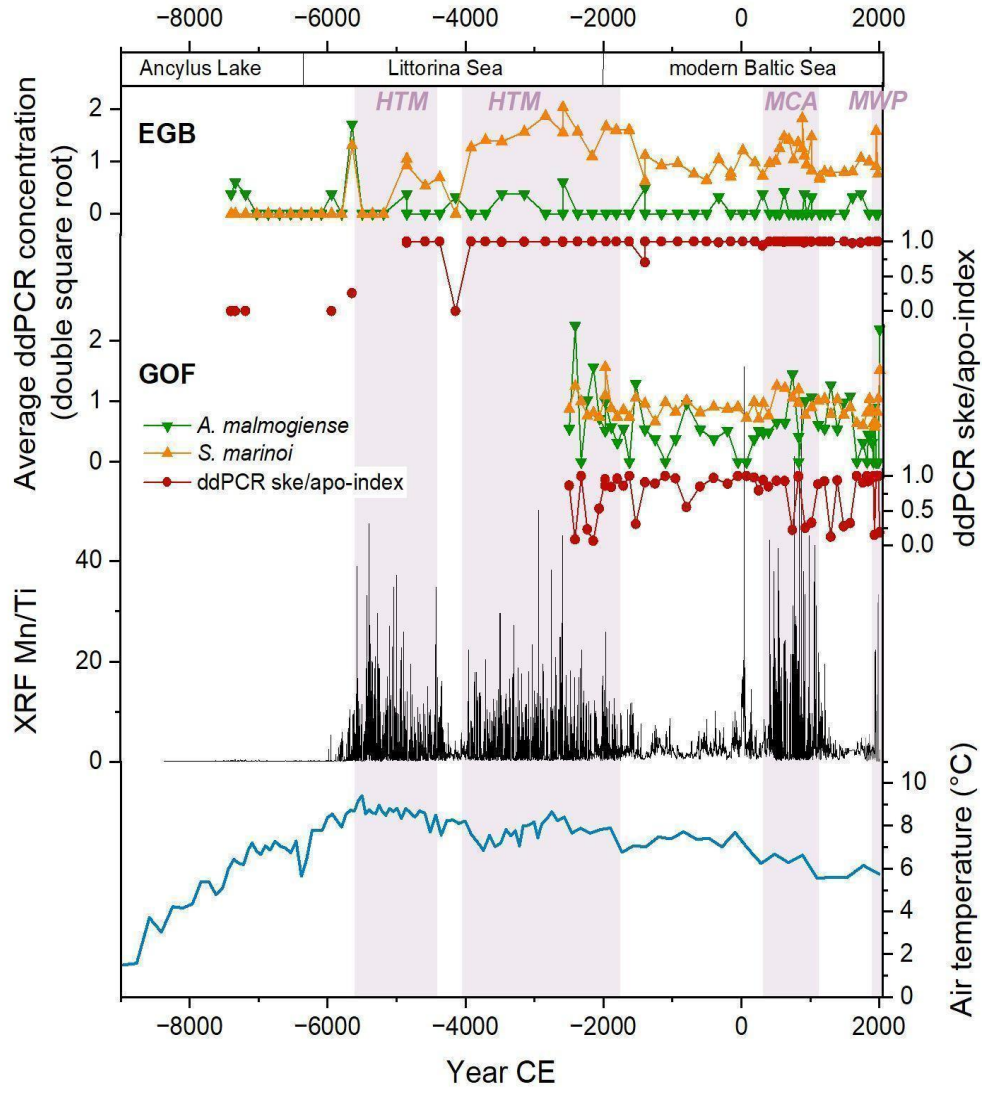

Fig. 6: Temporal development of the double square root transformed ddPCR concentration (expressed in copies/µL) at two locations: the Eastern Gotland Basin (EGB) and the Gulf of Finland (GOF). The ddPCR ske/apo-index over time is highlighted in red. The air temperature is shown in blue and based on data from Lake Flarken, Sweden (Seppä et al., 2005). The XRF Mn/Ti data, proxy for oxygen levels, from core EMB262/6-30GC is pin black. Red bars are used to indicate the primary anoxic phases in the central Baltic Sea (Andrén et al., 2011; Rosentau et al., 2017). Main phases of the Baltic Sea are used from Andrén et al. (2011) and Rosentau et al. (2017). Climatic phases: HTM: Holocene Thermal Maximum, MCA: Medieval Climate Anomaly, MWP: Modern Warm Period.

4. Discussion

4.1. Validation of Methods and Extension of Monitoring Records into the past - ske/apo-index

We compared three different methods to investigate diatom and dinoflagellate dominance: available biomonitoring data from ICES (2022), which dates back to the 1980s, DNA metabarcoding of eukaryotes, and species-specific ddPCR reactions. The molecular data showed consistent results across replicates, both in terms of community composition and in quantity of single species reads or ddPCR copies. The observed ddPCR copies are independent from each other, as evidenced by the lack of observed correlation between *A. malmogiense* and *S. marinoi* copy numbers. This independence is crucial as it allows us to avoid attributing changes in species-specific copy numbers to broader fluctuations in sedaDNA concentrations. By checking for this, we ensure a more accurate and species-specific trend analysis, thereby enhancing the reliability of our subsequent ske/apo-index analysis. It is essential to acknowledge that while our methodology offers valuable insights, it is not without limitations that could potentially impact the interpretability of the developed ddPCR index. For instance, the low correlations between the ddPCR reads and monitoring for the diatom might affect the utility of the index. Despite these challenges, we believe that the ddPCR index, when interpreted with an understanding of these limitations, can provide meaningful and concise insights into ecological dynamics. Furthermore, it is noteworthy that dry weight normalization (Siano et al., 2021) could be advantageous if the emphasis were shifted to absolute quantification. However, given that our index is based on the ratio of two organisms, this type of normalization is not a decisive factor, as both concentrations would be normalized in a similar manner.

Metabarcoding demonstrated its capability to detect changes in both phyla and species, although it was not as sensitive as ddPCR to changes in the biomonitoring data when it came to *A. malmogiense* or the diatom phylum. Diatoms were also underrepresented. While metabarcoding is effective in reflecting biomonitoring-based dynamics, it is of particular importance to note that it not only focuses on spring bloom organisms crucial for the Dia/Dino index. A broad metabarcoding primer is needed for investigating different phyla, typically resulting in a loss of taxonomic resolution, such that retrieved sequences can often not be identified to species level (Bunse et al., 2016; Haraguchi et al., 2023; Jaanus et al., 2006). Moreover, we cannot ascertain whether the V7 eukaryotic metabarcoding primer amplifies both groups equally or preferentially amplifies one of the two phyla, thereby biasing the conclusions drawn for the Dia/Dino-index. In combination with the limited temporal resolution of

sediment core samples, which typically include more than a year, this prohibits the recovery of season-specific dynamics. In contrast, targeting specific, seasonally distinct species with ddPCR, i.e. spring dinoflagellates and diatoms, such as *A. malmogiense* and *S. marinoi*, provides some seasonal dynamics. While it is likely that most of this DNA detected in sediment stems from spring blooms, particularly *S. marinoi* is present in the system throughout the year and can bloom at other times as well. *S. marinoi* has also been observed in autumn phytoplankton communities. The retrieved signal from each sample will likely stem from multiple spring blooms, but might also include records of other seasons. Quantitative approaches for single species from eDNA are by now well established, and both ddPCR and real time quantitative PCR (qPCR) have been utilized for many years (e.g. Doi et al., 2015; Takahara et al., 2012). In some qPCR studies (Knudsen et al., 2019; Spear et al., 2015), no clear links between DNA concentrations and biomass estimates were established, whereas ddPCR is suggested to be more accurate, especially at low DNA concentrations (Doi et al., 2015), where it shows higher detection rates compared to qPCR, possibly due to its resistance to inhibition (Dingle et al., 2013; Rački et al., 2014).

The two species were found across many segments of the core, but the patterns of appearance differed: In the case of *A*. *malmogiense*, changes in copy numbers were particularly pronounced, as many samples were negative. In *S. marinoi* we observed more gradual changes between samples. An explanation for this could be different taphonomy of the sedaDNA of the two species, as the fate of the two target species differs substantially once the bloom ceases. While diatoms such as *S. marinoi* primarily sink to the sea floor, where many of them disintegrate and some transform into a stage of physiological resting, cells of *A. malmogiense* partly demineralize in the euphotic zone (Wasmund et al., 2017) or encyst. When deposited in anoxic sediment after the bloom, their DNA is retained in a resistant resting propagule (Spilling et al., 2018). *S. marinoi* not only occurs in the spring bloom, but throughout the year, with a second smaller bloom in September. Considering this, the probability of finding *S. marinoi* DNA in the sediment core is generally higher than of encountering *A. malmogiense* (Saravanan and Godhe, 2010). The findings of *A. malmogiense* DNA in the sediment thus point to a significant increase in dinoflagellates in the study area.

Our study underscores the potential of molecular tools in decoding historical events and long-term environmental trend dynamics through sedaDNA, and including organismal signals that are not present in the visual fossil record. Here, we use simple species-specific reactions, including a quantitative aspect, i.e. highly sensitive ddPCR. This can simplify data in comparison to metabarcoding or metagenomics (Gielings et al., 2021; Nguyen et al., 2023). Our analyses suggest that this method can provide insights into ecological changes by narrowing the focus on ecologically relevant key species

that are used as proxies and in indices for ecosystem status. It remains to be evaluated how strong the ecological conclusions from our case-study ske/apo index can be, but such simple approaches could be implemented in monitoring and management relatively easily. While our current research has primarily centred around duplex ddPCR, it is crucial to acknowledge the emerging potential of multiplex ddPCR as a promising approach for the future. By extending the available fluorescent channels to accommodate up to six distinct targets, researchers can now detect and quantify multiple targets simultaneously within a single assay (Wainman et al., 2024). For instance, in the context of the molecular Dia/Dino-index, it becomes feasible to select three target spring bloom species per taxa, thereby creating a more complex index that does not lose temporal resolution. In the case of the molecular Dia/Dino-index we test here, such assays would allow to include more than the two model species used in this study. These additional assays would enhance our understanding of functional diversity across different taxa. Specifically, during the Baltic spring bloom, both diatoms and dinoflagellates play a crucial role. They consist of several key species, each possessing distinct traits that contribute to the index (Haraguchi et al., 2023). In the central and northern parts of the Baltic Sea, the dinoflagellate community is dominated by three cold-water species: *Apocalathium malmogiense* (Peridiniales), *Biecheleria baltica* (Suessiales), and *Gymnodinium corollarium* (Gymnodiniales) (Kremp et al., 2009). These species, although similar in habit, exhibit significant phylogenetic and ecological differences. By incorporating these diverse species through appropriate assays, we can enhance the complexity and informative value of the index.

The possibility to retrieve palaeoecological records from a number of locations, i.e., from different regions of the Baltic Sea, as done here, enables a more precise validation of local ecological indices and the establishment of region specific values for good ecological status (GES). For the original Dia/Dino index, Wasmund (2017) investigated historical count data from the first half of the 20th century, to verify that the index was stable prior to eutrophication and to establish regional values for GES. The data indeed indicated a pre-eutrophication stability, and demonstrated that values for GES are not uniform across the Baltic Sea. They suggested regionally different values for GES, which are currently only established for the southern and central Baltic Sea (HELCOM, 2023), not for e.g., the GOF.

Our sediment core analyses indicate that the GOF was much less stable regarding the dominance of diatoms and dinoflagellates on millennial scales (Fig. 6). However, ske/apo-index values for the past century demonstrate that the current anthropogenic eutrophication is captured very well by changes in this index (Fig. 5). The instability of the index in the GOF, as well as the historical drops in the relatively stable EGB record (Fig. 6) rather points to the spring bloom composition being sensitive to both eutrophication and warming. Overall, the data highlights the importance of defining a GES for each

region, as the optimal index value is significantly influenced by the unique environmental conditions in each region.

4.2. Trends over the past Decades and Centuries

Monitoring records suggest that diatoms were more abundant than dinoflagellates in the past, making diatom dominance the preferred status, reflecting a GES of the Baltic Sea (Wasmund, 2017; Wasmund et al., 2017). According to Wasmund et al. (2017), the Dia/Dino-index points to a regime shift at the end of the 1980s in the central Baltic Sea. This is also recorded in our data using the ddPCR-based ske/apo-index, but in particular the GOF and the LD show earlier drops in the dominance reversal during the 20th century (Fig. 5). In the GOF, ske/apo-index values have remained low since the 1960s, and in both the LD and the GOF, an initial massive drop is recorded in the first half of the century, slightly predating the Second World War, which aligns with the introduction of artificial fertilizers in the early 20th century (Treitel, 2015). Results from the EGB and the LD show shifts around the 1980s when eutrophication was nearing its peak in the Baltic Sea.

The massive eutrophication in the open Baltic Sea started after the Second World War and reached its peak in nutrient concentrations (phosphorus and nitrogen) in the 1980s and 1990s (Gustafsson et al., 2012; HELCOM, 2021; Murray et al., 2019). Murray et al. (2019) conducted a comprehensive study, modelling the past, present, and future eutrophication status of the Baltic Sea. They identified a shift from a healthy state to a state with eutrophication issues occurring in the late 1950s and early 1960s. Our results align with this timeline, showing a noticeable drop in the ske/apo-index during the 1980s and 1990s. Interestingly, we also observed a similar drop in the 1950s and 1960s specifically in the GOF. Consequently, our data suggests that changes in the trophic status may trigger species-level responses, particularly evident in the increased copy numbers of *A. malmogiense*. Hence, the ske/apo-index built with sedaDNA ddPCR copies is a promising tool to estimate changes in the trophic state of the past beyond the instrumental period.

4.3. Trends over Millennia

Our study also examines the millennial dynamics of key phytoplankton species by analysing long cores from the EGB and GOF. This allows us to investigate the influence of various factors on phytoplankton species over long time scales. Our findings indicate that temperature increases can enhance the abundance of dinoflagellates, but not diatoms. Salinity shifts may also alter the competitive dynamics

between the two species. However, the temporal dynamics of the two species are not fully congruent across the examined cores.

The EGB core exhibited a relatively stable dominance of *Skeletonema* prior to the 20th century, with only two notable shifts in the ske/apo-index. In contrast, the GOF exhibited a more dynamic history, with numerous shifts among the two species across millennia.

These shifts are associated with periods of ecosystem change and are not directly linked to a specific condition among the abiotic factors, including oxygen level, temperature, and salinity. Conversely, any significant alteration in these parameters can prompt a response in the ske/apo-index. The long-term data of the two species suggest that *A. malmogiense* is competitively superior during periods of ecosystem instability, while *S. marinoi* populations experience a drastic decline. In conclusion, the observed difference in the stability of the ske/apo-index between the two sites highlights the complex dynamics of the Baltic Sea marine ecosystem and underscores the necessity for further research. The observed difference in stability of the ske/apo-index between the two sites is striking. The stability across millennia in the EGB in conjunction with the late drop of the ske/apo-index compared to the GOF and LD in the past century, could be considered an indication of a highly resilient ecosystem in the Eastern Gotland Basin and a high tolerance to environmental change. However, the variability in the GOF is mostly caused by a high prevalence of *A. malmogiense* throughout the core, and the less striking dominance of *S. marinoi*. This could also be a reflection of the specific adaptation of *A. malmogiense* to cold-water habitats (Logares et al., 2007). The species is often associated with sea ice in coastal waters, and the conditions in the GOF are generally more conducive to its establishment. This underscores the complexity of the dynamics of the Baltic Sea marine ecosystem and highlights the need for further research.

5. Conclusion

Using species-specific ddPCR reactions allowed us to reconstruct species-level responses to past ecosystem perturbations caused by anthropogenic pressures, such as eutrophication. Our analysis resulted in a new index derived from the existing Dia/Dino-index, the ske/apo-index, which provides an attainable historical perspective on shifts in species dominance over time. This methodology enabled us to develop a time series of shifting species dominance extending back multiple millennia, and thus reaching much further back than modern biomonitoring, which only began in the 1980s. Our findings indicate that certain areas of the Baltic Sea showed a stable relationship of the proxy diatom

and dinoflagellate species across millennia, that was altered only very seldomly, as in the 20th century. This affirms that we can use an index, which is derived from observing shifts in dominance between diatoms and dinoflagellates, to deduce GES in these regions. Furthermore, it demonstrates the utility of molecular palaeoecological analyses to develop simple indices for biomonitoring and extend time series. Our analysis shows that the initial changes in the Baltic Sea during the 20th century, which notably predate standard biomonitoring programs, began around or slightly before the Second World War. This change to aligns with the early 20th century intensification of urbanisation, industrialisation, and the introduction of artificial fertilizers (Treitel, 2015). In summary, the change is co-eval with the start of the “Great Acceleration” of anthropogenic pressure on Earth (Steffen et al., 2015), highlighting consequences of this pressure in the sedimentary stratigraphy of marine phytoplankton.

6. Supplementary Information

6.1. Acknowledgements

This study was funded by the K314/2020 grant of the Collaborative Excellence Programme of the Leibniz Association. MB is supported by the Hessian Ministry of Higher Education, Research and the Arts through the LOEWE Centre for Translational Biodiversity Genomics. We thank Matthias Moros for performing radionuclide measurements of the GOF MUC. For help with the initial primer design we thank Ilayda Ayyuce Kocak and Kim Isabelle Mayer. For setting up the ancient DNA clean laboratory in Frankfurt where all DNA extractions were done by AS and JR, we thank Leonie Schardt. EA was financially supported by a grant provided by The Foundation for Baltic and East European Studies (42/19).

6.2. Data Availability Statement

Metabarcoding sequencing data can be accessed at EMBL (Project PRJEB74483). Unix shell scripts, R scripts and data are available at GitHub (https://github.com/Alex-132/EI_paper_rep).

6.3. Supplementary data

Supplementary data can be accessed via the publication:

https://doi.org/10.1016/j.ecolind.2024.112494

Chapter 2 – Multi-Millennial Genetic Resilience of Baltic Diatom Populations disturbed in the past Centuries

In review at *Global Change Biology*

Alexandra Schmidt[1,2*], Sarah Bolius[3], Anna Chagas[1], Juliane Romahn[4,5,6], Jérôme Kaiser[3], Helge W. Arz[3], Miklós Bálint[4,5,6], Anke Kremp[3], Laura S. Epp[1*]

1. Environmental Genomics, Department of Biology, University of Konstanz, Constance, Germany
2. international Max Planck Research School Quantitative Behaviour Ecology & Evolution, Constance, Germany
3. Leibniz Institute for Baltic Sea Research Warnemünde, Rostock, Germany
4. Senckenberg Biodiversity and Climate Research Centre, Frankfurt am Main, Germany
5. Loewe Center for Translational Biodiversity Genomics (LOEWE-TBG), Frankfurt am Main, Germany
6. Institute for Insect Biotechnology, Justus Liebig University, Giessen, Germany

* Correspondence

Abstract

Little is known about the genetic diversity and stability of natural populations over millennial time scales, although the current biodiversity crisis calls for heightened understanding. Marine phytoplankton, the primary producers forming the basis of food webs in the oceans, play a pivotal role in maintaining marine ecosystems health and serve as indicators of environmental change. This study examines the genetic diversity and shifts in allelic composition in the diatom species *Skeletonema marinoi* over ~ 8000 years in the Baltic Sea by analysing chloroplast and mitochondrial genomes. Ancient environmental DNA (aeDNA) from sediment cores demonstrates stability and resilience of genetic composition and diversity of this species across millennia in the context of major climate events. Accelerated change in allelic composition is observed from historical periods onwards, coinciding with times of intensifying human activity, like the Roman Empire, the Viking Age, and the Hanseatic Age, suggesting that anthropogenic stressors have profoundly impacted this species for the last two millennia. The data indicate a very high natural stability and resilience of the genomic composition of the species and underscore the importance of uncovering genomic disruptions caused by human impact on organisms, even those not directly exploited, to better predict and manage future biodiversity.

1. Introduction

Human activities have had a profound impact on natural environments, resulting in the endangerment of a multitude of ecosystems, including marine environments (Goulletquer et al., 2014). Phytoplankton forms the basis of marine food webs, and, as crucial components of marine ecosystems, changes in this group are central to understanding ecosystem shifts (Panja et al., 2024). Among the phytoplankton, diatoms, which play a significant role in global biogeochemical cycles, are sensitive bioindicators (Benoiston et al., 2017), as the different species have specific requirements. *Skeletonema marinoi*, a prominent marine diatom species (Johansson et al., 2019), is influenced by temperature, migration, and human activities (Nakweya, 2023). For this species, changes in bloom timing (Hjerne et al., 2019) and optimal growth temperature (Hattich et al., 2024) have already been observed as a result of recent climate change. Investigation of marine phytoplankton response to current global changes are mostly based on taxonomic diversity, but recent studies show that diatoms also quickly adapt to environmental changes through acclimation and genetic adaptation on a population genomic level (Rynearson et al., 2022). In fact, intraspecific genomic variation is an important factor in the response and resilience of diatoms to environmental perturbations (Godhe and Rynearson, 2017). Thus, investigating the genomic

composition of diatom populations and its changes is crucial for understanding their adaptability to environmental changes.

The Baltic Sea, despite its relatively brief history, has undergone significant transformations, making it suitable for studies addressing organism responses to environmental changes. For the last ~ 10,000 years, these transformations include a transition between fresh and brackish water around 8,500-8,000 years ago (Björck, 2008) and major climate changes since then (Zillén et al., 2008). Due to its shallow enclosed nature and a steep salinity gradient, modern biodiversity is relatively low (Ojaveer et al., 2010). Current pressures such as pollution, eutrophication and global warming present significant challenges to the biota (Reusch et al., 2018).

Sediments can serve as ecological archives when undisturbed, offering insights into past environmental changes and biodiversity shifts (Smol, 2010), and provide long time series of phytoplankton dynamics. Phytoplankton archives in sediments include biomarkers, microfossils, (living) resting stages, and sedimentary ancient DNA (sedaDNA). These remains contain information on biodiversity and can reveal changes in adaptive traits (Ellegaard et al., 2020; Hattich et al., 2024). Here, we develop a time series of population-level responses of the diatom *S. marinoi* in the Baltic Sea, by employing a targeted approach to enrich chloroplast and mitochondrial genomes using hybridization enrichment. This allows us to analyse genetic diversity and shifts in allelic composition across complete organellar genomes. We investigate 1) the degree of stability, response and resilience of *S. marinoi* to natural environmental changes in the Baltic Sea over the last ca. 8000 years, and 2) identify changes in the genetic composition of populations in recent centuries of heightened anthropogenic activities.

2. Results

We analysed sediment samples of two cores located in the Baltic Sea, the Eastern Gotland Basin (EGB) and the Gulf of Finland (GOF) (Fig. 7A), covering a time span up to approximately the Ancylus Lake (extrapolated age: 8148 cal yr BP) (BP; with present = 1950 Common Era) (Fig. 7B). We investigated the population structure of *S. marinoi* over this period at the two locations by focusing on Single Nucleotide Polymorphisms (SNPs) on the organelle genomes (Fig. 7C). This was achieved by target enrichment of the chloroplast and the mitochondrion.

This study provides an analysis of the genetic dynamics of *S. marinoi* populations in the EGB and GOF over millennia, revealing insights into the effects of both natural environmental changes and anthropogenic activities. The use of RNA baits for hybridization enrichment resulted in a higher degree

of resolution, enabling the identification of a greater number of SNPs in both mitochondrial and chloroplast genomes. The analysis revealed that climate events exert an influence on genetic variations, with specific climate events aligning with temporal variations in the population's genetic makeup, but that the genetic composition reverted back to a state that was mostly stable across millennia in between these events. Longer lasting patterns of genetic change over time were observed at both sites in the past two millennia, coinciding with periods of intensified anthropogenic activities. These findings imply high resilience of *S. marinoi* populations across time and climate events across millennia, along with more recent changes.

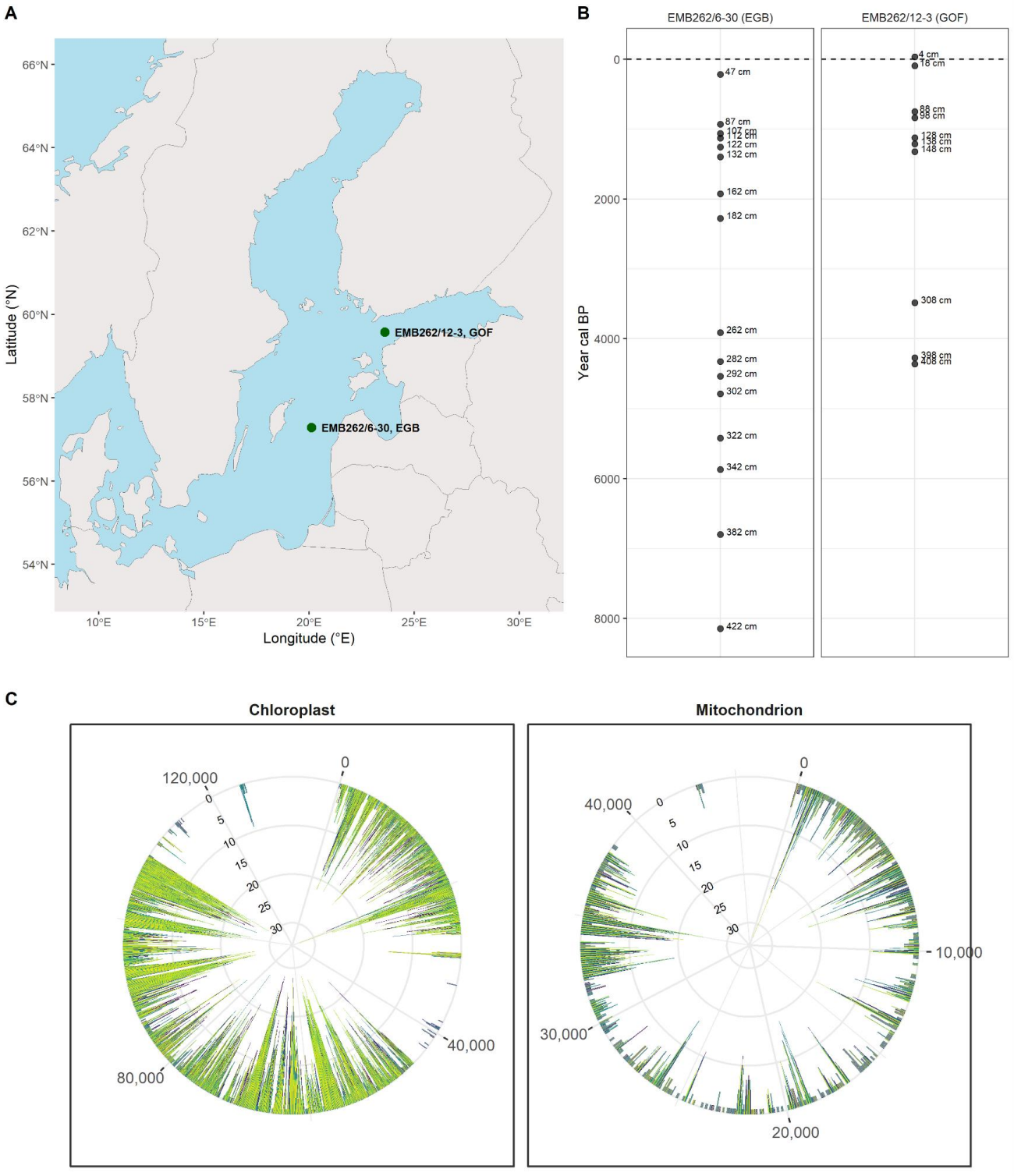

Fig. 7. A) Location of the coring sites and corresponding cores in the central Baltic Sea. Gulf of Finland (GOF; core EMB262/12-3GC) and Eastern Gotland Basin (EGB; core EMB262/6-30GC). B) Sample distribution (in cm) in the sediment cores and corresponding ages. C) Number of SNPs called per 100 base pairs across the genome for each sample. The mitochondrion and chloroplast genomes of *S. marinoi* are shown. The gaps indicate the repeat masked regions.

2.1. General Data Assessment

2.1.1. DNA vs. RNA Baits

In order to study past *S. marinoi* population dynamics in the EGB and GOF, we employed DNA and RNA baits for hybridization enrichment of full chloroplast and mitochondrial genomes. The use of RNA baits resulted in a higher degree of resolution. Following trimming, DNA and RNA baits yielded 34.9 million and 51.8 million sequences, respectively. Of these, 34.8 million (DNA) and 35.3 million (RNA) reads were successfully mapped to reference organelles (Fig. 8A and Supplementary Table S1). In the case of RNA baits, 6.41 million reads were mapped to the mitochondrion, while 28.9 million were mapped to the chloroplast. For DNA baits, the numbers were 6.27 million and 28.6 million, respectively. The RNA baits identified a greater number of SNPs in both the mitochondrial (301 vs. 92) and chloroplast (1716 vs. 403) genomes. Consequently, the analysis focuses on the results obtained from RNA baits. The results of the DNA bait analysis can be found in the supplementary section 2.1.

The average coverage achieved for the experimental samples was generally high, with an average mitochondrial coverage of 78.77 and an average chloroplast coverage of 575.5. This coverage demonstrates the efficiency of the hybridisation capture approach and ensures reliability of the genomic data collected. Furthermore, the Extraction Blanks and Library Blanks produced low to non-meaningful coverage (see Supplementary Table S2). All information about the samples, corresponding age, location and other metadata is available in Supplementary Table S3.

2.1.2. Ancient DNA – Damage Pattern

We analysed damage patterns in ancient DNA (aDNA) samples, focusing on C-to-T substitutions. These substitutions are key aDNA authentication markers due to their pervasiveness in aDNA and their function in indicating cytosine deamination, a prevalent form of DNA damage. No significant correlation ($R = 0.18$, $p = 0.315$) was found between mapping coverage and substitution frequency, suggesting that damage pattern does not affect the mapping coverage. The degree of damage was found to significantly increase with the age of the samples ($R = 0.65$, $p = 0.0003$, Fig. 8B). A greater number of sequences were mapped to the chloroplast due to its larger genome size. This allows a greater number of reads to be used to detect damage patterns, increasing the overall value of the data.

2.1.3. Genetic Diversity ad Spatial Differentiation

A comparison of the genetic differences between *S. marinoi* retrieved from the EGB and the GOF respectively, revealed that while the two locations exhibited some differences, they also shared a considerable degree of similarity. This was evidenced by the FST value, a measure used in population genetics to quantify the genetic differentiation between populations, which ranged between 0.05 and 0.1 (see Supplementary Material, Fig. S6). Notably, the genomic data from EGB shows a higher level of genetic diversity than the one from GOF. This is presented by different measures of distinct variant diversity, with significant differences observed (p-values: 0.002, 0.005, 0.006, see Fig. 8C-E).

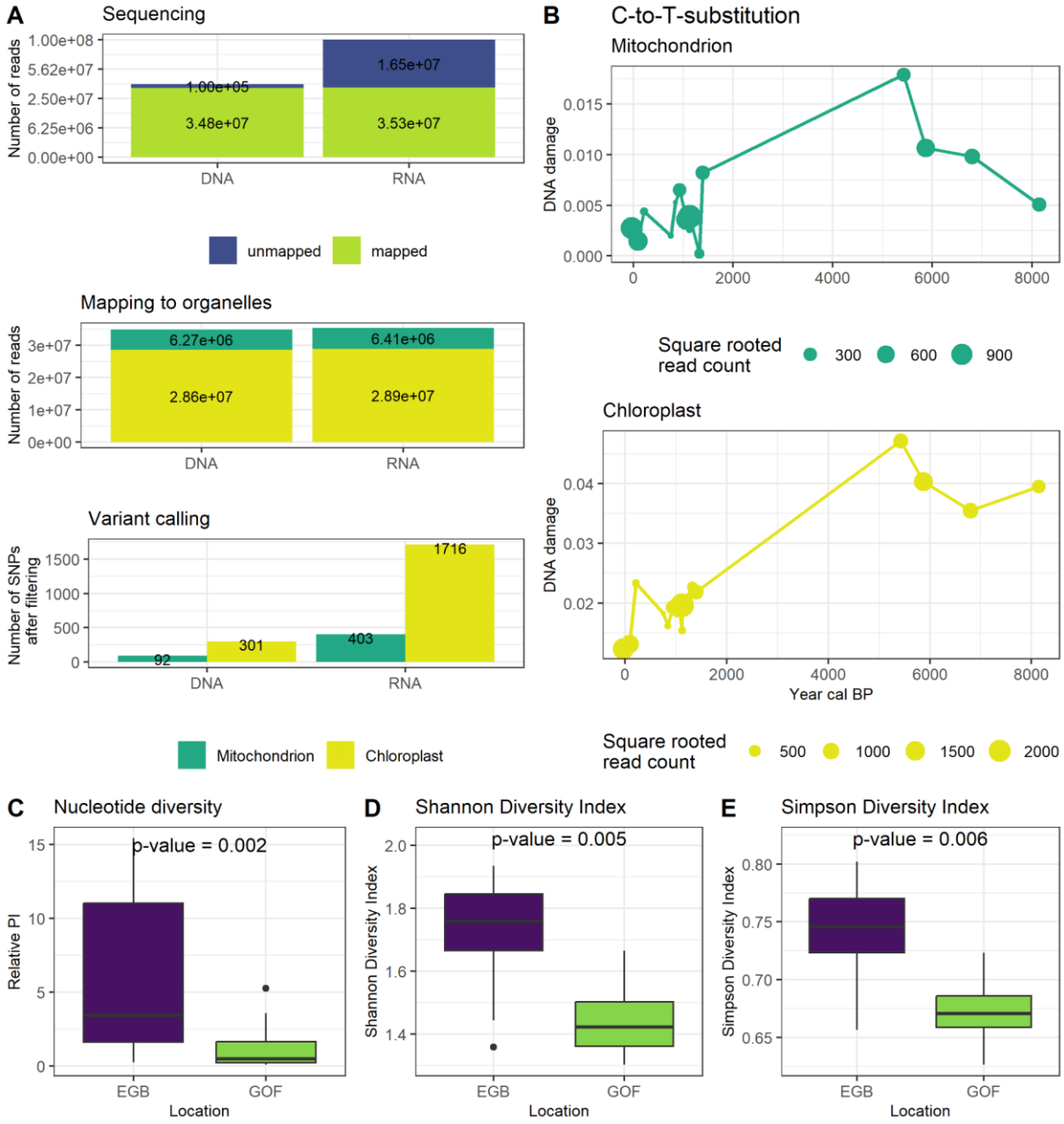

Fig. 8: Data assessment: A) Sequencing Yield and Single Nucleotide Polymorphisms (SNPs): Post-trimming sequencing yield for DNA and RNA baits, and the number of SNPs detected in mitochondrial and chloroplast genomes for each bait set. B) C-to-T Substitution: Analysis of C-to-T substitutions over time for each organelle from all data (GOF & EGB). C-E) Comparative analysis of normalized nucleotide diversity and diversity indices. C) Relative Nucleotide Diversity (PI), D) Shannon, and E) Simpson between Eastern Gotland Basin (EGB) and Gulf of Finland (GOF). Each bar graph shows data for both sites with significant p-values.

2.2. Effects of Environmental Change

Our study on *S. marinoi* organelles at two locations in the Baltic Sea reveals that across several millennia the genetic composition of the population remained stable, punctuated by differences in specific periods, putatively coinciding with environmental changes. A Principal Component Analysis (PCA), conducted to investigate differences in allelic composition between sediment layers, showed that the majority of samples are situated within a primary cluster, but a number of smaller clusters are found that correspond with periods marked by specific environmental events (Fig. 9A). Together, PC1 and PC2 describe 42.6% of the data variance.

The retrieved specific clusters (Fig. 9A) correspond to specific climate periods, including the Holocene Thermal Maximum (HTM, 10,000-6,000 cal yr BP), Little Ice Age/Late Antique Little Ice Age (LIA, 550-100 cal yr BP/LALIA, 1,414-1,290 cal yr BP), Medieval Climate Anomaly (MCA), and Modern Warm Period (MWP). We verified that existing variation in allelic composition is not a result of the C-to-T-substitution rate by performing a PERMANOVA, which showed that the C-to-T-substitution rate accounted for approximately 6.7% of the variation in the PCA space ($R^2 = 0.067$, $p = 0.19$), though this was not statistically significant (see Supplement, Fig. S5). Additionally, we performed a PERMANOVA to assess the impact of Total Organic Carbon (TOC) on the variation in the PCA space, which showed that TOC accounted for approximately 4.5% of the variation ($R^2 = 0.045$, $p = 0.328$), also not statistically significant.

In the EGB, the period under consideration extends to the Ancylus Lake (extrapolated age: 8,148 cal yr BP), and responses are observed during the HTM, LALIA, MCA, and LIA periods. The GOF record, which covers the last ca. 4,300 years, displays changes during the LIA and MWP periods. During periods without profound climate events, we observe a consistency in allelic composition, which points to resilience of the algal populations over time. However, within warm periods (e.g. HTM and MCA), there is a notable degree of variability, particularly for EGB. This pattern of long-term stability and resilience, interrupted by distinct variation, is visualized in Fig. 9B, depicting changes along PC1 in both cores. This shows temporal variations that align with specific climate events, suggesting that the population's genetic makeup is undergoing changes in response to these events. Additionally, nucleotide diversity increased significantly during the transition from the Littorina Sea to the Modern Baltic Sea, around 4,000 cal yr BP, and then successively decreased again (Supplementary Material, Fig S7).

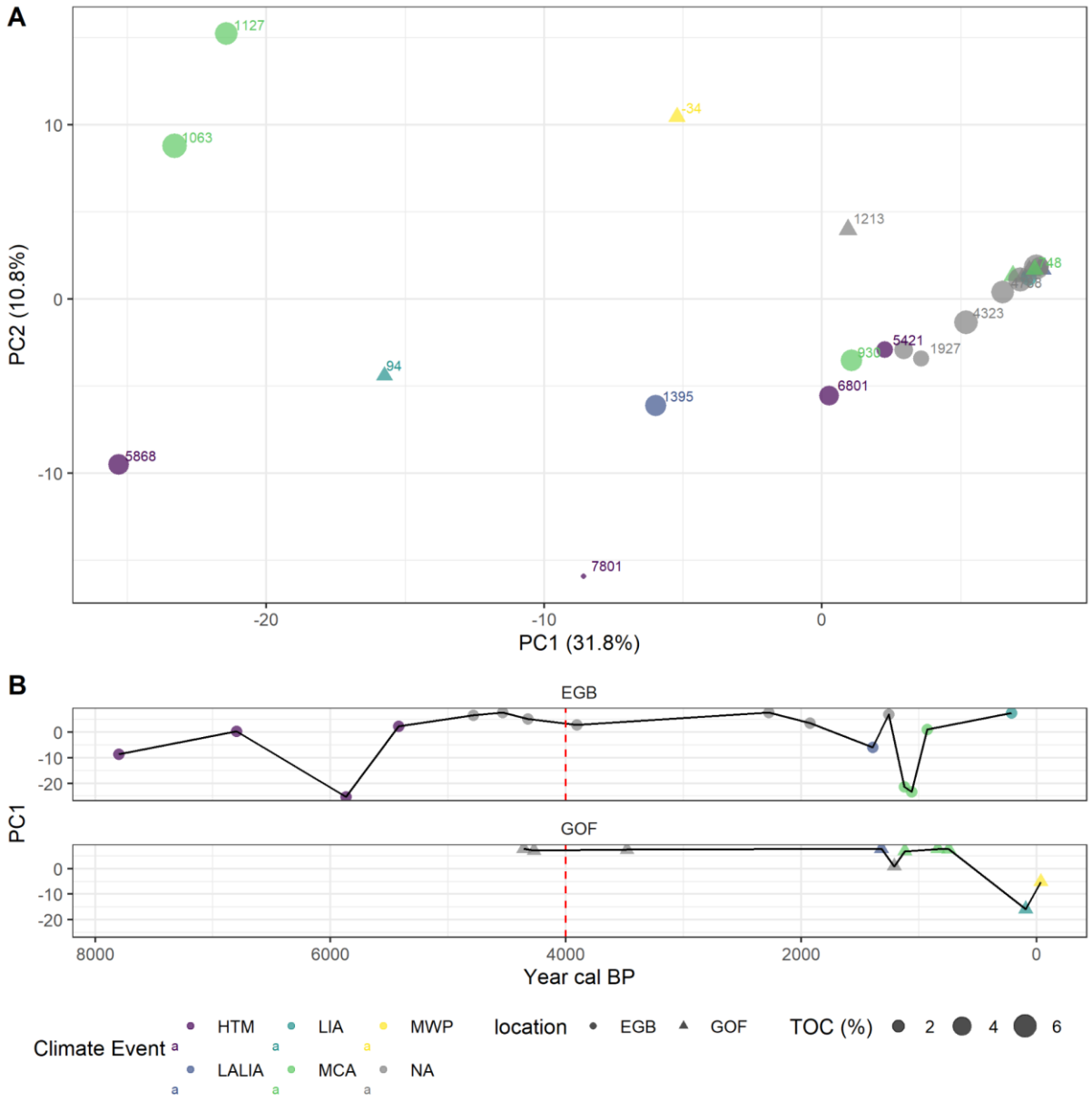

Fig. 9: Principal component analyses of the allelic composition of *Skeletonema marinoi* organelles, demonstrating the genetic diversity and population structure. A) PC1 and PC2 are categorized by climate events, total organic carbon (TOC) and the age of each sample is given for both sites. B) PC1 versus time for both sites. The red dashed line marks the transition from the Littorina Sea to the Modern Baltic Sea. Climate events include the Holocene Thermal Maximum (HTM), Late Antique Little Ice Age (LALIA), Medieval Climate Anomaly (MCA), Little Ice Age (LIA), Modern Warm Period (MWP) and NA = No event

Allele turnover, a measure of the rate at which new alleles replace old ones, provides insights into genetic variation over time. Figure 10 displays patterns of genetic change over time. A Generalized Additive Model (GAM) was used to analyse allele turnover as a function of time.

At EGB, an increase in allele turnover is observed around 1,500-1,000 cal yr BP, indicating a potential for heightened genetic change from the Medieval period onwards. At GOF, turnover remains consistent

until around 94 to -34 cal yr BP. While industrialization and anthropogenic impacts may be associated with recent genetic shifts, establishing a causal link between these changes and earlier historical periods requires caution.

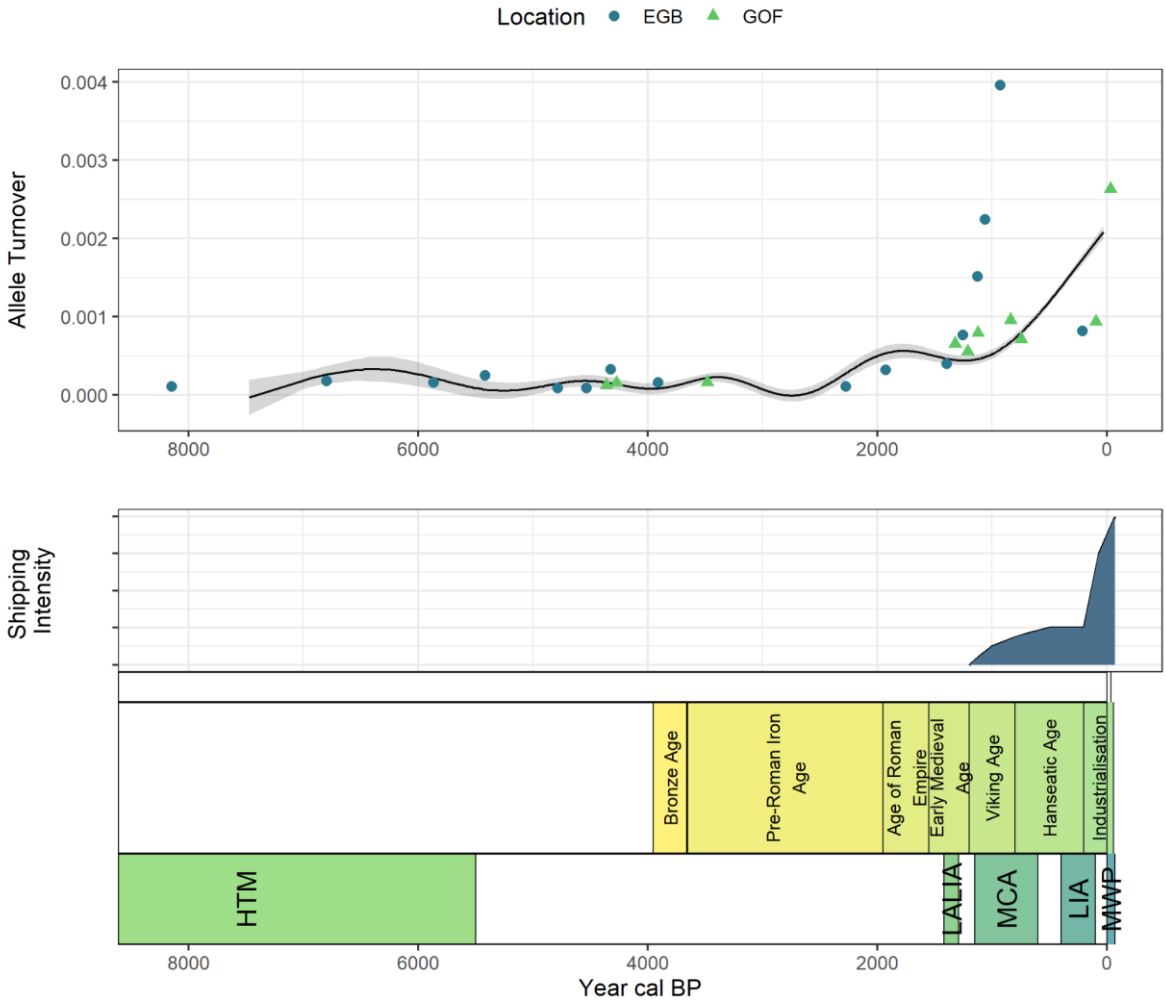

Fig. 10: Temporal Analysis of Allele Turnover in *Skeletonema marinoi*. This figure presents the Generalized Additive Model (GAM) fitted to the allele turnover over time (BP). The data includes both Eastern Gotland Basin (EGB) and Gulf of Finland (GOF) sites. Key environmental events, such as the Holocene Thermal Maximum (HTM), Late Antique Little Ice Age (LALIA), Medieval Climate Anomaly, Little Ice Age (LIA), and Modern Warm Period (MWP), are noted. Additionally, anthropogenic periods and associated shipping activity estimated from literature sources are shown.

3. Discussion

The millennial-long stability, spanning from the Ancylus Lake (approx. 8,148 calibrated years BP), to Littorina Sea and Modern Baltic Sea to 1,500–1,000 cal yr BP in the Eastern Gotland Basin (EGB), and from around 4300 to 94–34 cal yr BP in the Gulf of Finland (GOF), aligns with findings from other phytoplankton studies, albeit with a different temporal perspective. The findings of these studies indicate that long-term genetic stability is frequently maintained in marine populations due to their substantial population size and high dispersal capacity, even in the face of significant environmental changes (Rengefors et al., 2017). These factors contribute to a buffering effect against genetic drift and local extinctions, allowing populations to maintain genetic diversity over long periods of time. The ability of *S. marinoi* to cope with changing environmental conditions through reversible shifts in genetic composition as shown here for the Baltic Sea further supports this stability. This resilience is a common trait among phytoplankton, allowing them to respond to environmental changes without losing overall genetic diversity (Laso-Jadart et al., 2023). The observed stability is expected given the ecological and evolutionary characteristics of phytoplankton populations. Large population sizes, high reproductive rates, and the potential for gene flow between populations contribute to the maintenance of genetic diversity and stability over long periods of time (Brand, 1980). Other findings, such as studies of diatoms and other phytoplankton species have reported patterns of genetic stability and adaptability of phytoplankton to environmental change (Salmaso and Tolotti, 2021). These findings imply that the observed stability in *S. marinoi* populations is not only expected, but also indicative of the broader ecological and evolutionary dynamics of marine phytoplankton. Our data demonstrates that this stability can be upheld across several millennia.

However, this stability is not without punctuated interruptions. Our results show that climatic events have an influence on the allelic composition of *S. marinoi* populations. For example, shifts in the genetic composition of the EGB population were observed during the Holocene Thermal Maximum, the Late Antique Little Ice Age, and the Medieval Climate Anomaly. Similarly, the GOF population showed changes during the Little Ice Age and the Modern Warm Period. These shifts highlight the ability of *S. marinoi* to cope with different climatic events. The discrepancy between EGB and GOF may be due to the slightly higher genetic diversity in the EGB (Fig. 8C-E), which may facilitate adaptation. The differences between the two sites can also be attributed to their distinct geographical characteristics. The EGB, located in the Baltic Proper, may experience an influx of migrants from other populations, increasing genetic diversity and resilience. In contrast, the genetic composition of the GOF population could be influenced by its unique environment, characterized by freshwater influx and lower salinity

levels (Myrberg and Soomere, 2013), as well as more extensive ice cover in the northern regions of the Baltic Sea (Leppäranta, 2023). In particular, samples from the Little Ice Age and the Modern Warm Period show more pronounced responses in the GOF. The allelic composition remains relatively constant in the absence of climatic events, indicating a stable genetic structure under stable conditions and once a shift has manifested.

After this long period of punctuated genetic stability, recent centuries reveal a novel pattern of rapid allelic turnover, and this coincides with increased human activity. This rapid turnover is particularly evident in the GOF during periods such as the pre-Roman Iron Age, the Viking Age, the Hanseatic Age, and the Industrial Revolution. These results highlight the possible impact of human activities on the genetic dynamics of *S. marinoi* populations. This increased activity is manifested by increased shipping activity in the Baltic Sea (Salimi et al., 2021; Wang et al., 2020). Ballast water exchange (Andrés et al., 2023) in the last two centuries could have been a specific agent of increased changes in haplotype composition, causing rapid translocation of populations. This rapid allelic turnover contrasts with the more gradual and reversible shifts observed in response to natural climatic events, highlighting the influence of anthropogenic factors on the genetic composition of these populations. Due to its coastal position, the GOF is more exposed to these changes, and perhaps the phytoplankton is also more directly affected.

The rapid allelic turnover observed in recent centuries, particularly in response to human activities, underscores the influence that anthropogenic factors may have on the genetic dynamics of populations. Our study on the population dynamics of *S. marinoi* populations provides valuable insights into the long-term resilience and adaptability of marine phytoplankton. The observed genetic stability over several millennia, punctuated by reversible shifts in response to climatic events, underscores the inherent resilience of these populations. However, the recent rapid allelic turnover highlights the potential impact of human activities on population dynamics and structure, and thus presents an opportunity for further investigation.

At the same time it is important to acknowledge the limitations of our study. The limited number of samples included may affect the overall strength and generalizability of our findings. In addition, the lack of precise tests limits our ability to draw definitive conclusions about long-term genetic trends and the full extent of human impact. To date, we also lack a sufficient understanding of the distribution and taphonomy of ancient eDNA, but in sediments, DNA of small organisms, such as phytoplankton, has been shown to give a representative signal of a water body (Wang et al., 2023). The data of the two cores display a high level of congruency, and despite the limitations, this indicates a link between

environmental change, human activity, and genetic dynamics. Future studies with larger sample sizes and e.g. more locations are needed to further explore the complex interactions between environmental change and genetic responses.

The results of our study provide a solid base for future research on the effect of current global change on the genetic composition of natural populations across millennia. By understanding the genetic stability and adaptability of *S. marinoi* populations, we can better understand ecological and evolutionary dynamics of marine phytoplankton. This knowledge is crucial for predicting how these populations may respond to current and future environmental changes, including climate change and direct anthropogenic pressures, and thus offer a valuable foundation for future conservation efforts. Our study provides novel insights into the genetic resilience and adaptability of *S. marinoi* populations. It demonstrates that these populations maintain stability over millennia and respond dynamically to both natural climate fluctuations and human-induced changes. In sum, it highlights the importance of considering both natural and anthropogenic drivers and their relative impacts when assessing and predicting genetic resilience of marine ecosystems.

4. Material & Methods

4.1. Study Area

The Baltic Sea is a relatively young and shallow brackish water system currently facing a significant risk of increasing hypoxia, a condition characterized by low oxygen levels and bottom water anoxia. This phenomenon is primarily attributable to the reduction in dissolved oxygen and nutrient discharge resulting from warmer water temperatures, which in turn promote the formation of algal blooms (Carstensen et al., 2014b). These blooms decompose and consume oxygen, leading to the development of hypoxia. In recent history, the Baltic Sea has experienced a notable increase in eutrophication, a process driven by the excessive input of nutrients such as nitrogen and phosphorus from agricultural runoff, wastewater discharge, and industrial activities (Reusch et al., 2018). The enrichment of nutrients has resulted in the proliferation of phytoplankton and algal blooms, which, upon decomposition, have further depleted oxygen levels in the water. The Baltic's unique position as a land-enclosed sea with high human and industrial activity, coupled with its low biodiversity, makes it particularly vulnerable to these changes.

The Baltic Sea's history is well known. It has undergone substantial environmental changes in the past. After the end of the last glaciation approximately 10,000 years ago, it went through several fresh- and

saltwater stages, starting as a large meltwater lake, then transitioning into various states of salinity due to glacial retreat and land rebound (Snoeijs-Leijonmalm and Andrén, 2017). These stages included the Baltic Ice Lake, the Yoldia Sea, the Ancylus Lake, and the Littorina Sea (Andrén et al., 2011). The Baltic Sea's temperature and salinity have fluctuated over time, with notable increases during the Holocene Thermal Maximum and the Medieval Climate Anomaly, and a recent increase since 1850 (Kabel et al., 2012; Seppä et al., 2009; Snoeijs-Leijonmalm and Andrén, 2017).

Within the Baltic Sea this study covers a time series of two distinct locations: The Eastern Gotland Basin, located in the Baltic Proper with a profound water depth of max. 249 meters, and the comparatively shallower Gulf of Finland, reaching a depth of 81 meters. At both locations, we collected sediment cores to inform on the effects of environmental changes on the population genetic composition of *S. marinoi*.

4.2. Sampling

Two sediment cores were taken (Fig. 7A) in April 2021, during expedition EMB262. 1) Eastern Gotland Basin (EGB, 57°17.004'N, 020°07.244'E, 241 m water depth, core EMB262/6-30GC); 2) Gulf of Finland (GOF, 59°34.443'N, 023°36.461'E, 81 m water depth, core EMB262/12-3GC). The cores (Fig. 7B, ca. 500 cm) were taken using a gravity corer (GC). The sampling for the EGB began at a depth of 32 cm. The cores were sampled on board of the research vessel using sterile syringes according to Epp et al. (2019) and immediately frozen for storage.

Core dating was performed as described in Schmidt et al. (2024). This was done by correlating organic carbon records from post-Littorina transgression sediments in the Baltic Sea. The chronology of the cores is determined by the relative S content and the Br/K ratio. The Br/K ratio reflects changes in the bulk organic carbon content of the sediments. The data are visually matched with XRF and organic carbon data from dated sediment cores from the same or nearby locations for different historical periods. For the sediment core from the Gulf of Finland, an independent Bayesian age model was constructed based on radiocarbon dates of bulk organic matter in the sediments. This allows the sediments to be accurately dated to around 2300 BC. The same core samples were used as in Schmidt et al. (2024) and in this study.

4.3. Laboratory Work

4.3.1. DNA Extraction

The PowerSoil Pro Kit from Qiagen (Hilden, Germany) was used to extract DNA from both sediment cores (n=26, see supplementary Table SX) covering various depths (ages). For the extraction 0.5 g of sediment was used per sample, resulting in 26 samples and 2 extraction blanks. The extraction process was carried out according to the manufacturer's protocol with some modifications including an overnight incubation at 56 °C with the addition of 20 µL proteinase K (20 mg/ml) to enhance sample lysis. The washing steps were performed using a Qiagen Vacuum Pump (Hilden, Germany). Subsequently, samples were centrifuged for 3 minutes. After centrifugation, the elution process was performed in two steps. Each part involved adding 75 µL of elution buffer to the sample, allowing it to incubate for 5 minutes, and then collecting the eluate. Both eluates were collected in the same tube.

4.3.2. Library Preparation

A single-stranded library approach according to Gansauge et al. (2020) was performed to maximize the retrieval of genetic information from these degraded sedaDNA samples. This method, optimized for fragmented DNA, has been shown to significantly increase the sequence yield and preserves unique molecules, thereby providing a more comprehensive genomic analysis. The library preparation was conducted with 40 ng input DNA. All 26 samples and 2 extraction blanks were processed in the library preparation. Additionally, 2 library blanks were created.

After the indexing PCR, each library was cleaned using the MinElute PCR purification Kit (50,250; Qiagen) resulting in a final amount of 20 µL per sample (For more detailed info, see Supplement Section 1.1.). The samples were then analysed on a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) using the Agilent Bioanalyzer High Sensitivity DNA Analysis Kit (Agilent Technologies, Santa Clara, CA, USA). Due to adapters still being present in the samples, we performed an additional purification using the HighPrep™ bead cleanup (MagBio Genomics Inc., Gaithersburg, MD, USA). 1.6x of HighPrep™ PCR reagent was used. The DNA concentration was measured using ds-DNA HS Assay Kit and the Qubit® 4 fluorometer (Invitrogen Thermo Fisher, Waltham, MA, USA). The libraries were combined into pools of four, each with the same DNA amount. The extraction and library blanks were incorporated in the same volume as the sample with the lowest concentration. The libraries were then further used for hybridization enrichment of chloroplast and mitochondrial genomes.

4.3.3. Hybridization Enrichment

Bait design

In this study, a hybridization enrichment approach was employed. Hybridization enrichment allows for targeted isolation of specific sequences, improving sequencing efficiency by reducing the presence of non-target DNA. Thus, providing a better representation of the studied species, enabling a more detailed and precise analysis of its genetic composition.

Enrichment

This bait design was then used for two different bait sets: 1) RNA baits (BioCat, Heidelberg, Germany); 2) DNA baits (Integrated DNA Technologies, IDT). The libraries were enriched with both RNA and DNA baits, resulting in two sets of libraries: 1) RNA baits enriched; 2) DNA baits enriched. General steps are explained in the following. More detailed information on the used protocols can be found in the supplementary data (section 1.2.).

The enrichment with RNA baits was conducted according to the myBaits v.5.02 manual. A two-round enrichment strategy was performed. The hybridization mix was prepared and transferred to a rotation oven once the 24-hour incubation at a hybridization temperature of 63°C started. In the following bead cleanup, the libraries were resuspended and amplified. Each 27.4 µL PCR reaction included the following nine components: 1) 3.45 µL of DEPC treated H_2O; 2) 2,5 µL 10X HiFi PCR Buffer (Invitrogen Thermo Fisher, Waltham, MA, USA); 3) 0.25 dNTPs (25mM); 4) 1 µL BSA (20 mg/ml); 5) 1 µL $MgSO_4$ (50 mM); 6) 0.2 µL Platinum™ Taq DNA-Polymerase High Fidelity (5 U/µL) (Invitrogen Thermo Fisher, Waltham, MA, USA); 7) 2 µL IS5_bridge_P5 (10 µM); 8) 2 µL IS5_bridge_P5 (10 µM), 9) 15 µL library pool. The amplification was run using the following settings: 1 minute initiation at 94°C, 14 cycles of 15 seconds denaturation at 94°C, 20 seconds annealing at 60°C and 1 minute extension at 68°C, followed by 2 minutes of final elongation at 68°C and afterward the sample was stored at a temperature of 20°C. Afterwards, the samples were purified with beads and eluted in 10 µL. The second round of enrichment was performed in a similar way. The PCR was conducted in 8 cycles and the pools were eluted in 17 µL after the bead purification (see more detailed in supplement section 1.2.2.).

Enrichment with DNA baits was also performed in two rounds following the IDT (Integrated DNA Technologies, Löwen, Belgium) xGen™ hybridization capture of DNA libraries protocol (option: AMPure XP Bead DNA concentration protocol), with the following modification. The hybridization temperature

was set to 63°C. Once this temperature was reached on the thermal cycler, the tubes were moved to a rotation oven and incubated for 16 hours (see more detailed in supplement section 1.2.1.).

All resulting pools were quantified using Qubit® ds-DNA HS Assay Kit and Agilent Bioanalyzer HS Kit. Afterwards, the pools enriched with RNA baits were collectively combined into a single pool, while those enriched with DNA baits were combined into another separate pool, each in equal DNA amounts. This resulted in two distinct library pools. Both of these pools were sent for Illumina sequencing (paired-end, 2 x 150 bp, MiSeq-v3) at Fasteris SA (Geneva, Switzerland).

4.4. Bioinformatics

The sequences were trimmed, mapped against references, and converted to finally calculate mapping coverage and for variant calling. First, sequence file names were modified to fit the sample identifier using python v3.10.13. Then raw sequence reads were processed using Autotrim v0.6.1, a tool that automates the quality control and trimming of high-throughput sequence data (Waldvogel et al., 2018). Autotrim employs Trimmomatic v0.39 (Bolger et al., 2014), to trim sequences based on quality and remove adapters, FastQC v0.12.1 (Andrews, 2021) to assess the quality of the raw sequence data, and MultiQC v1.14 (Ewels et al., 2016) to aggregate the results into a single report. Following the initial processing, the trimmed reads were mapped to the reference chloroplast and mitochondrial genomes (GenBank: PRJNA493755) using BWA-MEM v0.7.17 (Li and Durbin, 2009). BWA is a software package for mapping low-divergent sequences against a reference genome, which is particularly useful for precisely aligning sequencing reads.

The mapped reads were then converted to bam format using Samtools v1.15 (Danecek et al., 2021), a suite of programs for interacting with high-throughput sequencing data. In addition, the per-sample mapping coverage was calculated. After careful consideration, we decided against deduplicating our sequence data as deduplication resulted in a significant loss of data. This is probably due to the nature of our short and fragmented sequences. In addition, each variation, even those present in duplicates, may hold biologically significant information. Therefore, removing duplicates may have eliminated critical genetic diversity, compromising the integrity of our analysis.

VCFtools v0.1.16 (Danecek et al., 2011) and BCFtools v1.19 (Danecek et al., 2021) were used for calling and filtering variants from the alignment. Variant calling is the process of identifying differences, such as single-nucleotide polymorphisms (SNPs) and insertions/deletions (indels), between the sequenced sample and the reference genome. Variants were filtered to include only those with a quality score (Q)

of 30 or higher. The identified variants were then phased into haplotypes using Beagle v5.4 (Browning et al., 2021). Haplotyping, or phasing, is the process of determining the distribution of alleles of multiple variants along each chromosome. In this context, it can be said it accounts for unique combinations of alleles at variant sites (distinct variations) across the chloroplast and mitochondrial genome.

The pattern of ancient DNA damage was assessed using mapDamage2 v2.2.2 (Jónsson et al., 2013). This tool quantifies patterns of DNA damage in ancient samples, which can provide insights into the preservation and authenticity of ancient DNA.

4.5. Analyses

The bioinformatic output was converted into a table format and for further analyses with R v4.3.1: The mapping coverage (i.e., the number of reads mapped against reference genome), allele frequencies, distinct variants, nucleotide diversity and C-to-T-substitution rate were combined with the sample metadata (Supplement, Tab. S3) using the R package “tidyverse” (Wickham et al., 2019). The data was visualized using the packages “tidyverse´”, “ggpubr” v0.6.0 (Kassambara, 2023) and “tidypaleo” v0.1.3 (Dunnington et al., 2022).

In order to facilitate a comparison of the genetic diversity between GOF and EGB, nucleotide diversity (resulting from Samtools) and distinct variants (resulting from Beagle) were normalized based on the mapping coverage for mitochondria and plastid. Second, Pearson's correlation was employed to verify the consistency of nucleotide diversity between the mitochondrion and chloroplast. For comparison, the dataset was subset to the overlapping time period of both locations. Subsequently, a t-test was applied to compare and test the significance of the nucleotide diversity (mitochondrial and chloroplast data combined) between the two sites. In addition, each distinct genetic variant was assigned a unique identifier for easy reference. These identifiers were utilized to calculate two diversity indices: the Shannon index and the Simpson index. These indices were compared between the EGB and GOF sites using a t-test,

To further identify spatial differences between GOF and EGB, we analysed allele frequencies (resulting from VCFtools & BCFtools) and calculated the Fixation Index (FST) using corresponding samples from both locations. The FST is a measure of population differentiation attributable to genetic structure. The FST value ranges from 0 to 1, with 0 indicating complete interbreeding (i.e., the two populations are freely intermixing) and 1 indicating that all genetic variation is explained by the population structure

(i.e., the two populations do not share any genetic diversity). We implemented a custom R function to calculate the FST over time. This process involved sorting the data by age, subsetting samples within a specified time window, and computing FST based on allele frequency variance. The allelic composition, derived from parsing VCFtools output, was analysed using Principal Component Analysis (PCA) to examine the genetic structure.. We also performed Pearson's correlation and PERMANOVA to assess the impact of C-to-T substitution rates and Total Organic Carbon (TOC) on the variation in the PCA space, specifically the first two principal components (PCs). These analyses helped determine the extent to which these factors influenced genetic composition. For PCA and PERMANOVA we also used the functions provided by the package "vegan" v2.6.4 (Oksanen et al., 2020).

The C-to-T-substitutions at the first position from the mapDamage2 output were examined to authenticate the ancient DNA (aDNA) and analyse the damage patterns. A Pearson's correlation test was conducted to assess the influence of mapping coverage on these substitutions. This was done to evaluate if coverage has an impact on the observed damage. The Baltic Sea Mn/Ti ratio data were also integrated into the analysis.

To assess the genetic diversity and adaptability of the population, we calculated the rate of allele turnover. This measure, derived from the ratio of significant allele changes (greater than 1%) to total generations, reflects the estimated rate of genetic change over time based on the present data. Given the low FST values, we inferred that the two sites, EGB and GOF, belong to a single population. The calculation covered all of the data, from the oldest sample from EGB to the youngest sample from GOF, providing insight into the evolving genetic makeup of the population.

To identify phases of change in the allele turnover across the entire dataset, a generalized additive model (GAM) was fitted using the "gam" function from the "mgcv" package (Wood, 2011). This model was applied to the complete dataset, including periods where we did not have direct samples, as the allele turnover was estimated based on the available data. We calculated the mean allele turnover for the present data points, which were then included in the GAM plot. Allele turnover was used as a response variable to fit a gam model as a function of "age".

Corresponding metadata to this analysis can be found in supplementary material (Table S3). Data on shipping activity in the Baltic Sea were obtained from three main sources: Kontny (2023), Mägi (2018), and Rytkönen et al. (2002). Kontny's work provided insights into maritime contacts during the Roman and Migration Periods (1st-7th centuries AD), while Mägi's research focused on the role of the Eastern Baltic in Viking Age communication across the Baltic Sea. Rytkönen et al.'s research on the statistical analysis of Baltic Sea shipping revealed a significant increase in maritime traffic during the late 20th

and early 21st centuries. These sources were used to establish a timeline of shipping intensity from 1200 cal yr BP to the present.

5. Supplementary Information

5.1. Acknowledgements

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5.2. Data Availability Statement

The supplementary data can be accessed via the following link: https://cloud.uni-konstanz.de/index.php/s/iBXMz3G5fMoikyb in folder “Chapter_2”. Additionally, the scripts and pipeline used for data processing and analysis are available in the subfolder named "chapter_2_github". This repository includes all the code necessary to replicate the study's findings. Furthermore, supplementary information is provided in the document "Supplement.docx”.

Chapter 3 – Back to Life after 6,800 Years: Resurrection of a Diatom from anoxic Baltic Sea Sediments

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Sarah Bolius[1*], Alexandra Schmidt[2,3], Jérôme Kaiser[4], Helge W. Arz[4], Olaf Dellwig[4], Ulf Karsten[5], Laura S. Epp[2], Anke Kremp[1]

1. Biological Oceanography, Leibniz-Institute for Baltic Sea Research Warnemünde, Rostock, 18119 Germany
2. International Max Planck Research School Quantitative Behaviour Ecology & Evolution, Konstanz, 78457 Germany
3. Department of Biology, University of Konstanz, Konstanz, 78464 Germany
4. Marine Geology**,** Leibniz-Institute for Baltic Sea Research Warnemünde, Rostock, 18119 Germany
5. Institute of Biological Science, University of Rostock, Rostock, 18051 Germany

* Correspondence

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Abstract

Dormancy is a wide-spread key life history trait observed across the tree of life. Many plankton species form dormant cells stages that accumulate in aquatic sediments and, under anoxic conditions, form chronological records of past species and population dynamics under changing environmental conditions. Here we report on the germination of a microscopic alga, the abundant marine diatom *Skeletonema marinoi* that had remained dormant for up to 6871 ± 140 years in anoxic sediments of the Baltic Sea and resumed growth when exposed to oxygen and light. Resurrected diatom strains, representing cohorts from 6 different time points of the past 6871 ± 140 years, are genetically differentiated and fundamental physiological functions such as growth and photosynthesis had remained stable through time, despite distinct environmental dynamics. Showing that resurrection and full functional recovery, in comparison to 3 ± 2 years of dormancy, is possible after millennial resting, we emphasize the relevance of dormancy and living sediment archives. For the future, sediment archives together with the resurrection approach would offer a powerful tool to trace adaptive traits over millennia under distinct climatic conditions and elucidate the underlying mechanisms.

1. Introduction

Across the tree of life, from bacteria to mammals, dormancy is a widespread key trait of life (Lennon et al., 2021). This allows organisms to survive periods of unfavourable and uncertain environmental conditions by shifting into a reversible state of reduced metabolic activity. Nonetheless, besides securing survival, dormant cells, which are often specialized, have a number of other important features and ecological functions: they contain specialized protective structures such as robust and impregnated cell walls or covers, and accumulate storage products to support reduced metabolic activity over long periods of time, thereby promoting survival under unfavourable conditions (Ellegaard and Ribeiro, 2018; Hendry et al., 1994).

Despite their common protective features, dormant cells are highly diverse in their morphologies, with varying shapes and sizes. Different phylogenetic groups build specific forms, like spores, cysts, akinetes, specific eggs or seeds. These dormant cells or tissues typically differ in morphology from active, vegetative cells (Ellegaard and Ribeiro, 2018). In many organisms, dormant cells are part of the sexual life cycle and facilitate recombination. Additionally, resistant dormant cells can be transported in ecosystems by various vectors, such as migratory birds, animals, and both winds and resuspension by

ocean currents, thereby expanding their geographic dispersal and distribution range (Allen, 2007; Fontaneto, 2019; Mestre and Höfer, 2021).

Sedimentation is a key process that enables dormant cells to form seed or propagule banks, serving as reservoirs of diversity over time and space (Lennon et al., 2021; Sundqvist et al., 2018). When dormant cells accumulate consecutively in undisturbed laminated sediments, e.g. under anoxic conditions, they represent distinct temporal cohorts of respective organisms. Such accumulations constitute so called 'sediment archives' that provide valuable information on past ecosystems, species, and population history, as well as on their response to environmental changes. "Resurrection" ecology (Kerfoot et al., 1999), referring to the specific germination of dormant cells from such sediment archives, and subsequent characterization of these organisms, has proven to be an effective approach to e.g. experimentally study functional traits and their adaptation in relation to environmental changes over time.

The longest dormancy period reported for resting stages buried in sediments so far, lasting for nearly 2000 years was found for seeds of the date palm *Phoenix dactylifera*, from an archaeological site (Gros-Balthazard et al., 2021; Sallon et al., 2020, 2008). Further reports of long-term dormancy of plants range from centuries, such as sedges (Leino and Edqvist, 2010; Vavrek et al., 1991) to 1300 years shown in Sacred Lotus (Shen-Miller et al., 1995). For aquatic habitats, the oldest record of a resurrected and subsequently growing species stems from the crustacean *Daphnia pulicaria* from a 700-years-old sediment layer of South Center Lake in Minnesota (Frisch et al., 2014). For phytoplankton, a number of studies reported germination after decadal to centennial periods of dormancy (Delebecq et al., 2020; Härnström et al., 2011; Hinners et al., 2017; Medwed et al., 2024; Stockner and Lund, 1970). Viable cells germinated from initially dormant cells of diatoms (spores) (Sanyal et al., 2022) and cyanobacteria (akinetes) (Legrand et al., 2019) were reported from 6600-years old anoxic Baltic Sea (Landsort Deep) and nearly 2000-years-old French lake sediments, respectively. However, after being released from dormancy, in both studies the dormant cells remained non-growing, due to unknown reasons. But it is reasonable to assume a trade-off between the longevity and viability of the spores/akinetes and their reproductive ability.

Due to recurrent hypoxic (< 2 mL L^{-1} O_2 (Diaz and Rosenberg, 2008)) to sulfidic (euxinic) conditions in bottom waters of its deep basins, the Baltic Sea is a particularly suitable system for the application of the sediment archive approach. These conditions, along with cold temperatures prevent degradation and preserve dormant cells (Ellegaard and Ribeiro, 2018). Several studies on dormancy, resurrection, and survival of phytoplankton dormant cells from Baltic Sea sediments emphasize its specific potential for phytoplankton resurrection after extended time periods (Härnström et al., 2011; Hinners et al.,

2017; Ribeiro et al., 2011; Wood et al., 2021). So far, resurrection studies on phytoplankton mostly addressed the period of environmental deterioration of the past ~ 100 years (Härnström et al., 2011; Hinners et al., 2017; Medwed et al., 2024; Ribeiro et al., 2011; Wood et al., 2021). However, phytoplankton residues like microfossils (e.g. diatom valves, resting spores) and smaller parts that still contain DNA are constantly found in even older sediment layers (Andrén et al., 2020, 2000; Schmidt et al., 2024), suggesting that viable phytoplankton cells may also be present for far longer periods of time.

Here we used an anoxic sediment archive from a deep (240 m water depth) basin of the Baltic Sea (Eastern Gotland Basin, Fig. 11 A) represented by a composite sediment core covering the past 7500 years to investigate how far back in time dormant phytoplankton may remain viable in a dormant state and keep its germination capability. The sediment core represents the mid- to late Holocene history of the Baltic Sea, the so-called Littorina Sea stage (Andrén et al., 2000), offering the possibility to search for viable phytoplankton dormant cells millennia back in time and specifically using layers that represent the different climate phases and respective conditions of the Holocene. To evaluate viability and functional recovery of resurrected strains, functional physiological traits such as growth rate and oxygen production were examined. We report resurrection of a common Baltic Sea spring bloom diatom, *Skeletonema marinoi* (Fig. 11 B), from millennial dormancy and demonstrate millennial viability of dormant strains.

2. Material & Methods

2.1. Sediment Sampling

During a cruise on RV Elisabeth Mann Borgese (EMB262), in April 2021, a short (54 cm, EMB2625/6-28) and long sediment core (547 cm; EMB262/6-30) were retrieved by a multicorer device and a gravity corer, respectively, from the deep Eastern Gotland Basin (EGB; 57°17.004' N, 020°07.244' E, 240 m water depth), Baltic Sea. To prevent the use of disturbed sediments from the uppermost part of the gravity core (~ top 40 cm), both cores were combined to a composite profile. The sediments were nearly entirely laminated and no signals of bioturbation could be visually identified (Supplemental Material Fig. 2). This finding agrees with the contents of redox-sensitive trace metals determined in sediments from a previous core obtained at the identical location in December 2018 (EMB201). Thus, the corresponding records of manganese and molybdenum indicate alternating hypoxic and euxinic bottom water conditions throughout the sampled brackish Littorina phase (Supplemental Material Fig. 1). Overall, this confirms the undisturbed state of the recovered sediment material used for this study.

To avoid sediment core alterations and contamination, the cores were immediately split and sub-sampled on-board, according to sampling procedures for sedimentary ancient DNA (Epp et al., 2019). Therefore, the surface of the split core-half was carefully cleaned and the outer sediment that had been in contact with the core liner (~ 0 - 0.5 cm) was discarded. For resurrection of phytoplankton, ca. 3 cm^3 of the sediment was sampled from one half of the core, using a sterile syringe, at a resolution of 1 to 2 cm on the short core and every 10 cm on the long core, and stored dark at 4-6°C.

2.2. Stratigraphy

The dating of the short core is based on an event stratigraphic approach using, e.g., activities of radiogenic ^{137}Cs and ^{241}Am from former nuclear bomb testing (onset 1954 and max. 1963) and the Chernobyl accident in 1986, Hg contents for the onset of industrial and modern pollution around 1900 and 1950, stable $^{206/207}Pb$ for maximum pollution from leaded fuel (1970 – 1980), respectively, and Mn contents indicating major Baltic inflows (MBIs) in 1994, 2003 and 2014 (Dellwig et al., 2018; Kaiser et al., 2023; Lin et al., 2021; Moros et al., 2017). The present short core was taken from the same location where two independently dated short cores from a previous cruise (EMB201, December 2018) were available (Kaiser et al., 2023; Lin et al., 2021) allowing age model transfer via core parallelization. Sediment core regions that are disturbed were not used for resurrection approach, as for example seen in cm 162 to 172 of EMB262/6-30 (GC; Supplemental Material Fig. 2).

The age assignment of sediment core EMB262/6-30-GC (EGB long core; Supplement Material Figure 3) is achieved through a detailed visual correlation of high-resolution XRF scanner elemental Br/K values. These ratios are compared with loss-on-ignition (LOI) and total organic carbon (TOC) from recently published central Baltic Sea sediment cores owning exceptionally well-dated radiocarbon chronologies. For the older, mid-Holocene part, the chronology of sediment core P435-2-1 cored at about the same location as EMB262/6-30 in the EGB (Fig. 11 A) was used as a reference (Warden et al., 2017). For the late Holocene part (last ~ 3000 years) sediment cores M86-1a/36 (Häusler et al., 2018) (Fig. 11 A) and MSM62-60 (Moros et al., 2020) (Fig. 11 A) from the western Gotland Basin served as reference chronologies. A precise age assignment of pre-Littorina-Stage sediments was not possible, but the lowermost about 50 cm of the core most likely belong to the late Ancylus Lake phase < 9500 calibrated years Before Present (cal yr BP, present = 1950 CE) of the Baltic Sea. The short and long cores were finally combined to one composite core (EMB262/6; text above, Fig. 12). Root mean squared errors were calculated to estimate chronological uncertainties. Uncertainties of the reference chronologies,

sample spacing of the reference and own records, and an extra uncertainty allowance for more ambiguous tie-points were considered (Grant et al., 2012).

2.3. Resurrection and Cultivation of *Skeletonema marinoi*

For the resurrection approach, sediments from 12 sampled and well defined layers of the core were selected to represent the main climate phases of the Baltic Sea Holocene history. Samples were taken from cm 1 (-68 ± 2 cal yr BP), 3 (-63 ± 1cal yr BP), 16 (-24 ± 4 cal yr BP), 20 (-13 ± 5 cal yr BP), 21 (-10 ± 5 cal yr BP), 35 (66 cal yr BP), 44 (110 ± 15 cal yr BP), 45 (115 ± 15 cal yr BP), 71 (740 ± 110 cal yr BP), 107 (1060 ± 110 cal yr BP), 232 (3340 ± 120 cal yr BP) and 382 (6800 ± 140 cal yr BP) and thus cover the Holocene Thermal Maximum (HTM; 8000 - 4000 cal yr BP) with a warmer climate; the Medieval Climate Anomaly (MCA; 1150 – 600 cal yr BP), warmer climate, but not as warm as HTM; the Little Ice Age (LIA; ~ 400 - 100 cal yr BP), a period of cold temperatures and the ongoing Modern Warm Period (MWP, ~ since 100 cal yr BP), with a fast increase in temperature until today (Zillén et al., 2008)(Supplemental Material Table 1).

Around 0.5 - 1 cm^3 of each sediment sample was suspended in 15 mL of sterile filtered and autoclaved Baltic Sea water (salinity of 7.5, taken from the Bornholm Basin) and sonicated (45 s at 80%). The resulting sediment slurry samples were diluted 1:100 with f/8 medium with silicate (Guillard, 1975)(diluted from f/2, f/2 silicate concentration: 106 µM; using sterile 0.2 µm filtered Baltic Sea water) and distributed in 24-well plates, with 2 mL in each well. For each sediment sample, 4 24-well-plates (96 replicates) were prepared and incubated at 4°C, 40 µmol photons m^{-2} s^{-1} photon fluence rate, and a 16:8 h light:dark cycle.

After 6 weeks to 3 months, the slurries were diluted 6-fold again with f/8 medium to provide the dormant cells with an additional “nutrient push”. The wells were checked for germinated cells every week under an inverted microscope (100 – 400 x magnification; Zeiss, Jena, Germany), after ~ 1 - 16 months, individual cells of *Skeletonema marinoi* were isolated and established from the wells using a thin glass capillary connected to a tube and fully established as cultures after 2-4 re-isolations. The well-plates were sealed with Parafilm to avoid any contamination through air. Furthermore, cross-contamination was excluded by DNA analyses in the course of the study (see Methods, Results). The re-isolations were performed to obtain clonal cultures and minimize bacterial contamination as much as possible. Subsequently, the strains were cultured in f/2 medium with silicate (106 µM) at 4°C, with a 16:8 h light:dark cycle and 40 µmol photons m^{-2} s^{-1} photon fluence rate.

2.4. DNA extraction an microsatellite analysis

To extract DNA, cultures of *S. marinoi* were centrifuged during their (mid) exponential growth phase, and the cell pellets frozen at –80°C until further processing. For extraction the Qiagen DNeasy Plant Pro Kit (Hilden, Germany) was used, following the manufacturers' protocol.

To lyse the cells, the frozen cell pellets were thawed, mixed with the lysis buffer, and subjected to an initial bead-beating for 30 s. Subsequently, 2 µL of proteinase K (Thermo Fisher Scientific, Waltham, MA, USA) was added and the cells were homogenized in two rounds of 45 s with a 5-minute break in-between on a FastPrep machine. The lysate was then incubated with rotation at 56°C over-night. The remaining extraction followed the manufacturers' suggestions and the DNA was stored at -20°C.

Genetic diversity, based on 8 microsatellites was analysed on a subset of 62 DNA extracts (Supplemental Material Table 1). These were diluted to a concentration of 20 ng μL^{-1}. The eight primer pairs (S.mar1-S.mar8 (Godhe and Härnström, 2010), Supplemental Material Table 2) were used in a 33 µM concentration. The forward primers were modified with fluorescent dyes at the 5' end. Modified forward primers were dissolved in a 10 mM Tris-HCl buffer (pH 7.5) and the reverse primers in DEPC treated water.

The eight primer pairs were combined in three different primer mix combinations: 1) S.mar1-4, 2) S.mar5-7, and 3) S.mar8. This resulted in a total of three PCR reactions per sample. Each reaction mix of 25 µL was transferred into a 96-well PCR plate and consisted of five ingredients: 1) 9.5 µL DEPC H_2O; 2) 12.5 µL AmpliTaq Gold 360 Master Mix; 3) 1 µL of forward primer mix; 4) 1 µL of reverse primer mix; 5) 1 µL of diluted DNA extract (20 ng μL^{-1}).

The PCR plate was transferred into a Primus 96 advanced PCR-Cycler (Peqlab, VWR, Radnor, PA, USA). The following cycling parameters were used: an initial denaturation for 10 min at 95°C, followed by 40 cycles of 95°C for 30 s, 65°C for 30 s, 72°C for 30 s, and a final elongation step at 72°C for 7 min. Afterwards the samples were stored at -20°C.

After performing all PCRs, the labelled products were sent to Microsynth SEQLAB (Göttingen, Germany) for fragment length analysis.

2.5. Ecological Traits

As proxies for a successful resurrection, proof of viability and "functionality" of *S. marinoi*, the growth rate and photosynthetic activity of the isolated strains from different length of dormancy were measured. We thereby consider the most recent strain from the youngest sediment layer (in this case -68 ± 2 cal yr BP or 3 ± 2 years of dormancy, from year of sediment coring 2021) as modern and reference respectively, according to other resurrection studies (Hattich et al., 2024; Hinners et al., 2017). These strains have only bloomed recently and under the currently prevailing environmental conditions.

From isolated clonal cultures of *S. marinoi* (stock cultures - see above) strains from 6 sediment ages (see Supplemental Material Table 1), inoculum cultures were set up during their exponential growth by adding 2 mL culture suspension into 40 mL f/2 medium with silicate in autoclaved Baltic Sea water (salinity 7.5). When these cultures reached their exponential phase, the experiment was started. The starting cell concentration was set up to a low concentration of 3500 cells mL^{-1} (± 427) based on triplicate fluorescence measurement (Plate Reader, infinite m plex Tecan, ext 450 nm, em 682 nm; ~ 3500 cells mL^{-1} ≙ 9 relative fluorescence units (rfu)) and dilution of the inoculum cultures. For each strain 4 replicates to follow growth were set up in 24-well plates (2 mL culture). The growth was recorded daily by chlorophyll-*a* (chl-*a*) fluorescence measurement until ca. 3 days after growth ceased (the stationary phase). On average the strains reached their mid exponential phase after 10 days and the stationary phase after 15 days. As the mid-exponential phase the middle in between start and end of the exponential growth (in days) was taken. The growth rate was measured as average exponential growth. The experiment ran at 4°C with a 16:8 h light:dark cycle and 40 µmol photons m^{-2} s^{-1} photon fluence. These were the same conditions as during germination process. During the growth experiment the well-plates were closed with breathable plate-seals (Breath-Easy, Sigma Aldrich, St Louis, MO, USA) to prevent any evaporation.

For the measurement of photosynthetic activity, 3 strains from 4 sediment layers were used (Supplemental Material Table 1) to examine photosynthetic activity under saturating light. Again, from stock cultures, inoculum cultures were set up during their exponential growth by adding 2 mL in 40 mL f/2 medium with silicate in autoclaved Baltic Sea water. To measure the photosynthetic activity of the cultures a so-called 'Photosynthetic Irradiance Box' (PI-Box) was used, following a widely applied methodology (Karsten et al., 2010; Prelle et al., 2019) and mimicking natural conditions. Under a light gradient from 0 (dark) to 1446 ± 83 µmol photons m^{-2} s^{-1} the oxygen production was measured using an optode as sensor, with 4 - 8 technical replicates per culture. The light was changed every 10 min

without any dark phases, whereby the first and last minute were not taken into account in the subsequent calculation. At the end of each measurement, particulate chl-*a* determination was undertaken on GF/6 filters (Whatman, 25 mm), to relate oxygen data to chl-*a* cell content. The chl-*a* was extracted in 4 to 10 mL 70°C hot ethanol for 10 min., centrifuged for 10 min. at 4840 x g (Heraeus Megafuge 1.0R, Hanau, Germany), and spectrophotometric measured (extinction of 665 and 750 nm; Shimadzu UV-2401 PC, Kyoto, Japan).

2.6. Calculations and Statistical Methods

The raw microsatellite data in the form of Fasta-files was analysed with the R package Fragman (Covarrubias-Pazaran et al., 2016). Resulting SSR call results were stored in an R data frame and then further analysed using the packages tidyverse (Wickham et al., 2019), polysat version 1.6 (Clark and Schreier, 2017), and vegan version 2.6-4 (Oksanen et al., 2020). An ANOSIM and PERMANOVA was performed to check if the SSR calls of the cultures differ from each other. For each time point, we calculated observed and expected heterozygosity, the Shannon diversity index, and allele richness across loci. We also evaluated allele size variation across each time point.

The growth rate μ was calculated from the start of the exponential growth to the start of the stationary phase, according to:

$$\mu = \frac{\ln(RFU_{ty}) - \ln(RFU_{tx})}{tx - ty} \qquad (1)$$

where RFU_{ty} is the fluorescence of the culture at the end of the exponential phase, RFU_{tx} is the fluorescence of the culture at the beginning of the exponential phase, ty is the day at which the culture is at the end of the exponential phase, tx the day when the culture is at the beginning of the exponential phase.

To obtain oxygen production data (Fig. 13 B and Supplemental Material Table 3), these raw data were fitted with a widely used model for photosynthesis by estimating from least- square regression curves to data (Walsby, 1997), using solver function in MS Office Excel 2016.

To test growth and oxygen production data for normal distribution and subsequent statistical analysis (ANOVA or Kruskal-Wallis) R was used.

3. Results

The core (EMB262/6) obtained in EGB for the resurrection approach covers the history of the Baltic Sea from a modern, recent time point (here -68 ± 2 cal yr BP/2018 CE/3 ± 2 years of dormancy) to ~ 7500 years ago (Fig. 12).

Of the 12 sediment layers analysed for resurrection capacity (see Methods), we were able to resurrect actively growing strains of the chain-forming diatom *Skeletonema marinoi* from 9 sediment layers (~ 70%; Supplemental Material Table 1). Resurrection success was relatively low though, as growing isolates could only be successfully established from ca. 10% of incubated sediment slurries. Nevertheless, the established strains from distinct sediment layers represent a time frame of nearly 7000 years, ranging from 3 ± 2 years (-68 ± 2 cal yr BP) to 6870 ± 140 years of dormancy (Fig. 12). Here, we consider the most recent strains (3 ± 2 years of dormancy) as modern strains, as they bloomed during modern times, and use that as comparison. Temporal cohorts of *S. marinoi* were successfully resurrected from sediment layers dated to -68 ± 2, -13 ± 5, -10 ± 5, 110 ± 15, 115 ± 15, 740 ± 110, 1060 ± 110, 3340 ± 120, and 6800 ± 140 cal yr BP (Fig. 12; Supplemental Material Table 1), which can be translated into dormancy length (from year of sediment coring 2021) of 3 ± 2, 58 ± 5, 61 ± 5, 181 ± 15, 186 ± 15, 811 ± 110, 1131 ± 110, 3411 ± 120, and 6871 ± 140 years. *Skeletonema marinoi* was the only phytoplankton species consistently found in the investigated sediment layers, whereas other species appeared mostly in the top layers (Supplemental Material Table 4).

To first ascertain that the successfully isolated strains were distinct, i.e. no repeated strains were established, and the results are not confounded by cross-contamination among the depths (Andersson et al., 2023), we subjected the established strains to microsatellite analyses. Analysis of the simple sequence repeats (SRR) markers (Supplemental Material Fig. 4) revealed that all strains were individually differentiated strains and formed temporal cohorts, providing evidence that we are working with individual strains. The PERMANOVA analysis yielded an R^2 value of 0.28 (P = 0.001) when age was considered as a variable, indicating a significant difference among age. Similarly, the ANOSIM test resulted in an R^2 value of 0.25 (P = 0.001), further confirming the observed differences as significant. These results provide evidence that the cohorts from different sediment layers are genetically different. Genetic diversity indices, including heterozygosity, Shannon index, and allele richness demonstrated consistent patterns over time, with minimal variation. Allele size exhibited slight shifts in position across the different length of dormancy (time points), but stable patterns within each age group indicate limited allele size variability within each age layer. We are thus confident that cross-contamination among the samples during sampling and laboratory work did not affect our results.

Second, as a proof of full functional recovery of the established strains the growth rate and oxygen production of the oldest established temporal cohorts were compared to the more recent cohort(s) under the culturing conditions. Growth is an important physiological process as it integrates all positive and negative influences on a cell.

For further analyses and characterization of the isolated strains, only a subset of temporal cohorts with more than 1 strain was considered, i.e. the strains with dormancy of 3 ± 2, 58 ± 5, 61 ± 5, 181 ± 15, 186 ± 15, 811 ± 110, 1131 ± 110, 3411 ± 120, and 6871 ± 140 years (Supplemental Material Table).

The growth rate of the temporal cohorts varied on average between 0.26 µ d^{-1} (58 ± 5 years of dormancy) and 0.31 µ d^{-1} (1131 ± 110, 3411 ± 120, 6871 ± 140 years of dormancy; Fig. 13 A) at 4°C and did not exhibit any significant variation among temporal cohorts (ANOVA, *P* = 0.37; Supplemental Material Table 5), suggesting a similar growth potential over dormancy length. Furthermore, in terms of photosynthetic activity, the strains length of dormancy of 3 ± 2, 1131 ± 110, 3411 ± 120, and 6871 ± 140 years showed a maximal oxygen production on approximate the same levels from on average between 136 ± 24 µmol O_2 mg^{-1} chl-*a* h^{-1} (mean and standard deviation; Fig. 13 B; 3411 ± 120 cal yr BP) and 224 ± 14 µmol O_2 mg^{-1} chl-*a* h^{-1} (mean and standard deviation; Fig. 13 B; 1131 ± 110 cal yr BP), averaging around 184 ± 66 µmol O_2 mg^{-1} chl-*a* h^{-1} (mean and standard deviation; Fig. 13 B; ANOVA, *P* = 0.281; Supplemental Material Table 5; further data in Supplemental Material Table 3), with variability among strains.

4. Discussion

In this study we provide evidence for millennial dormancy, viability, and full physiological recovery with uncompromised fitness of a diatom, *S. marinoi,* after 6871 ± 140 years of dormancy in Baltic Sea sediments. This represents, to the best of our knowledge, the longest sedimentary dormancy period reported so far for a eukaryotic organism.

A potential for long-term dormancy, exceeding decadal and even centennial time scales has been indicated recently for phytoplankton (Sanyal et al., 2022): germination of a 6600-years-old diatom *Chaetocerus muelleri var. subsalsum was shown* from permanently anoxic laminated sediments from the Landsort Deep, a comparable basin within the Baltic Sea. However, while metabolically active cells germinated in their study (Sanyal et al., 2022), viable growing strains were never established. Nevertheless, their results strongly support our findings, suggesting that long-term survival through resistant dormant propagules may be more common in phytoplankton than expected so far. The

resurrected cells of both studies originated from sediment deposited during the Holocene Thermal Maximum, a period of relatively warm climate and high diatom productivity (van Wirdum et al., 2019; Warden et al., 2017). More (comparative) studies on diatoms, and other phytoplankton species with different ecophysiological traits are needed to get deeper insights into the survival potential of phytoplankton in general. In the present investigation we did not analyse length of dormancy longer than 6871 ± 140 years, thus *S. marinoi* and other phytoplankton species could even have potentially longer dormancy periods than shown here or for *Chaetocerus muelleri var. subsalsum* (Sanyal et al., 2022).

Although the physiological basis of extended dormancy is still largely unknown, there is evidence for physiological activity in the dormant cells. Respective studies have reported that during dormancy, *S. marinoi* assimilates nutrients through heterotrophic capacities and transforms them into organic matter, even under dark and anoxic conditions (Stenow et al., 2024, 2020) which might explain their ability to remain viable over such a long time span.

Even though germination after long-term dormancy is observed, not much is known about how dormant life under anoxic conditions affects the respective organism after resurrection. All strains tested here grew and performed photosynthesis with only slight differences between modern strains, and those germinated after multimillennial dormancy. Thus, the present results demonstrate that an extended dormancy period, compared to short dormancy periods of 3 ± 2 years, does not necessarily have detectable impacts on the physiological performance of resurrected cells. The example of *S. marinoi* reported here, shows, that a complex metabolic machinery can apparently re-establish after prolonged periods of significant metabolic reduction and suggests that organisms can resume full functioning after extended dormancy periods, comparable to that of modern individuals. This emphasizes the ecological significance of dormancy and its assumingly complex mechanisms. More studies among different species, are again necessary.

Phases of resting over short periods of time are an appropriate survival strategy in seasonally variable habitats, such as the Baltic Sea. As a consequence of long-term dormancy periods that extend beyond seasonality, and under successive sedimentation, dormant cells are cut off from the possibility of resuspension. However, randomly occurring mixing events could potentially lead to resuspension of ancient dormant cells and could potentially secure survival during catastrophic events, as suggested by Ribeiro et al. (2011), demonstrating an enormous resilience potential of phytoplankton. Moreover, sediment archives, dormancy, and subsequent resurrection of species over a millennial time period as

shown here, present a significant opportunity to study, adaptation, and evolution over far longer than previously possible time periods *in vivo*.

Under the conditions tested here (4°C, salinity 7.5) all revived strains exhibited similar trait value ranges, primarily proving strain viability after extended dormancy and indicating growth capacities comparable to modern individuals, despite different conditioning by past warm or cold periods. During the Holocene history, globally (McLauchlan et al., 2013; Wanner et al., 2015, 2008) and in the Baltic Sea (Table 1, see Methods and (Emeis et al., 2003; Harff et al., 2011; Zillén et al., 2008)), phases of varying environmental conditions, specifically for temperature, salinity and nutrient availability have occurred along with increasing human-induced changes (Kirch, 2005). Despite being isolated from sediment layers representing different climatic conditions, significant differences in trait value ranges among temporal cohorts were not detected, for the one tested laboratory condition, suggesting that significant changes of the specific traits examined here were not altered beyond the indicated ranges over 6871 ± 140 years of dormancy. Though somewhat unexpected, this is in line with observations of resurrected phytoplankton over an up to 100-year time span (Hattich et al., 2024; Ribeiro et al., 2013), under one condition. While currently difficult to explain, our results might be related to the fact that the experimental conditions, which represent current distribution patterns of *S. marinoi* in the Baltic Sea, are close to their actual minimum temperature (Hattich et al., 2024). Using the resurrection approach under the conditions defined here, most likely represents only a snapshot of the actual niche of the *S. marinoi* population in the Baltic Sea in the intended years. It is likely, that selection by laboratory conditions contributed to the outcome in terms of strains and traits being selected originally. As the here used controlled abiotic conditions were also identical during germination and culturing, acclimation to these particular parameters could have taken place. Another factor with implications for selection is seasonality, an aspect that might have shaped temperature adaptations of the respective resurrected cohorts in our study. It is impossible to resolve seasonal bloom patterns of the past that might have driven adaptation to temperature. Over an environmental gradient, trait values for the strains from respective prevailing conditions likely would differ, as recently shown for *S. marinoi* over a period of 60 years (Hattich et al., 2024). The observed indicated differences in oxygen production are mainly due to variability among the different strains of temporal cohorts. The cohorts with length of dormancy of 1131 ± 110 and 3411 ± 120 years show less variability than strains of the other temporal cohorts.

Taking all our results together and considering the careful handling of all steps from sampling to cell isolation, we are confident of our approach and time frame. The mostly alternating hypoxic/euxinic conditions (Supplemental Material Fig. 1), and the consequently resulting absence of bioturbation as

also indicated by pronounced lamination, emphasize that sediment mixing had not occurred throughout the core (Supplemental Material Figs. 1 and 2). Further support comes from the microsatellite analysis, confirming significant delineation of distinct temporal cohorts Supplemental Material Figs. 4).

While the data shown here report an extraordinary capacity of life, we are fully aware that substantial research is needed to elucidate the underlying mechanisms of extremely long dormancy, survival, and subsequent germination in phytoplankton and living organisms in general. Nevertheless, the system we describe here for *S. marinoi* can be used to further explore fundamental mechanisms of adaptive evolutionary processes and factors shaping their outcome. First-studies offer insights into metabolic processes facilitating centennial dormancy and activation after extended periods of resting (Stenow et al., 2020).

In summary, our study demonstrates the (1) biological significance of dormancy and an enormous potential of phytoplankton to persist over long time scales and survive unfavourable conditions. In addition, it emphasizes (2) a significant resilience of phytoplankton towards climatic fluctuations. The system established here, could provide a powerful tool to trace potential ecological and genetic adaptation patterns on a millennial time scale over periods of altered climatic conditions offering valuable information on these organisms as a model species and predictions for future climatic changes.

5. Supplementary Information

5.1. Figures

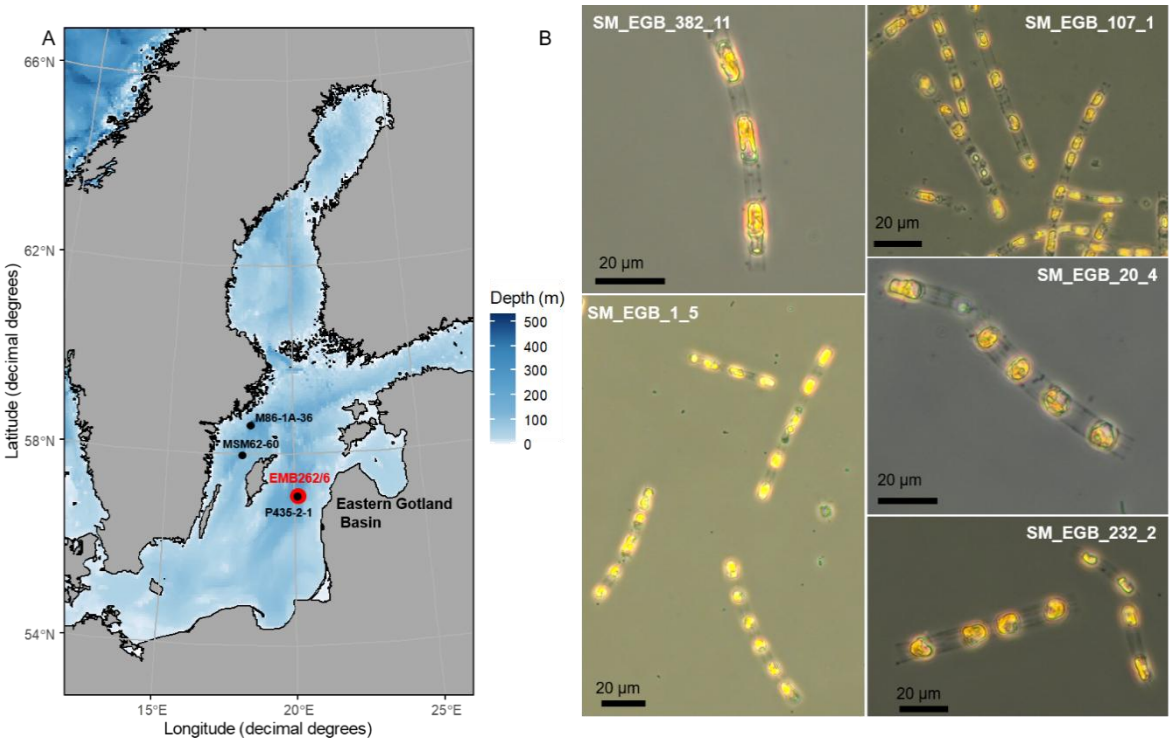

Fig. 11: Location of sediment sampling and pictures of resurrected species *Skeletonema marinoi*. (A): Map of the Baltic Sea (northern Europe) with the location of sediment core sampling (EMB262/6; 240 m water depth) in the Eastern Gotland Basin (red point). Additional sampling points of stratigraphy reference cores (black points): M86-1a/36 [35], MSM62-60 [36], and P435-2-1 [34]. (B): Pictures of different Lugol-stained strains of *Skeletonema marinoi*. Strain SM_EGB_382_11 from 6871 ± 140 years of dormancy, , SM_EGB_1_5 from 3 ± 2 years of dormancy, SM_EGB_107_1 from 1131 ± 110 years of dormancy, SM_EGB_20_4 from 58 ± 5 years of dormancy , and SM_EGB_232_2 from 3411 ± 120 years of dormancy.

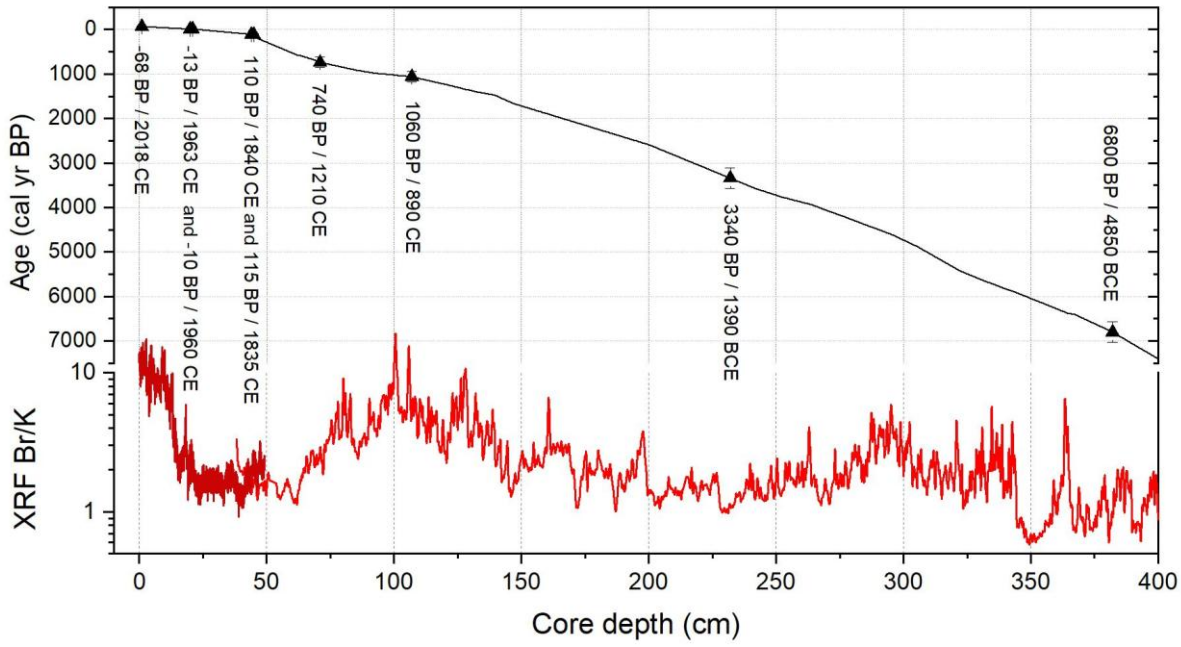

Fig. 12: Age-depth model of core EGB262/6 from the Eastern Gotland Basin with age of resurrected temporal cohorts of *Skeletonema marinoi* (triangles). The Br/K ratio of the sediments reflects the relative organic matter content of the sediment (Ziegler et al., 2008), with high (low) Br/K values indicating high (low) contents. Calculated years ± root mean squared errors. Years in (calculated years= cal yr) BP (before present = 1950) and B/CE (Before the/Common Era) respectively.

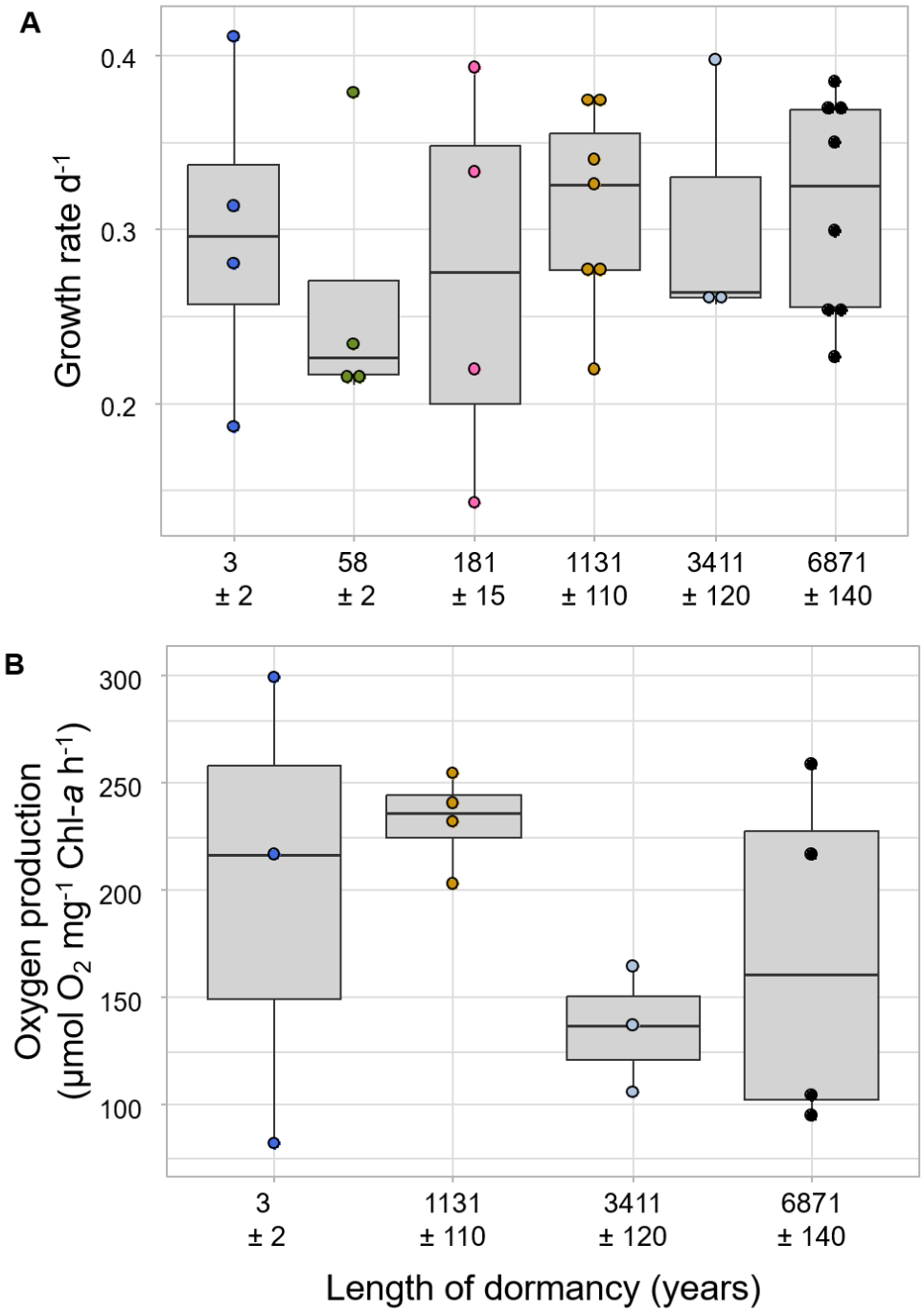

Fig. 13: A: Mean growth rate of *Skeletonema marinoi* temporal cohorts from 3 ± 2 (n = 4), 58 ± 5 (n = 4), 181 ± 15 (n = 4), 1131 ± 110 (n = 7), 3411 ± 120 (n = 3), and 6871 ± 140 (n = 8years of dormancy at 4°C. B: Mean oxygen production of *S. marinoi* temporal cohorts from -3 ± 2 (n = 3), 1131 ± 110 (n = 4), 3411 ± 120 (n = 3), and 6871 ± 140 (n = 4) years of dormancy at 4°C

5.2. Acknowledgements

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5.3. Data Availability Statement

Trait data that support the findings of this study are openly available at: https://doi.org/10.5281/zenodo.12723950. The supplementary data can be accessed via the following link: https://cloud.uni-konstanz.de/index.php/s/iBXMz3G5fMoikyb in folder “Chapter_3”. Supplementary information is provided in the document "Supplement.docx”.

Chapter 4 – Millennial-Old Genomes from Revived *Skeletonema marinoi* Resting Cells uncover Temporal and Spatial Patterns in the Baltic Sea

Alexandra Schmidt[1,2,*], Sarah Bolius[3], Juliane Romahn[4,5,6], Jérôme Kaiser[3], Helge W. Arz[3], Miklós Bálint[4,5,6], Markus Pfenninger[4,5,7], Anke Kremp[3], Laura S. Epp[1,*]

1. Environmental Genomics, Department of Biology, University of Konstanz, Constance, Germany
2. International Max Planck Research School Quantitative Behaviour Ecology & Evolution, Constance, Germany
3. Leibniz Institute for Baltic Sea Research Warnemünde, Rostock, Germany
4. Senckenberg Biodiversity and Climate Research Centre, Frankfurt am Main, Germany
5. Loewe Center for Translational Biodiversity Genomics (LOEWE-TBG), Frankfurt am Main, Germany
6. Institute for Insect Biotechnology, Justus Liebig University, Giessen, Germany
7. Institute for Molecular and Organismic Evolution, Johannes Gutenberg University, Mainz, Germany

* Correspondence

Keywords: Phytoplankton, diatom, *Skeletonema marinoi*, resurrection ecology, genomics

Abstract

The Baltic Sea has undergone profound environmental changes throughout its history. Since 6,800 cal yr BP, it has transformed from a freshwater system to a brackish one, influenced by climate change and the increasing anthropogenic impacts from human settlements over time. Millennia-old diatom resting cells from the Baltic Sea have been brought back to life, revealing a surprising convergence in phenotypes over time. Despite genetic differences, the revived *Skeletonema marinoi* strains exhibit similar traits, which is likely due to the influence of shared selective pressures or environmental conditions. Here we analyse full genomes of these resurrected strains, as well as strains cultured from modern samples across geographical locations in the Baltic Sea. The study of these ancient diatoms revealed insights into the ways in which *S. marinoi* adapted to historic climate shifts and human impact. Overall, we find a high level of genetic diversity. Spatially, the modern populations exhibit little genetic differentiation, pointing to a high degree of coherences and a single large population within the Baltic Sea. Temporally, we also find a high stability, but higher levels of allele turnovers in comparison to the spatial sampling. We uncover a substantial shift from early Medieval times to the past century, falling both within a time of climatic changes and increased anthropogenic activity. Our study underlines a high diversity and high degree of population coherence across both space and time, but also reveals that environmental factors can influence marine phytoplankton populations across millennial time periods.

1. Introduction

Aquatic ecosystems, including limnic and marine systems, take approximately 71% of Earth's surface (Tockner, 2021). They provide a wide range of important ecosystem services, such as carbon sequestration (Ferreira et al., 2023), food supply, and biodiversity support (Tockner, 2021). Unfortunately, these ecosystems are critically endangered due to various anthropogenic pressures, including pollution, overfishing, habitat destruction, and climate change (Ward et al., 2022).

Phytoplankton are primary producers in aquatic ecosystems, playing a fundamental role at the base of the food web. They contribute significantly to the carbon cycle and act as valuable indicators of ecosystem health due to their sensitivity to environmental changes such as temperature fluctuations (Dokulil and Qian, 2021). Additionally, phytoplankton are capable of fixing essential nutrients like nitrogen and phosphorus. However, when there is an excess of these nutrients in the system, blooms may occur, leading to oxygen depletion and the development of hypoxic conditions (Otero et al., 2020; Trombetta et al., 2019). Within the broader group of phytoplankton, diatoms are particularly

important. They play a central role in the biochemical cycling of silica and carbon, contributing up to 50% of marine primary production and serving as key drivers of the biological carbon cycle (Bach and Taucher, 2019).

Despite their ecological importance, phytoplankton communities display a surprising level of diversity, which contradicts traditional ecological theories that predict competitive exclusion. This phenomenon, known as the paradox of phytoplankton, arises from the unexpectedly high number of coexisting species within these communities (Hutchinson, 1961). Such diversity may contribute to the resilience of phytoplankton populations in fluctuating environments (Borics et al., 2021). Understanding the factors that drive this diversity is crucial for assessing the stability and productivity of aquatic ecosystems.

In recent decades, the Baltic Sea has come under intense anthropogenic pressure from activities such as eutrophication, overfishing, and pollution, which have significantly altered the ecosystem's structure and function (Reusch et al., 2018). These ongoing human impacts, combined with the region's history of natural variability, make the Baltic Sea an exemplary system for studying the compounded effects of both climate-driven changes and human activities on aquatic ecosystems. The Baltic Sea, due to its semi-enclosed nature and unique sub-regions (the Gulf of Finland, Gulf of Bothnia, Gulf of Riga, and the Baltic Proper), provides an ideal case study for exploring spatial and temporal differences in ecosystem dynamics. Each of these sub-regions exhibits distinctive environmental conditions such as salinity gradients, temperature variations, and different depths (HELCOM, 2024). This heterogeneity shapes species distribution, resilience, and the extent of eutrophication and hypoxia (Lehmann et al., 2022; Murray et al., 2019). Therefore, it is crucial to investigate how these spatial differences influence ecosystem functioning and adaptability.

The Baltic Sea has undergone several major transitions throughout its relatively young history, including changes in salinity and ecosystem structure. It began as a freshwater Ice Lake (14,600–12,300 cal yr BP), transitioned to brackish conditions in the Yoldia Sea (12,300–11,500 cal yr BP), reverted to freshwater in the Ancylus Lake (11,500–10,000 cal yr BP), evolved into a marine state during the Littorina Sea phase (9,500–4,000 cal yr BP) with the highest documented salinity levels, and finally stabilized into its current brackish state known as the Modern Baltic Sea (4,000 cal yr BP–Present) (Andrén et al., 2011; Weckström et al., 2017). These transitions were accompanied by significant climatic events, such as temperature fluctuations and changes in sea level (Zillén et al., 2008). The combination of historical transitions and spatial heterogeneity makes the Baltic Sea a unique system for investigating ecosystem responses to environmental changes.

An opportunity to explore adaptation and resilience in these varying environments lies in studying phytoplankton species like the diatom *Skeletonema marinoi*, which exhibits high adaptability and phenotypic plasticity (Jing et al., 2024). *S. marinoi* forms resting stages that enable it to survive unfavourable conditions through dormancy, hatching once conditions improve (Thakur and Ojha, 2020). This ability to form resistant stages contributes to their resilience and adaptability in the face of environmental changes (McQuoid et al., 2002; Thakur and Ojha, 2020). However, it is important to note that resurrection from resting stages might also contribute to maladaptation, such as gene flow from populations that are not locally adapted (Gechev et al., 2021). Resting stages that remain dormant can become incorporated into the sediment and preserved (McQuoid et al., 2002). Such stages serve as a historical archive, allowing researchers to access past populations and assess both phenotypic and genomic characteristics. It was observed that revived millennia-old *S. marinoi* from resting cells exhibited phenotypic similarity but genetic divergence (Bolius et al., 2024). This suggests phenotypic convergence, which contrasts with the previously mentioned phenotypic plasticity, indicating that perhaps only specific phenotypes are capable of building robust resting stages. This convergence may arise not only from selective pressures but also from the interplay of reproductive strategies such as asexual and sexual reproduction.

Skeletonema marinoi is a diploid diatom that primarily reproduces asexually through mitotic cell division, which is usually clonal (Johansson et al., 2019). As a primarily diploid organism (Bulankova et al., 2022), it also undergoes occasional sexual reproduction, which is typically triggered when cells reach a critical size threshold (Bilcke et al., 2022). This sexual phase involves the formation of gametes and subsequent fertilisation, leading to the creation of larger initial cells called auxospores (Bilcke et al., 2022). The rate of reproduction can vary depending on environmental conditions, but the ability to switch between asexual and sexual reproduction allows *S. marinoi* to maintain genetic diversity and adapt to changing environments (Johansson et al., 2019).

This interplay of reproductive strategies not only ensures adaptability in changing environments but also enhances the species' capacity to contribute to long-term evolutionary studies. By employing resurrection ecology, which involves reviving dormant cells from the sediment, it is possible to investigate the evolution of *S. marinoi* populations over different temporal scales (Weider et al., 2018). In diatoms, resting cells have been documented to successfully revive after up to approximately 6,600 years for the species *S. marinoi* (Sanyal et al., 2022). This capability opens a window for studying adaptation and differentiation in genetically distinct populations across regions of the Baltic Sea with varying environmental conditions. This underscores the evolutionary importance of dormancy as both an adaptive strategy and a tool for understanding historical population dynamics.

In this study, we utilize both ancient and modern strains of *S. marinoi* collected from different sub-regions of the Baltic Sea to: (1) investigate spatial differences in population structure and dynamics, (2) analyse how genomic structure and gene functions are structured over time at a single location, and (3) explore how physiological and functional traits are linked to genomic structure. Our aim is to understand how *S. marinoi* adapts and evolves in response to spatial environmental heterogeneity and over temporal scales, which can provide insights for managing aquatic ecosystems amid ongoing environmental changes.

2. Material & Methods

2.1. Samples

Skeletonema marinoi is a chain-forming diatom and main component during Baltic Sea spring bloom. To analyse the genetic and ecological diversity of *S. marinoi* in the Baltic Sea, we established a comprehensive spatial and temporal series. *S. marinoi* is crucial for the marine ecosystem, significantly contributing to primary production and supporting the food web during the spring bloom. We use cultures obtained from samples across different locations and times to investigate population structure and adaptive mechanisms of the organism.

Modern strains from different geographical areas of the Baltic Sea serve to establish a baseline of today's (spatial) diversity (Fig. 14). The strains were either isolated from water samples or sediment. Methods according to (Godhe & Härnström, 2010; Hattich et al., 2024).

S. marinoi can form dormant cells that sink to the sediment and build up sediment archives. Based on this, for the temporal data set re-germinated *S. marinoi* strains isolated from a sediment core of the Eastern Gotland Basin, Central Baltic Sea were used (Schmidt et al., 2024). The strains cover points in time from 6800 to -68 cal yr Before Present (calibrated years Before Present (BP=1950)) with distinct climatic conditions .

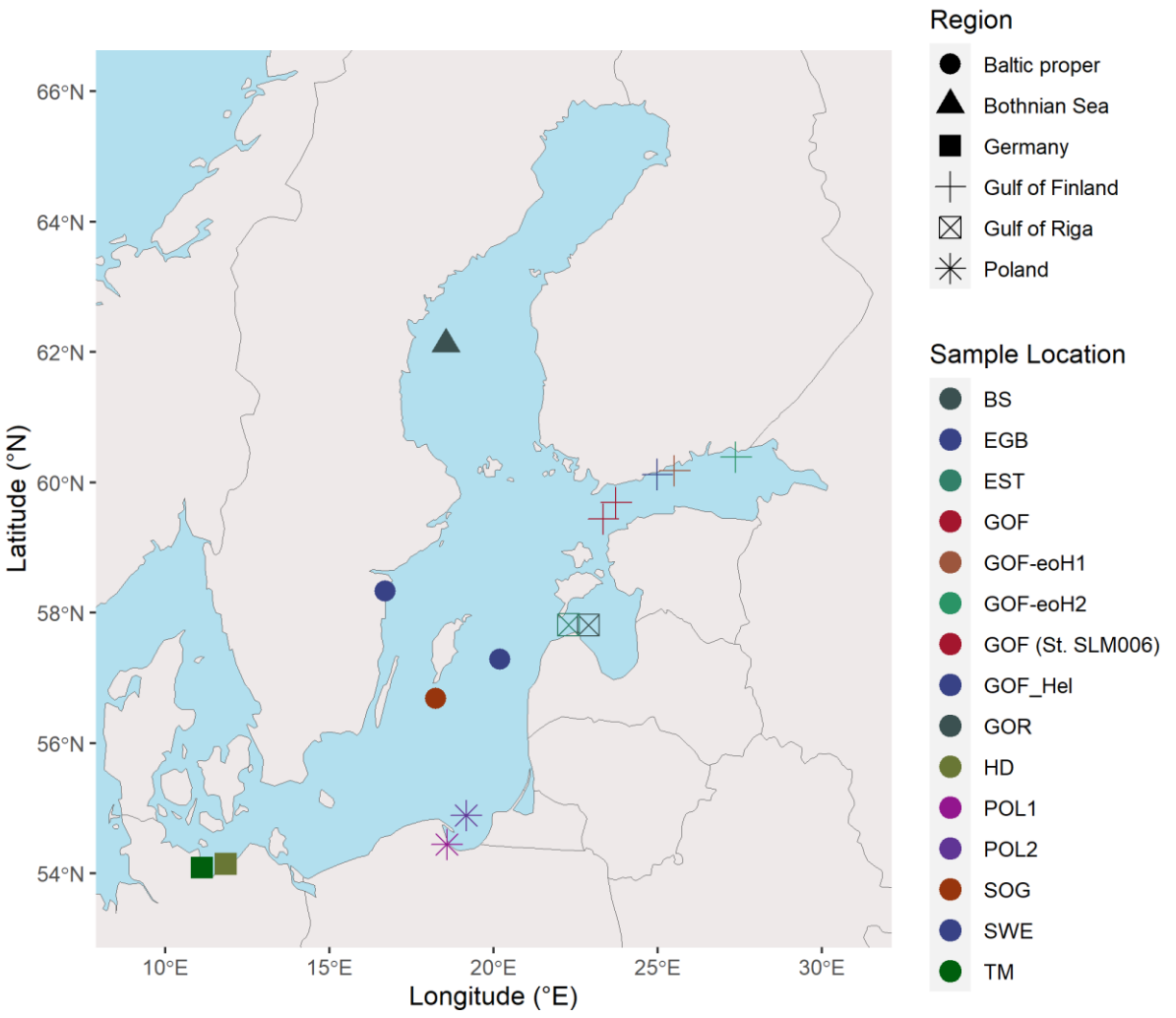

Fig. 14: Map showing various sampling locations across the Baltic Sea region. The sampling sites include the Eastern Gotland Basin (EGB) and the Gulf of Finland (GOF), featuring time series data from sediment archives. Additional spatial sampling locations are distributed around Germany (HD), Estonia (EST), Sweden (SWE), Poland (POL1, POL2), and the Bothnian Sea (BS). All samples are taken from sediment or water.

2.2. Laboratory Work

2.2.1. Culturing

For this study, analysed *S. marinoi* strains were isolated from either water or sediment samples (Table S1) and established as clonal cultures. All strains were cultured in f/2 medium with silicate (Guillard, 1975) at PSU 7.5 or 14, under a 16:8 h light:dark cycle with ~40 photons m^2 s^{-1}, at either 4°C or 10°C.

2.2.2. Phenotypic Measurements

For phenotypic trait measurements, all strains were cultured at 10 °C under the same conditions as stock cultures. The strains initially cultured at 4 °C (Table S1) were acclimatized to 10 °C for 7 days before the start of the experiments. The experimental cultures were inoculated during exponential growth.

To analyse the trait/ecological diversity of *S. marinoi*, we measured several parameters, that included: a) growth rate , b) carrying capacity, c) production of reactive oxygen species (ROS) at 10 and 20 °C, d) the effective quantum yield of photosystem II (PSII) and e) cell biovolume and f) filament length as morphological traits. using a high-throughput approach. For each strain, six wells in 24-well plates (2.1 ml culture) were prepared as replicates at low cell densities (~3500 cells/ml) from stock cultures during their exponential growth phase (day 4 – 10). All subsequent measurements were conducted using these samples.

For the growth rates, the growth of each strain (4 replicates/wells) was monitored in vivo using a plate reader (infinite m plex Tecan; chl-*a* Ext. 450 nm, Em. 682 nm) until three days into the stationary phase. The growth rate (μ) was calculated as the average exponential growth, using the following formula:

$$\mu = \frac{\ln(RFU_{ty}) - \ln(RFU_{tx})}{tx - ty} \qquad \text{(I)}$$

where RFUty is the fluorescent of the culture at the end of the exponential phase, RFUtx is the fluorescent the culture at the beginning of the exponential phase; ty and tx are the according days.

The carrying capacity was determined from the growth data as the highest measured relative fluorescence unit (RFU) at the end of exponential growth phase. This value was averaged from four replicates for each strain. The RFU serves as a proxy for biomass.

The effective quantum yield of PSII (Y(II)) was measured with an Imaging-PAM (Pulse Amplitude Modulated, Walz) and corresponding software (Imaging Win v2.47) during the exponential growth phase, for 6 replicates (6 wells). The PAM settings were adjusted separately for each well/strain to a chlorophyll fluorescence (F) of 0.1 – 0.2 before the light pulse by modifying the settings of intensity, frequency and gain. The area of interest was set to cover a large area within the well, excluding the well plate borders. Just before the measurements the cultures were carefully shaken to ensure homogeneity of cells in each well. The values for Y(II) were measured after a saturation pulse and calculated by the software. The entire measurement process took no more than 30 second. The culture plates were kept in an isolating box until the measurement to prevent any effects of changing

conditions on the *S. marinoi* cultures. The effective quantum yield of PSII is the efficiency of how absorbed light is used for photochemistry (Murchie and Lawson, 2013).

For the reactive oxygen species (ROS) measurement, cultures were taken from their exponential phase, with 2 replicates for each strain. ROS forming was analysed at 10 °C (culture conditions) and an elevated temperature of 20 °C. The measurement and calculations followed the protocol of Argyle *et al*. 2021 (Argyle et al., 2021) (Protocol: dx.doi.org/10.17504/protocols.io.ewov14qopvr2/v1). The measurement was then related to measured RFU (as above for growth) as a biomass proxy. ROS serves as a stress indicator of cells (McInnes et al., 2019).

Samples of strains for morphological traits were also taken during exponential growth phase, using a mixed sample of 2 replicates. The samples were fixed with Lugol's solution for subsequent measurement. Cell length and width were measured and the cells per filament was counted (mind. 30 cells/filaments) from the fixed cells using an inverted light microscope (Axio Zeiss S100), camera (Olympus) and software (Olympus Stream Essential 2.4). The biovolume for *S. marinoi* was calculated assuming a cylindrical cell shape (Hillebrand et al., 1999).

2.2.3. DNA Extraction and Sequencing

Prior to DNA extraction, cultures were inoculated with cells in their exponential phase, allowed to grow until they reached the exponential phase again, and then harvested.

The DNA extraction was conducted using the Qiagen Dneasy Plant Pro Kit (Hilden Germany). The cell pellets (n=88) were initially frozen at -80°C to preserve the DNA. Upon thawing, they were mixed with lysis buffer, which facilitates the breakdown of the cell walls to release the DNA. The samples then underwent an initial bead-beating for 30 seconds to mechanically disrupt the cells, further aiding in lysis. 2 µL of proteinase K (20mg/ml) were added to digest proteins and purify the DNA. The cells were homogenized twice for 45 seconds each, with a five-minute pause in between, using a FastPrep-24™ 5G machine (M.P. Biomedicals, LLC Santa Anna CA, USA). This ensures a thorough breakdown of the cells. Afterward, the samples were incubated overnight at 56°C with rotation to allow complete digestion of cellular material. Finally, the manufacturer's protocol was followed.

For the genomic analysis, 3 µg of DNA extract was utilized for sequencing for each sample. A PCR-free library preparation (plant and animal whole genome library preparation (350 bp)) and sequencing

(Illumina NovaSeq X Plus, PE150 platform) were conducted at Novogene. Sequencing depth was 25X per sample.

2.3. Bioinformatics

2.3.1. Annotation of the Reference Genome

The genome annotation of *S. marinoi* (GenBank PRJNA493755) was conducted using a combination of BUSCO and AUGUSTUS tools to ensure comprehensive and accurate gene predictions. The initial assessment of the genome assembly's completeness was conducted using BUSCO v5.2.2 (Manni et al., 2021) with the eukaryota_odb10 database. This analysis identified and quantified the presence of conserved single-copy orthologs in the genome, thereby providing a measure of the assembly's completeness and quality.

Subsequently, gene prediction was conducted using AUGUSTUS v3.2.3. (Stanke et al., 2006). The reference genome (GenBank PRJNA493755) was utilized as the input for this analysis. The gene predictions were based on the Saccharomyces cerevisiae S288C species model, which was selected for its well-characterized gene structure and annotation accuracy. The predicted genes were output in GFF format, providing a comprehensive annotation of the genomic features.

2.3.2. Sequencing Data of the Resurrected Strains

To ensure that only high quality reads were retained for downstream analyses, raw sequencing data was first processed using Autotrim v0.6.1 (Waldvogel et al., 2018) to remove low quality sequences, adapter contamination and quality control. In order to achieve this, Autotrim employs Trimmomatic v0.39 (Bolger et al., 2014) FastQC v0.12.1 (Andrews, 2021) and MultiQC v1.14 (Ewels et al., 2016).

After preprocessing, the high-quality reads were aligned to the reference genome (*Skeletonema marinoi*, GenBank PRJNA493755) using BWA v0.7.17 (Li and Durbin, 2009) with the mem algorithm. This tool was chosen for its efficiency and accuracy in aligning short reads to a reference genome, which is essential for accurately identifying the genomic locations of the sequences.

Once the reads were mapped, Samtools v1.15 (Danecek et al., 2021) was used to convert the SAM files to BAM format, sort and deduplicate the BAM files, and index them. Coverage depth was then calculated to assess the depth of sequencing across the genome. Using R, the modal value of coverage per sample was determined, and a threshold of 15x coverage and a quality score of 30 were set for

variant calling. This step was critical to ensure that only high confidence variants were considered, thereby minimizing the likelihood of false positives. Variants were then called using the Bcftools v1.19 (Danecek et al., 2021) integrated into Samtools. The variant calling criteria were based on the coverage analysis to ensure that only variants with sufficient coverage (≥15x) and quality (Q≥30) were included. This step is essential to identify genetic variation within the samples. For further analysis of the data, Vcftools v0.1.16 was used to calculate allele frequencies and nucleotide diversity.

To explore the genetic structure of the genomes, we utilized PLINK v1.90b6.21 (Purcell et al., 2007) for linkage disequilibrium (LD) analysis and LD pruning. These methods help to identify regions of the genome in LD, which may suggest selection or other evolutionary processes. Pairwise LD was calculated using PLINK. Due to high LD ($r^2 > 0.2$), pruning was not feasible despite parameter adjustments. The full dataset was used for subsequent analyses. Subsequently, we conducted admixture analysis with ADMIXTURE v1.3.0 (Alexander et al., 2009) to infer population structure and estimate the ancestry proportions from various populations.

For the purpose of variant annotation, SnpEff v5.2 (Cingolani et al., 2012) was employed. This tool is used for the annotation and prediction of the effects of genetic variants on genes and proteins. The gene predictions described in the Annotation section of the manuscript were utilized as the reference for annotation. SnpEff was utilized to annotate the filtered VCF files, thereby providing functional information about the variants. This step is of great importance for the comprehension of the potential impact of the identified variants on the organism's biology. For pathway analysis of these genes, we employed the EggNOG-mapper v2.1.12 (Cantalapiedra et al., 2021). This tool maps the annotated variants to gene regions, extracts CDS sequences, translates them to protein sequences, and performs functional annotation and pathway analysis.

2.4. Analyses

The genomic structure of our samples was analysed using ADMIXTURE results. The Q matrix, which represents ancestry proportions, was merged with the metadata to create a comprehensive dataset. The metadata includes various attributes such as sample ID (libid), year in Common Era (year_CE), years Before Present (year_BP), location, larger regions, latitude (lat), longitude (lon), water zone, isolation source, PS_yield, ROS (at 10°C and 20°C), growth rate (gr), cell biovolume (cellbv), carrying capacity (carcap), filament length, and culture salinity (calture_salinity_PSU). A principal component analysis (PCA) was conducted using the package “vegan” v2.6 (Oksanen et al., 2020) on the ancestry proportions

to reduce data dimensionality and identify patterns. The first two principal components were plotted to illustrate genetic variation among individuals, with colours indicating data type (temporal or spatial) and isolation source (water or sediment). An Analysis of Similarities (ANOSIM) using the "vegan" package was performed to determine if the genetic variation between temporal and spatial data types was significant.

To calculate allele turnover, we analysed allele frequency data for significant changes (over 1%) and determined the total number of generations. Allele turnover was the ratio of significant changes to generations. Based on the BCFtools output, we assessed allelic diversity using the Shannon index, which measures allele frequency and evenness, and nucleotide diversity (pi), representing average nucleotide differences per site. These metrics were compared between temporal and spatial datasets to understand genetic structure and dynamics. A t-test was performed for each metric to identify significant differences between the datasets.

The mean ancestry proportions were calculated for each year_BP and subsequently normalized to ensure that the total proportions summed to 100%. To identify temporal patterns in genomic structure, we employed the Constrained Incremental Sum of Squares (CONISS) clustering method using the "rioja" package (Juggins, 2015). This hierarchical clustering approach groups samples based on their similarity, constrained by their temporal order, and thus facilitates the identification of significant changes in genomic structure over time.

To analyse the spatial distribution of genetic structure, the dataset was filtered to include only spatial data from -72 to -60 cal yr BP. World map data was obtained using the "rnaturalearth" (Massicotte and South, 2024) and "rnaturalearthdata" (South et al., 2024) packages. The spatial data was then prepared by converting PC values (PC1, PC2, PC3) into a long format using the "tidyverse" package (Wickham et al., 2019). A map was created with the world map as the base layer, and PC values were plotted as points, coloured by genetic structure. The map was divided by PC to show the spatial distribution of the first three principal components. ANOSIM was used to assess how much genetic structure is explained by geographic regions.

EggNOG-mapper results were used to identify biological processes that correspond to the resulting protein families (PFAMs). PFAM is a database of conserved evolutionary units, which can be used to explore domains and other functional regions in a protein, providing insight into the function of that protein (Mistry et al., 2021). In this study, PFAMs were identified and analysed to understand their distribution across various biological processes over time. CONISS was performed on the composition of biological processes, to identify shifts and clustering patterns in the data over time. To test for

stratigraphic effects, we included sedaDNA copy numbers, which represent species-specific copy numbers obtained from droplet digital PCR (ddPCR) analysis, from Schmidt et al. (2024).

Redundancy Analysis (RDA) was employed using “vegan” to investigate the correlation between genomic structure and trait variables, including PS_yield, ROS_10, ROS_20, gr, cellbv, carcap, and filament_length. The RDA plot was color-coded by year_BP and larger regions, with environmental factors represented as arrows indicating their influence on the genomic structure.

To evaluate the impact of trait variables on the Shannon diversity index, a metric of genomic diversity, we employed Generalized Additive Models (GAMs) using the “mgcv” package (Wood, 2011). The smooth terms for each variable were plotted to visualize their influence on genomic makeup, thereby providing insights into the extent to which different factors contribute to genetic diversity. Variable importance scores were calculated as the inverse of the p-values from the GAM summary statistics. Higher scores indicate greater significance in predicting genomic diversity changes. The regional effect on the Shannon diversity index was quantified through the coefficient values of the regions factor from the GAM. These coefficients were extracted from the summary of the GAM model and plotted to highlight the regional impacts on genomic diversity. The Baltic Proper was chosen as the reference region, and hence its coefficient is set to 0. This means that the effects of other regions are interpreted relative to the Baltic Proper. By setting the Baltic Proper as the baseline, we can assess how genomic diversity in other regions deviates from this reference point.

3. Results

3.1. Spatial vs. Temporal Structure

The Principal Component Analysis (PCA) of ADMIXTURE compositions, illustrated in Figure 15A, reveals no significant (ANOSIM: R = 0.00291, p = 0 .407) distinction between spatial and temporal samples across principal components PC1 (10.3%) and PC2 (9.4%). Also, there are no significant (ANOSIM: R = 0.046, p = 0.151) differences between samples isolated from water or sediment (Supplement, Fig. S1).

Additionally, the analysis compares allele turnover, allelic diversity (Shannon Index), and nucleotide diversity between the spatial and temporal data types. The data are presented for each of these measures (Figures 15B, 15C, and 15D), which illustrate the observed variations across locations in the Baltic Sea (spatial) and over time in the EGB (temporal). It is notable that the temporal dataset shows

a significantly higher allele turnover (p = 0.009), while no significant differences are observed in allelic diversity (p = 0.148) and nucleotide diversity (p = 0.374) between the spatial and temporal data types.

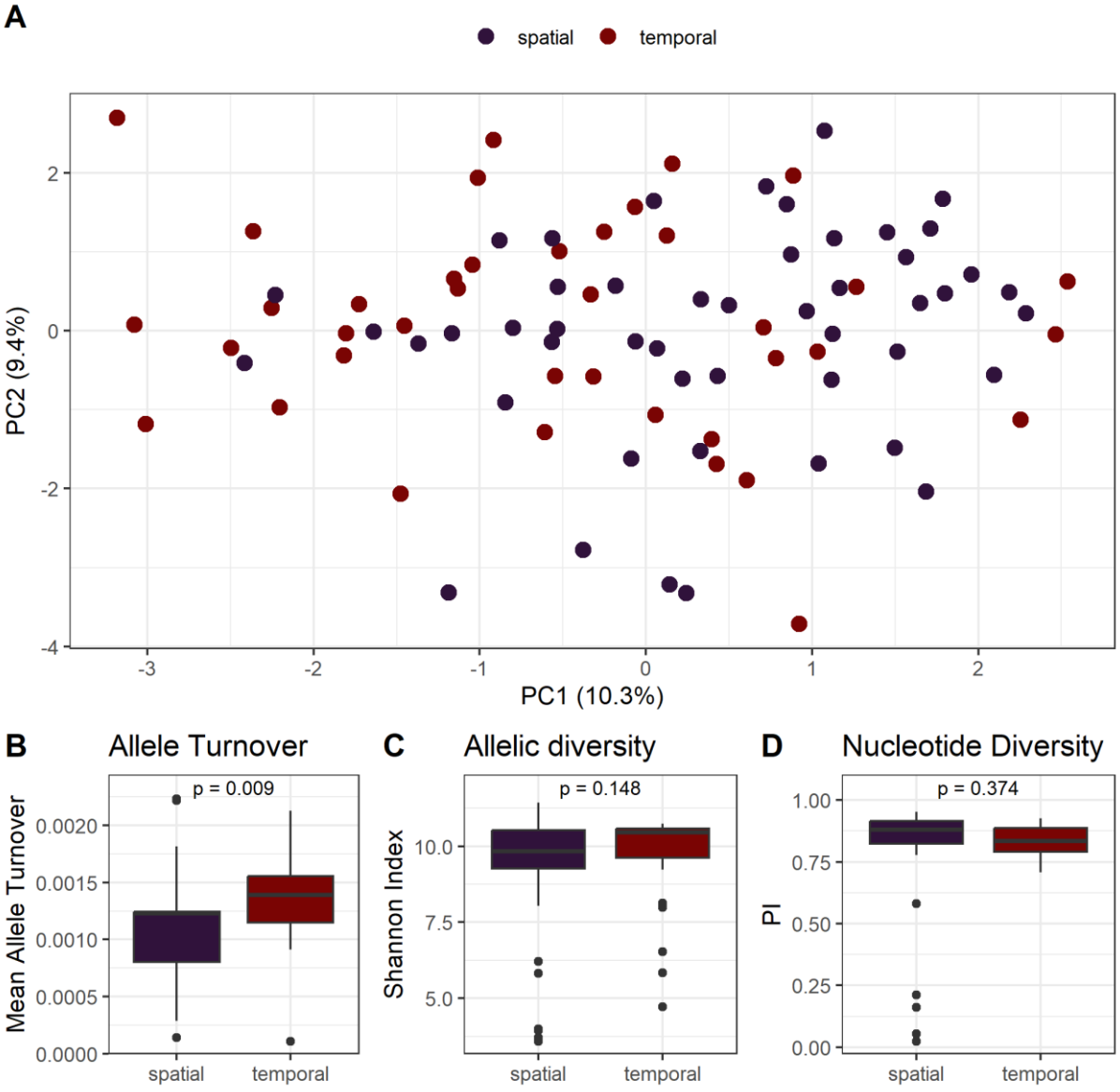

Fig. 15: Comprehensive analysis of spatial and temporal data types. A) Scatter plot illustrating the distribution of two data types, spatial and temporal, along the first two principal components (PC1 and PC2) (ANOSIM: R = 0.00291, p = 0.407). B) Allele turnover (t-test: p=0.009) C) Shannon Index (allelic diversity, t-test: p = 0.148) and D) Nucleotide diversity (t-test: p = 0.374) by data type (spatial and temporal), emphasizing the variation across locations in the Baltic Sea and over time in the EGB.

To further investigate these temporal patterns, we employed the CONISS clustering method, which revealed changes over time. As illustrated in Fig. 16A, the mean ancestry proportions for each cal yr BP were calculated and normalized, demonstrating fluctuations in genomic composition across disparate

time intervals. The dendrogram serves to further highlight these temporal patterns by grouping samples based on their similarity and temporal order, with the dashed red line indicating the most significant shift between 742 and 110 cal yr BP.

The investigation was extended to examine the spatial distribution of genetic structure, employing principal component analysis (Fig. 16B). The maps, grouped by PC1, PC2, and PC3, demonstrate the spatial distribution of genetic variation across different Baltic Sea locations. Each point on the maps represents sample locations, coloured by genetic structure scores. These scores indicate genetic variation across different regions. The genetic structure is slightly explained by the region across the first three principal components (PC1, PC2 and PC3) (ANOSIM: R = 0.1959, p = 0.002).

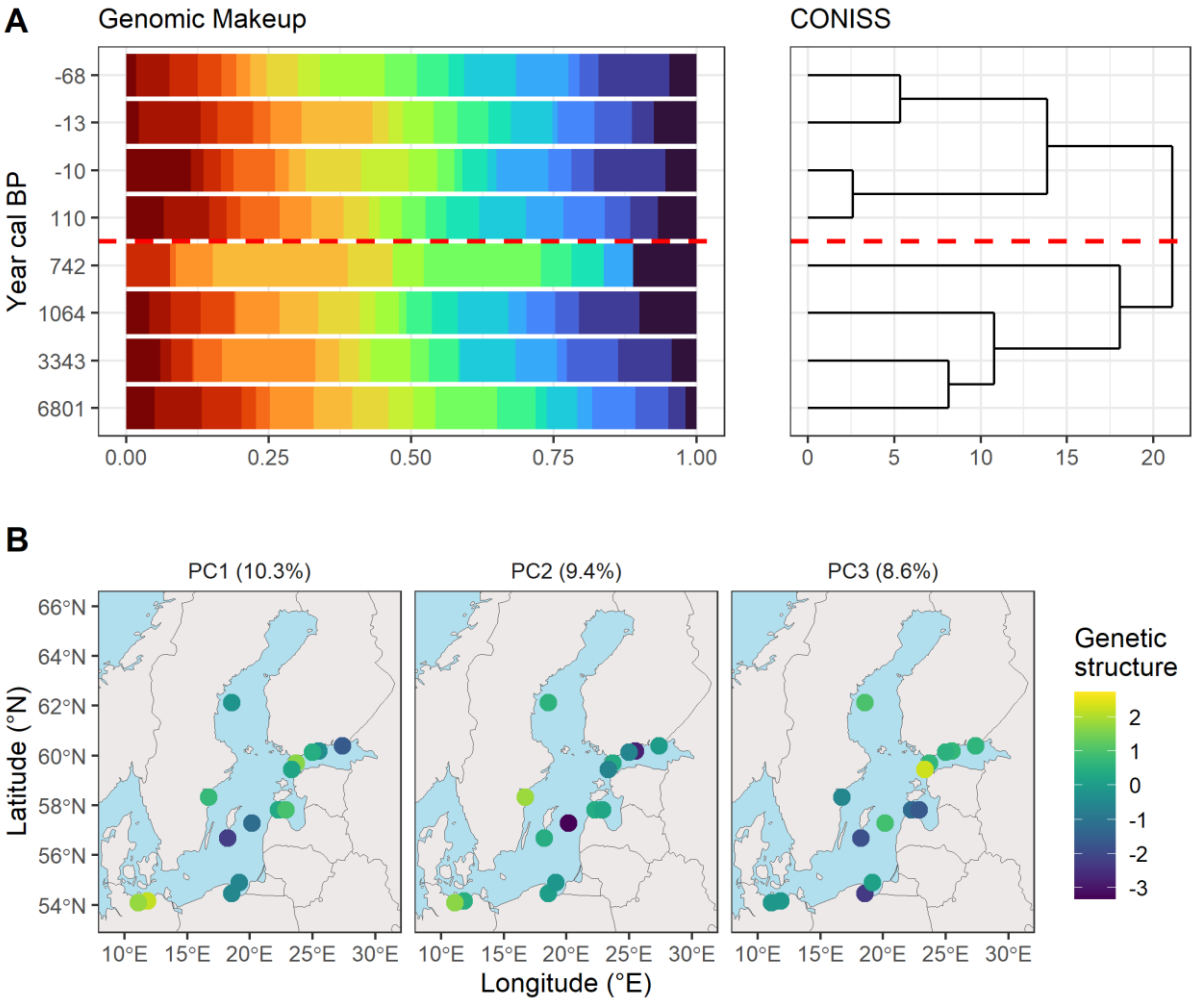

Fig. 16: Temporal and Spatial Analysis of Genetic Structure. A) depicted is the temporal analysis, which is represented by a bar plot illustrating the composition of ancestral proportions over time and a dendrogram generated by the CONISS clustering method. The dashed red line indicates the most significant shift between 742 and 110 yr cal BP. B) Baltic Sea maps corresponding to PC1 (10.3%), PC2 (9.4%), and PC3 (8.6%), which illustrate the spatial distribution based on principal

component analysis for samples between -72 and -60 cal yr BP across Baltic Sea geographic coordinates. Each map has points plotted at various locations, coloured according to their genetic structure score (ANOSIM: R = 0.1959, p = 0.002).

3.2. Functional Changes over Time

To gain further insight into the implications of these genetic patterns, we investigated whether the temporal changes observed in genetic structure are also reflected in functional aspects. The analysis reveals distinct temporal patterns in the distribution of PFAMs across different biological processes, as evidenced by changes in mean counts over time (Fig. 17). In particular, certain protein families show fluctuations, with the most pronounced shift observed at 742 cal yr BP. During this period, variants observed in genes involved in DNA and protein processes was notably reduced compared to other periods. This shift does not correspond to the lowest sedaDNA copy number (Schmidt et al., 2024). More detailed information on the specific PFAMs and related processes can be found in Supplementary Table S2.

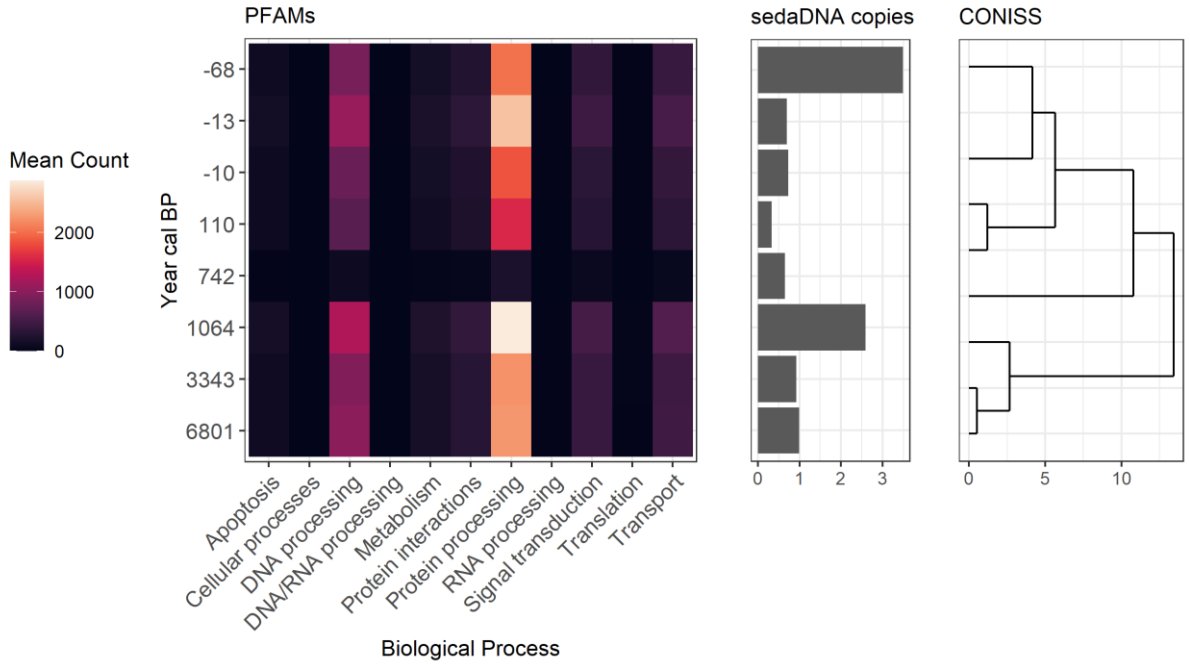

Fig. 17: Temporal distribution of PFAMs across different biological processes over time. Heatmap of mean counts per biological process (left), sedaDNA species specific ddPCR copy numbers over time (Schmidt et al. 2024) (middle) and CONISS dendrogram illustrating clustering patterns (right).

3.3. Drivers of Genomic Variation

In order to gain insight into the mechanisms behind these patterns, it is essential to understand how physiological and phenotypic traits, in addition to age and location of the samples, influence genomic structure. The RDA analysis in Fig. 18A reveals that certain factors, especially photosynthesis efficiency towards the samples from 6801 cal yr BP, have directional influences over time as indicated by vector lengths and orientations. In Fig. 18B, the series of GAM smooths for each variable against Shannon diversity index suggests complex relationships where some factors, like age (year_BP), show clear trends while others exhibit more nuanced patterns within their confidence intervals. Variable importance scores (Fig. 18C) are calculated as inverse p-values from GAM summary statistics; higher scores indicate greater significance in predicting genomic changes. The factors stress response (ROS) at 20 °C ($p = 0.00308$), photosynthesis efficiency (PS_yield) ($p = 0.01008$), sample age ($p = 0.02134$) and filament length ($p = 0.04048$) have a significant impact on the genomic diversity. Finally, Fig. 18D quantifies regional impacts through coefficient values, demonstrating that specific locations contribute differently to genomic diversity. While the overall influence is marginal, samples from the Poland region show the strongest effects when the Baltic Proper serves as the reference.

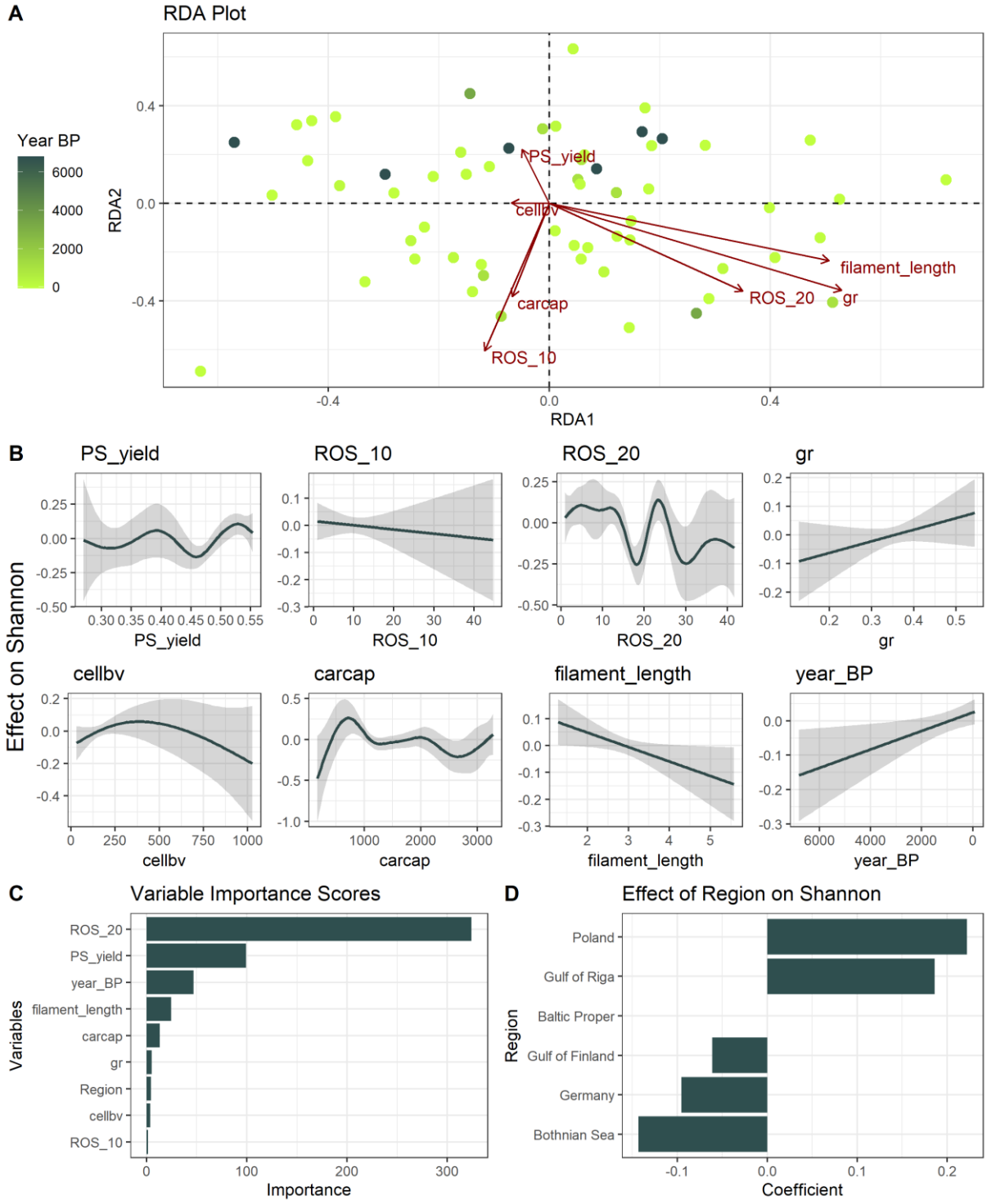

Fig. 18: Integrated analysis of admixture results using redundancy analysis (RDA) and generalized additive models (GAM) to assess population structure. A) illustrates an RDA plot correlating various environmental factors with sample age (Year cal yr BP). B) displays GAM smooth terms for each factor's effect on Shannon diversity index with confidence intervals. Significant p-values are observed for PS_yield (p = 0.01008), ROS_20 (p = 0.00308), year_BP (p = 0.02134) and filament_length (p = 0.04048), C) shows variable importance scores derived from inverse p-values of GAM smooth terms including regional effects. D) highlights regional effects on biodiversity through coefficient values associated with larger

regions. The Baltic Proper is used as the reference region, hence its coefficient is set to 0, allowing for the comparison of other regions relative to this baseline.

4. Discussion

Our study reveals that the overall genomic composition of *Skeletonema marinoi* populations remains relatively consistent across both spatial and temporal scales, suggesting a high degree of genetic coherence in the Baltic Sea. Despite this coherence, our analysis identified subtle regional differences that hint at localized environmental influences. For instance, the ANOSIM results ($R = 0.1959$, $p = 0.002$) indicate minor but detectable differences in genomic structure between regions, with a slight effect on Shannon diversity observed in samples from Poland. These localized differences could reflect adaptations to specific environmental conditions, though the overall lack of strong regional effects suggests a high degree of gene flow and connectivity among populations. Such connectivity likely diminishes the impact of spatially varying environmental gradients, a pattern consistent with the findings of Sexton et al. (2014) and Slatkin (1987) on the homogenizing effect of gene flow across environmental gradients.

The Baltic Sea's dynamic environmental conditions, characterized by fluctuations in salinity, temperature, and nutrient levels (Viitasalo and Bonsdorff, 2022), could promote genetic diversity and facilitate population connectivity. This aligns with the "paradox of the plankton" (Hutchinson, 1961), where diverse plankton species coexist despite limited resources and competitive pressures. Thus, while *S. marinoi* populations are genetically coherent at broader spatial scales, localized influences are still present, albeit to a minor extent.

A striking results of this study is the unexpected phenotypic convergence observed among *S. marinoi* strains of the temporal dataset, even among genetically distinct populations. This is consistent with findings by Bolius et al. (2024), suggesting that shared selective pressures in the Baltic Sea may be driving these traits, overriding genetic divergence. Such convergence underscores the resilience of *S. marinoi*, likely reflecting the survival advantage of maintaining specific phenotypes capable of withstanding environmental variability or forming robust dormant stages. However, the reliance on dormancy as a resilience mechanism could also introduce maladaptation. Gene flow from dormant populations that are not locally adapted may undermine the selective advantage of locally optimized traits, as highlighted by Gechev et al. (2021). his interplay between resilience and maladaptation emphasizes the complex role of dormancy and gene flow in shaping long-term population dynamics.

Shifting focus to temporal patterns, our results indicate significantly higher allele turnover in the temporal dataset, suggesting that *S. marinoi* has undergone more dynamic genetic changes over historical time scales compared to modern spatial scales. This dynamism is likely facilitated by the species' ability to switch between asexual and sexual reproduction, enabling both rapid clonal expansion and periodic bursts of genetic diversity (Johansson et al., 2019). Historical environmental transitions, such as the shift from the Littorina Sea to the Modern Baltic Sea, appear to have driven these changes by introducing variations in salinity and water levels. While this adaptability reflects *S. marinoi*'s evolutionary resilience, it also entails trade-offs: dormancy-driven gene flow may reintroduce maladaptive traits from populations less suited to prevailing local conditions, potentially compromising population fitness. The transition from historical environmental variability to modern anthropogenic influences, such as eutrophication and pollution (Reusch et al., 2018), is likely to intensify these pressures, further shaping the genomic structure of *S. marinoi*.

The GAM analysis provides additional insights into these temporal patterns, indicating that factors such as sample age, oxidative stress response, and photosynthetic efficiency had a more substantial impact on genetic diversity than regional differences. The RDA results (Fig. 18) further highlight the role of photosynthetic efficiency (PS_yield) in driving genomic changes, particularly in samples from around 6800 cal yr BP during the Littorina Sea period. This period was characterized by higher water level, salinity and stable marine conditions, which likely favoured efficient photosynthesis and influenced genomic composition.

For example, our study identifies that oxidative stress response (ROS regulation), photosynthetic efficiency, and filament length significantly influenced the genomic diversity of *S. marinoi* populations. These findings align with previous research by Graff Van Creveld et al. (2016) and Siauciunaite et al. (2019), which demonstrated the importance of ROS regulation in maintaining cellular homeostasis under environmental stress. Additionally, the influence of filament length on nutrient acquisition likely enhances surface area and facilitates nutrient transport in moving fluids (Musielak et al., 2009), indicating its role in ecological interactions.

A notable shift in genomic structure and the associated PFAMs (protein families) occurred between samples from 742 cal yr BP and 110 cal yr BP. These samples correspond to key climatic events: the Medieval Climate Anomaly (MCA, 1150–600 cal yr BP), characterized by warmer temperatures, and the Little Ice Age (LIA, 400–100 cal yr BP), characterized by cooler conditions and altered nutrient dynamics (Niedźwiedź et al., 2015). Samples from around 6800 cal yr BP, during the Littorina Sea period, represent a stable marine habitat with higher salinity and sea levels, providing optimal conditions for

S. marinoi. In contrast, samples from 3343 cal yr BP belong to the Modern Baltic Sea, which features lower salinity due to significant freshwater inflow (Weckström et al., 2017), likely presenting different ecological challenges to these populations.

In conclusion, these findings demonstrate the adaptability of *S. marinoi* in response to both spatial and temporal environmental variability. While the genetic coherence of modern populations suggests a high degree of stability, the higher allele turnover over time indicates underlying evolutionary dynamics driven by changing environmental conditions. This adaptability appears to be driven more by the intrinsic genetic diversity and plasticity of the species, rather than by significant genetic differentiation across regions. This resilience may be crucial for the species' long-term stability and survival in the face of ongoing climate change and anthropogenic impacts.

5. Conclusion

The study provides an in-depth look at the evolutionary patterns of *Skeletonema marinoi*, a diatom found in the Baltic Sea, over thousands of years. By resurrecting ancient strains, the research shows that these diatoms have maintained genetic diversity despite spatial and temporal environmental changes. While genetic diversity is high, regional variation is minimal, indicating a connected population. Interestingly, despite significant genetic differences in the temporal dataset, the strains show phenotypic convergence, with similar phenotypic traits emerging in genetically distinct lineages. This suggests that *S. marinoi* has adapted to maintain beneficial traits that favour survival in different environments. The results show that genetic shifts are more influenced by historical environmental changes than by modern spatial differences, highlighting the resilience of *S. marinoi* to past climatic events and human impacts. This work contributes to our understanding of the evolution of phytoplankton and their potential to withstand future ecological change.

6. Supplementary information

6.1. Acknowledgements

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6.2. Data Availability Statement

The supplementary data can be accessed via the following link: https://cloud.uni-konstanz.de/index.php/s/iBXMz3G5fMoikyb in folder “Chapter_4”. Additionally, the scripts and pipeline used for data processing and analysis are available in the subfolder named "chapter_4_github". This repository includes all the code necessary to replicate the study's findings. Furthermore, supplementary information is provided in the document "Supplement.docx”.

General discussion

The findings presented in this study underscore the importance of genetic and physiological diversity in maintaining resilience within phytoplankton populations, particularly in dynamic environments like the Baltic Sea. Genetic diversity ensures adaptability by enabling populations to withstand fluctuating environmental conditions, such as salinity and nutrient shifts. The transition from a single-marker analysis to a comprehensive genomic and physiological investigation reveals both patterns of stability and change across spatial and temporal scales. By focusing on *Skeletonema marinoi* as a model organism, this research aims to show how genomic monitoring can reveal the resilience mechanisms that drive ecosystem stability and adaptability, providing valuable insights for mitigating climate change and human-induced impacts.

The resilience and adaptability of phytoplankton populations in the Baltic Sea, more precisely during the spring bloom targeted in this research, serve as critical indicators of ecosystem health. My research delves into this intricate relationship by examining population dynamics, genetic diversity, and genetic responses over millennia.

To begin with, we reconstructed population sizes and ecosystem status, developing a molecular index that revealed profound historical shifts in the dominance of key spring bloom species (Chapter 1). The ske/apo index is a molecular proxy that measures shifts in diatom and dinoflagellate dominance, linking phytoplankton dynamics to nutrient levels and providing insights into ecosystem health. In particular, these shifts showed transitions between diatoms and dinoflagellates, reflecting wider ecological changes. The spring bloom is of particular importance as it represents the period with the highest annual primary production and sinking of organic matter to the sediment (Hjerne et al., 2019). These shifts were found to align with major climatic events, underscoring the importance of understanding past population dynamics to anticipate future alterations.

Our genomic analysis of *S. marinoi* populations at two Baltic Sea locations over time highlighted the impact of environmental changes on genetic composition (Chapter 2). Periods of climatic stability, such as those not affected by major climate events like the Holocene Thermal Maximum (HTM), Medieval Climate Anomaly (MCA), Little Ice Age/Late Antique Little Ice Age (LIA/LALIA) or Modern Warm Period (MWP) (Fig. 1), were marked by genetic resilience, while notable environmental events led to increased genetic variability. This pattern indicates that phytoplankton populations can maintain stability over extended periods, yet they are also capable of genetic shifts in response to environmental stressors. Recent studies, such as Sjöqvist (2022), have highlighted high levels of genetic diversity in

phytoplankton populations, providing them with the adaptive potential to respond to rapidly changing environmental conditions.

Evolutionary changes were found to be significantly influenced by specific traits and temporal factors, affecting genetic diversity within populations. In addition, through resurrection ecology, we demonstrated that phytoplankton can survive and remain viable after thousands of years in anoxic sediments. Resurrection ecology involves germinating dormant cells from historical sediments to study genetic and phenotypic traits of ancient populations, providing a window into past environmental responses. This finding not only extends the known limits of dormancy but also provides insights into evolutionary responses to long-term environmental changes. Our investigation revealed that specific traits and temporal factors significantly shape the genomic structure of phytoplankton populations. While there is no clear distinction between spatial and temporal data types, the temporal dataset showed higher allele turnover rates, indicating dynamic evolutionary processes over time. This higher allele turnover, reflecting changes in the frequency of genetic variants within populations, highlights the capacity of phytoplankton to adapt to environmental pressures through evolutionary responses. These findings underscore the role of temporal dynamics in shaping genetic diversity across millennia.

Together, these findings offer crucial insights into the resilience and adaptability of phytoplankton populations, on the example of the Baltic Sea, providing a broader perspective on the interplay between ecological and population/genomic processes affecting the health and future trajectory of marine ecosystems in the context of ongoing environmental change.

Genomic Tools in Ecosystem Monitoring: Broader Applications and Implications

This research not only unravels historical phytoplankton dynamics in the Baltic Sea, but also establishes a robust framework for genomic ecosystem monitoring with broad applications. Genomic indices from sedimentary DNA provide high-resolution records across temporal and spatial dimensions, uniquely addressing the complex challenges of coastal and marginal seas worldwide. Recent advances in sedaDNA methodologies have demonstrated its capacity to reconstruct long-term ecosystem dynamics, offering insights into historical and modern ecosystem shifts under changing environmental conditions (Armbrecht et al., 2021; Armbrecht, 2020; Zimmermann et al., 2023). By using molecular tools, as highlighted in Chapters 1, 2 and 4, this study captures changes in the ecosystem that align with known environmental changes and historical trends, extending its utility to other impacted marine

environments such as the North Sea and Black Sea (Moullec et al., 2021). The North Sea and the Black Sea have also been significantly affected by a range of human activities, including pollution, overfishing, and climate change (Micheli et al., 2013).

In Chapter 1, a single genomic marker approach using droplet digital PCR (ddPCR) allowed a targeted reconstruction of ecosystem changes. This approach effectively distinguished shifts in the dominance of diatom and dinoflagellate populations over millennia, specifically focusing on *S. marinoi* and *Apocalathium malmogiense*. These patterns align with environmental data, highlighting the potential of molecular indices for reconstructing broad ecosystem trends. While limited to a single aspect of community structure, this method serves as an accessible starting point for establishing baseline trends and supports monitoring efforts across diverse marine systems.

Chapter 2 extends this exploration by enriching sedaDNA specifically for organelle DNA, allowing a more nuanced examination of population dynamics at the level of mitochondrial and chloroplast genomes. By focusing on this organelle DNA enrichment, Chapter 2 reveals population stability in *S. marinoi* that spans millennia, suggesting resilience to past environmental fluctuations. However, this raises questions about potential responses to recent anthropogenic pressures. This deeper genomic perspective serves to advance our understanding of population structure, moving beyond the scope of simple species ratios to reveal the presence of intraspecific diversity and historical patterns within populations. This approach could be applied to other marginal and coastal seas to assess long-term population shifts in response to both natural events and human activities, thus identifying resilient populations and potential conservation targets.

The study of dormant resting cells in Chapter 3 bridges past and present by contrasting ancient and modern populations, revealing surprising phenotypic stability alongside genomic divergence (under one condition). These findings highlight the enduring adaptive traits of phytoplankton and improve our understanding of how populations withstand environmental stress over time. Resurrection ecology thus provides a living archive for direct physiological and genomic comparisons across millennia.

In Chapter 4, the study examines both ancient and modern *S. marinoi* strains from various Baltic Sea regions, revealing patterns of genomic coherence across spatial scales with subtle regional genetic differences influenced by localized environmental factors. While the overall genomic structure of *S. marinoi* populations remains relatively stable, minor variations emerge between regions, indicating the influence of environmental factors on local adaptation. This lack of significant spatial differentiation may also be attributed to high connectivity among different regions, facilitating gene flow and maintaining genetic coherence. For example, Chapter 2 indicates that populations in the Eastern

Gotland Basin exhibit slightly elevated genetic diversity relative to those in the Gulf of Finland. This discrepancy may be attributed to distinctive regional conditions or variations in historical nutrient dynamics. These observations are consistent with Hutchinson's "paradox of the plankton" demonstrating that diverse planktonic genotypes can coexist under fluctuating environmental conditions. These findings align with studies highlighting the role of gene flow in buffering genetic variability and enhancing resilience in phytoplankton populations (Dokulil and Teubner, 2024; Sjöqvist, 2022).

Temporal analysis indicates an increase in allele turnover over centuries, particularly during periods of significant environmental transition, such as the shift from the Littorina Sea to the modern Baltic. Research has shown that diatoms like *S. marinoi* are shaped by both rapid environmental changes and long-term evolutionary pressures, which influence their adaptive trajectories and genetic diversity (Nakov et al., 2019; Rengefors et al., 2017). This historical variability suggests that while *S. marinoi* demonstrates considerable genetic stability in the face of spatial gradients, temporal environmental shifts, driven by both natural and anthropogenic factors, have shaped its evolutionary trajectory.

The collective scope of these chapters offers a comprehensive perspective on genomic monitoring, integrating broad ecosystem trends with intricate genetic and physiological analyses. The framework presented here, which progresses from single markers to comprehensive physiological and genomic comparisons, provides a foundation for a multi-layered approach to ecosystem monitoring that can be adapted to other marine systems facing similar threats. By integrating molecular monitoring into conservation policies, high-resolution genetic baselines and trait-based insights become indispensable tools for adaptive management. Emerging omics tools, such as eDNA metabarcoding and population-scale genomics, further enhance ecosystem monitoring by revealing genetic diversity and adaptive capacity, supporting both conservation and sustainable resource use (Jeffery et al., 2022). This approach not only provides a retrospective view but also offers future monitoring efforts the means to capture, understand, and protect the full spectrum of marine biodiversity. The progression from single-marker analyses to comprehensive genomic studies ensures that molecular monitoring captures the full spectrum of biodiversity, thereby providing an indispensable approach for protecting marine ecosystems in a rapidly changing world.

The advent of genomic tools, including sedimentary ancient DNA (sedaDNA) analysis and whole genome sequencing of revived resting cells, has transformed our capacity to monitor long-term population dynamics. These tools provide previously undocumented insights into the genetic and phenotypic resilience of phytoplankton, revealing how historical populations responded to

environmental pressures. SedaDNA has demonstrated its utility in reconstructing ecosystem dynamics and tracking biodiversity changes over time (Mejbel et al., 2021). In parallel, resurrection ecology enables the revival and genomic study of dormant life stages, providing unique insights into phenotypic stability and adaptation (Sjöqvist, 2022; Sjöqvist and Kremp, 2016). These tools not only reveal past responses to environmental pressures but also inform conservation strategies aimed at sustaining marine biodiversity. The integration of genomic approaches with ecological monitoring enables the uncovering of patterns of adaptation, thereby informing the development of conservation efforts designed to address the specific needs of diverse marine systems.

Detection of Environmental Change Through Phytoplankton Dynamics and Genetic Structure

Marine ecosystems, particularly marginal seas like the Baltic Sea, are characterized by a complex and dynamic relationship between resilience and vulnerability in the face of environmental variability (Reckermann et al., 2022). The ske/apo index, as described in Chapter 1, is a novel molecular measure that monitors shifts in diatom and dinoflagellate dominance using abundant spring bloom species as proxies for their respective groups. The ske/apo index tracks species dominance ratios over time, offering a method to detect early ecosystem changes linked to environmental change. This novel molecular tool offers a robust, adaptable approach to the detection of ecosystem change, while simultaneously addressing limitations in historical monitoring capabilities.

The index effectively links phytoplankton dynamics to anthropogenic nutrient inputs, providing critical insights into ecosystem changes under nutrient stress. By extending monitoring capabilities beyond modern observations, the ske/apo index reveals early shifts in nutrient levels that began to alter patterns of phytoplankton dominance well before the late 20th century. Results from other systems, such as the Great Lakes, also show shifts in phytoplankton composition associated with nutrient inputs, highlighting the broader applicability of these molecular tools for assessing ecosystem health (Taberlet et al., 2018; Valentini et al., 2016). Furthermore, the index provides a framework for integrating molecular data with conservation strategies, thereby ensuring the generation of actionable insights into ecosystem trajectories.

In the Baltic Sea, the genetic makeup and adaptive responses of phytoplankton reveal how environmental shifts have shaped communities over millennia (Listmann et al., 2021). As demonstrated in Chapter 1, the ske/apo index provides a cost-effective tool for identifying early nutrient-driven

changes, extending the scope of monitoring beyond observational data. Chapter 2 expands on this by analysing sedimentary DNA archives to document genetic resilience and disruptions caused by anthropogenic pressures in *S. marinoi* populations. This research documents the resilience and sensitivity of these communities using environmental genomic evidence and genomic insights from resurrection ecology. Furthermore, it explores the impact of long-term evolutionary pressures on the adaptive capacity of Baltic Sea phytoplankton, thereby providing a broader ecological context for their persistence. This approach highlights how phytoplankton serve as important indicators of ecological stress and resilience.

The sedaDNA extracted from cores taken from three sites of the Baltic Sea (Chapter 2, Fig. 2) provides a genomic archive that allows for the tracking of both natural and anthropogenic influences on the structure of phytoplankton communities. As outlined in Chapters 1 and 2, the molecular signals that have been preserved in sediment cores document responses to periods of change. The genetic composition of *S. marinoi* remained remarkably stable over millennia, despite periodic environmental shifts such as the Holocene Thermal Maximum and the Medieval Climate Anomaly (Chapter 2). However, as outlined in Chapter 2, recent impacts, including eutrophication and warming, have resulted in a notable shift in genetic composition, indicating that modern pressures may exceed the natural resilience thresholds established through millennia of relative stability. This observation aligns with findings from similar semi-enclosed ecosystems, such as the Chesapeake Bay, where anthropogenic disruptions to nutrient cycles have dramatically altered ecosystem structure (Shen et al., 2020). Similar patterns are observed in the Gulf of Mexico (Karnauskas et al., 2015) and the Black Sea (Akoglu, 2023; Tokarev and Shulman, 2007).

The population dynamics presented in Chapters 1, 2, and 4 offer further insights into the process of adaptation. The salinity gradient of the Baltic Sea has resulted in the development of slightly localized adaptations in *S. marinoi*, which have led to the emergence of distinct genetic dynamics across the Gulf of Finland and the Eastern Gotland Basin. These variations reflect regional adaptation to environmental conditions, thereby demonstrating that even in low-diversity systems, adaptive diversity can persist through local differentiation. Studies from other coastal regions, including the Chesapeake Bay and the Gulf of Mexico, demonstrate comparable genetic differentiation in response to salinity and temperature gradients, indicating a generalizable pattern of resilience through localized adaptation in marine phytoplankton (Wang et al., 2024). Nevertheless, the data from the full genomes of resurrected *S. marinoi* cultures demonstrate that rising anthropogenic pressures disrupt these dynamics, underscoring the vulnerability of adapted populations to additional stressors (Chapter 4). Similar findings from freshwater systems, such as those in the Cerrado biome of Brazil, indicate that

eutrophication and pollution can diminish diversity in phytoplankton communities, potentially undermining resilience in systems adapted to variable conditions (Machado et al., 2023).

Although we observe significant genetic shifts and potential adaptations over time, the spatial component in *S. marinoi* populations across the Baltic Sea reveals a surprising lack of strong heterogeneity. As outlined in Chapter 4, even sub-regions such as the Gulf of Finland and the Eastern Gotland Basin exhibit genetic uniformity, indicating the presence of extensive connectivity between populations. Minor regional genetic differences are shown, underscoring the coherence of these populations despite environmental heterogeneity across sub-regions. Studies have demonstrated that high levels of genetic connectivity, as seen in other phytoplankton species, can buffer populations against localized pressures and maintain resilience across variable environments (Sjöqvist, 2022). This genetic connectivity may reduce the potential for strong spatial adaptation, even in areas with distinct salinity and nutrient profiles. This may suggest that gene flow across regions can buffer populations against localized pressures, maintaining genetic coherence and possibly enhancing resilience at larger scales. Conversely, this high connectivity could limit the capacity for localized adaptation, potentially leaving populations vulnerable to regionalized stress events.

The resurrection ecology analysis presented in Chapter 3 contributes to our understanding of phytoplankton adaptability across extensive temporal scales. The germination of dormant *S. marinoi* cells from Baltic sediments, some of which had been preserved for up to 6,800 years, demonstrates that diatoms possess robust dormancy mechanisms that enable their survival across diverse climate phases. This capacity suggests the existence of something like a "genetic memory" that contributes to long-term resilience, with dormant cells acting as archives of genetic diversity. Similarly, comparative studies in Arctic and sub-Arctic systems demonstrate that dormant diatoms serve as a genetic reserve against environmental extremes, emphasizing the pervasive ecological importance of dormancy as a resilience mechanism (Ellegaard et al., 2020). However, these mechanisms alone may not suffice to withstand the compounding effects of modern anthropogenic stressors.

In conclusion, this study demonstrates that while Baltic Sea phytoplankton species, such as *S. marinoi,* have historically demonstrated resilience, recent data suggest that rates of environmental change that are greater than previously observed may be testing the adaptive limits of phytoplankton species. The observed patterns in *S. marinoi* offer a potential indication of vulnerability in the face of rapid anthropogenic change. This aligns with evidence showing that diatom populations can experience abrupt shifts in response to changing environmental pressures, such as nutrient enrichment and warming, which may amplify stress in marine ecosystems (Gardoki et al., 2023; Hattich et al., 2024).

Further research, particularly the integration of both genomic and phenotypic data, will be essential to facilitate an understanding of these vulnerabilities in the context of a rapidly changing world. Nevertheless, the findings offer preliminary insights into how marginal marine ecosystems globally may be impacted by ongoing pressures. These observations underscore the need for targeted conservation strategies that address the combined challenges of climate change and nutrient loading in coastal and semi-enclosed seas.

Phytoplankton as Indicators of Baltic Sea Ecosystem Health: Historical Baselines for Future Conservation

Phytoplankton are an integral component of aquatic ecosystems, functioning as sensitive bioindicators due to their responsiveness to environmental factors, including temperature fluctuations, differing nutrient levels, and salinity changes (Souza Araujo et al., 2022). The influence of phytoplankton extends beyond the immediate ecosystem, as they play a role in global nutrient cycles and carbon sequestration (Hutchins and Tagliabue, 2024). In the Baltic Sea, which is characterized by its brackish waters and thus relatively low species diversity, these organisms provide valuable insights into environmental health, particularly in light of the increasing pressures from anthropogenic influences that have been observed in recent decades. The monitoring of shifts within phytoplankton communities and more specific populations thus reveals both natural and human-induced impacts on the ecosystem, making them ideal indicators in this nutrient-sensitive and semi-enclosed sea (Chapter 1; Wasmund et al., 2017).

The Baltic Sea's unique temperature, salinity and nutrient dynamics present particular challenges for species survival, with phytoplankton showing sensitivity to these variables (Savchuk, 2018). Diatoms and dinoflagellates, two dominant phytoplankton groups, display pronounced responses to shifts in nutrient availability and seasonal temperature changes (Wang et al., 2022). The structure of these two groups reflects nutrient cycling processes, thus providing a measurable indicator of the ecosystem's nutrient load and health. In Chapter 1, the dominance patterns of two spring bloom species, the diatom *Skeletonema marinoi* and the dinoflagellate *Apocalathium malmogiense*, provide insights into nutrient-driven eutrophication that precede observable changes in higher trophic levels. While the general shift from diatoms to dinoflagellates under increased nutrient input is well documented, Chapter 1 shows that even focusing only on these two species reveals this pattern. This highlights their use as effective proxies for their respective taxa in our index.

Diatoms and dinoflagellates play a pivotal role in the Baltic's nutrient and carbon cycles, with their populations showing fluctuations in response to nutrient influxes, which are often driven by human activities (Spilling et al., 2018). Chapter 1 provides evidence that increased nutrient inputs, such as nitrogen and phosphorus from agricultural runoff, coincide with a shift in spring bloom dominance from diatoms to dinoflagellates. This shift reflects the different nutrient use strategies of these groups, with diatoms thriving under silica-rich conditions and dinoflagellates proliferating in environments with elevated nitrogen-to-phosphorus (N/P) ratios (Devlin and Brodie, 2023; Zhang et al., 2024). Phosphorus limitation further inhibits diatom growth under these conditions, enhancing the competitive advantage of dinoflagellates (Zhang et al., 2024).

These changes in bloom dynamics are indicative of eutrophication processes and their cascading effects on the ecosystem, such as reduced oxygen levels in bottom waters, disruptions to the food web, and altered biogeochemical cycling (Ellegaard et al., 2020). For example, the dominance of dinoflagellates can exacerbate hypoxic zones by slowing the sinking of organic matter and prolonging decomposition in surface waters (Eom et al., 2024). As a result of their role in the cycling of nutrients, these phytoplankton populations offer an early indication of eutrophic conditions within the Baltic Sea, highlighting their value in the monitoring of ecosystem processes (Mack et al., 2020).

While current monitoring provides valuable information on ecosystem health, our findings demonstrate that changes in the Baltic Sea ecosystem occurred earlier than previously assumed, with shifts detected as far back as 1,000–1,500 years ago, based on sedimentary archives (Chapter 2). Understanding these historical responses to natural climatic changes enhances our ability to assess the scope of recent anthropogenic impacts.

In Chapter 2, sediment archives are used to reconstruct long-term genetic population dynamics of *S. marinoi*, capturing baseline ecosystem states over millennia Analysis of chloroplast and mitochondrial genomes reveals both stability and resilience in genetic diversity, even during major climatic events such as the Holocene Thermal Maximum and the Little Ice Age. However, the genetic composition displays notable shifts in the last two millennia, coinciding with intensified human activity (Chapter 2, Fig. 10). These changes align with anthropogenic stressors, including increased agricultural runoff, industrialization, and maritime activities during the Hanseatic and Industrial ages (Kontny, 2023; Mägi, 2018). These anthropogenic stressors coincide with observed patterns of genetic turnover in *S. marinoi*, suggesting a direct impact on the genetic resilience and stability of the species during this period. This temporal pattern of genetic disruption underscores the influence of human activities on

the genetic stability of diatom populations, highlighting a transition from natural resilience to more profound anthropogenically induced changes (Rytkönen et al., 2002).

The sediment archives preserved in the anoxic conditions of the Baltic Sea basins serve as such a record of past phytoplankton dynamics, thereby allowing for the reconstruction of the ecosystem's natural baseline states. These sedimentary records provide insights into major climatic events, including the Holocene Thermal Maximum, the Little Ice Age, and the Modern Warm Period, and reveal long-term shifts in phytoplankton populations over millennia (Chapter 2). For example, such archives provide crucial context for understanding nutrient-driven shifts in community composition, illustrating how modern eutrophication alters previously stable ecosystems (Berthold and Campbell, 2021). The Baltic Sea is susceptible to nutrient accumulation due to its semi-enclosed nature and limited exchange with the North Sea, which exacerbates eutrophication. As shown in Chapter 1, long-term records from sediment cores can be used to compare historical baselines with recent nutrient loads, offering insights into the impact of modern nutrient loads and industrialization on natural patterns. This provides a context for conservation efforts and emphasizes the need to balance present-day impacts with an understanding of the ecosystem's inherent variability.

Establishing a meaningful reference period for conservation requires a balance between historical ecosystem variability and modern anthropogenic impacts. Historical ecology provides critical long-term insights that help define ecological baselines, capturing the natural variability of ecosystems and the cumulative effects of human impacts (McClenachan et al., 2024). This research suggests that the last 2,000 years provide a valuable baseline, capturing both natural pre-industrial variability and early human-induced changes. The shift around 1,500-1,000 cal yr BP marks a critical phase where natural dynamics intersected with anthropogenic impacts, providing important insights into ecosystem robustness and response. Hence, focusing on baselines within the last 2,000 years aligns historical context with actionable management goals.

Chapters 1 and 2 both demonstrate the value of sedaDNA analysis in capturing genetic data from past phytoplankton populations. Chapter 2 focuses on mitochondrial and chloroplast DNA, providing a broad overview of the historical composition of phytoplankton populations. The observation of genetic shifts in these typically conserved organellar regions (Yu et al., 2022) highlights the extend of evolutionary and ecological responses associated with environmental transitions over millennia. By establishing a timeline of natural variability, these archives help distinguish between recent anthropogenic impacts and natural ecosystem dynamics. The combination of sedaDNA and environmental data makes it possible to track ecosystem shifts, such as nutrient inputs during early

agricultural activities or industrialisation (Chapter 1). This distinction is crucial for developing effective conservation strategies tailored to the Baltic Sea's unique ecological and historical context. Integrating molecular and ecological data, the findings highlight the extent of human-induced changes and provide actionable insights for managing future ecosystem responses.

Similarly, Chapter 3 demonstrates that phenotypic similarity in *S. marinoi* strains, observed across nearly 6,800 years of dormancy, arises despite significant genetic differentiation between temporal cohorts. This phenotypic convergence is supported by physiological traits such as growth rates and photosynthetic activity, which remain stable across millennia, emphasizing the species' resilience (Härnström et al., 2011). While Chapter 3 provides evidence of stable phenotypic traits across temporal cohorts, Chapter 4 adds a genomic dimension, illustrating how environmental pressures reinforce these traits across spatial and temporal scales. Building on these findings, Chapter 4 uses whole-genome analyses to reveal how shared selective pressures, such as photosynthetic efficiency and oxidative stress responses, drive this convergence.

These findings complement Chapter 2's focus on organelle DNA, offering a complete view of adaptation mechanisms. These pressures, observed across both temporal and spatial scales, highlight the interplay between genetic variation and phenotypic consistency, reinforcing the role of stabilizing selection in maintaining key survival traits (Pham and McConnaughay, 2013; Reusch and Boyd, 2013). This reflects an ecosystem "memory" in which the genetic and phenotypic resilience of *S. marinoi* is shaped by millennia of environmental variation. Studies on phytoplankton communities, like Baho et al. (2021), support this concept by highlighting the influence of historical contamination on ecological responses. This memory provides a framework for predicting how ecosystems might respond to future stressors by identifying traits and dynamics that have historically supported resilience. Recognizing these patterns is critical for designing conservation strategies that reinforce natural adaptive capacities. Trait stability plays a pivotal role in maintaining ecosystem balance, even under fluctuating environmental conditions. These insights emphasize the importance of monitoring genetic and phenotypic shifts to identify thresholds of stability, ensuring that ecosystems can continue to adapt to anthropogenic pressures. However, this memory may be at risk as anthropogenic pressures increase, potentially pushing these populations beyond their adaptive capacity. By identifying thresholds of adaptation, conservation strategies can better ensure that ecosystems retain their capacity to respond to modern pressures.

In addition to historical baselines, the genetic diversity within phytoplankton populations provides insight into their adaptive capacity and thus resilience. Chapter 2 explores the genetic resilience of *S.*

marinoi, revealing patterns of stability that indicate how these organisms have adapted over millennia to both natural environmental changes and recent anthropogenic pressures. This genetic capacity provides a baseline for understanding how phytoplankton populations maintain their ecological roles in response to varying environmental conditions. The establishment of these baselines is invaluable for distinguishing natural resilience mechanisms from those altered by human activity. Genetic diversity supports ecological resilience, enabling *S. marinoi* and possibly other diatoms to stabilize despite fluctuating environmental conditions. Experimental studies with diatoms, including S. marinoi, demonstrate that higher genetic diversity enhances ecological functions such as nutrient cycling and primary production, even under environmental stress (Jacob et al., 2024; Sjöqvist and Kremp, 2016). This suggests that genetic variation is a foundational element in maintaining the stability and adaptability of diatom populations across variable conditions. In particular, Chapter 2 demonstrates that *S. marinoi* shows genetic stability across millennia, suggesting an adaptive capacity that has enabled them to withstand historical environmental fluctuations and climate events. This genetic stability reflects a "memory" within the ecosystem, though it may now be at risk due to growing anthropogenic pressures.

Notably, Chapter 3 demonstrates that the resting stages of *S. marinoi* can remain viable in sediments for nearly 6,800 years, contributing to their resilience by acting as a genetic reservoir. This evidence of selective genetic shifts, discernible only through temporal resolution, highlights the necessity for long-term monitoring to capture the gradual decline in resilience that may occur with sustained human impact. Without sufficient genetic diversity, the future adaptive capacity of these populations could be constrained, increasing their vulnerability to rapid environmental changes.

The study of dormant resting stages in sediment archives, when combined with advanced genomic and physiological analyses, offers a comprehensive understanding of the resilience and adaptability of phytoplankton populations in the Baltic Sea. In contrast to sedaDNA, resurrection ecology provides higher-quality DNA and detailed information about phenotypes and physiological traits. This enables functional annotation and a more precise representation of the genomic constitution and admixture of populations over time. Furthermore, while sedaDNA encompasses a more diverse range of individuals, resurrection ecology concentrates on isolated and cultivated individuals from specific time points, which may exhibit genetic similarities or even identity. Both approaches can be valuable for distinguishing between natural and anthropogenic influences on populations. Research has shown that resting stages of phytoplankton exhibit long-term viability, allowing them to survive decadal timescales and catastrophic environmental disturbances such as prolonged darkness, highlighting their role in ecosystem resilience and recovery (Ellegaard and Ribeiro, 2018; Ribeiro et al., 2011). This knowledge is

essential for anticipating future shifts in phytoplankton populations and their cascading effects on the Baltic Sea ecosystem. Such insights will be instrumental in developing effective conservation and management strategies to address the challenges posed by ongoing environmental change.

This combined approach underscores how dormant stages and resurrection ecology not only reveal past resilience but also reveal the nuanced genetic adaptations that occur in the face of environmental change. For instance, studies utilizing sediment cores and revived diatom strains have demonstrated that resting stages can persist for centuries, providing a valuable genetic archive to track historical population dynamics and adaptive shifts (Härnström et al., 2011). In particular, insights into phenotypic convergence would not be possible without the resurrection approach. Chapters 3 and 4 build on these findings by analysing resurrected *S. marinoi* and their full genomes to provide further insights into genetic shifts over time. The analysis reveals subtle genetic changes likely driven by modern anthropogenic factors, underscoring the importance of maintaining genetic diversity to support adaptation. Moving forward, integrating long-term ecological records with advanced genomic analyses will be essential for predicting and mitigating the impacts of climate change and human activity on phytoplankton populations.

Comparative Adaptability and Resilience of Phytoplankton Across Temporal Scales

Baltic Sea *S. marinoi* populations demonstrate resilience and adaptability, responding dynamically to natural climatic fluctuations over millennia. However, the historical record indicates a critical shift: natural changes now overlap with intensified human activity, resulting in a distinct interplay between natural and anthropogenic pressures. While these species possess resilience, their recent responses highlight emerging sensitivities to modern stressors (Hattich et al., 2024). Short-term studies have further shown that *S. marinoi* can acclimate to fluctuating environmental conditions such as temperature and pCO_2 changes, although these responses do not capture long-term evolutionary dynamics (Briddon et al., 2023).

The historical resilience of diatom populations, particularly *S. marinoi*, in response to natural climatic cycles across millennia (Chapters 2-4) presents a notable contrast with their recent responses to anthropogenic pressures. For example, during the Medieval Climate Anomaly and the Modern Warm Period, diatom productivity fluctuated in response to changes in salinity and nutrient inputs, as demonstrated in Chapter 4. In particular, Chapter 2 reveals that allelic composition changes during

these periods are likely linked to adaptive responses to climatic variability, manifesting as changes in genetic diversity. However, modern shifts, such as those in nutrient loading and salinity influenced by human activities, have introduced new selective pressures, resulting in observable changes in genetic diversity across Baltic diatom populations (Weckström et al., 2017). Recent studies, such as those on soil ecosystems, suggest that it is the cumulative number of stressors, rather than their specific nature, that leads to significant ecosystem impacts (Rillig et al., 2023). While this work focuses on terrestrial ecosystems, similar dynamics may apply to aquatic systems, emphasising the compounding effects of multiple stressors on diatom populations.

As outlined in Chapter 4, periods of intensified anthropogenic activity have been identified to coincide with genetic differentiation in diatom populations. This differentiation is likely due to increased selective pressures exerted by these environmental stressors, which can lead to rapid evolutionary changes (Collins et al., 2014). This suggests a transition from historically stable genomic configurations, characterized by relatively consistent genetic structures, to altered states during periods of accelerated modern change. Such shifts in the genomic structure of *S. marinoi* populations highlight the impact of intensified anthropogenic pressures on genetic stability. This transition reflects a shift from natural evolutionary processes driven by climatic cycles to rapid changes induced by intensified human activities, such as eutrophication. Specifically, the genomic shifts observed in diatom populations under these conditions may be indicative of adaptive responses to the altered nutrient and chemical landscapes (Häder and Gao, 2015).

This adaptability is further supported by the dual reproductive strategies of *S. marinoi*, which alternates between clonal asexual reproduction and occasional sexual reproduction. While asexual reproduction ensures rapid population growth, sexual reproduction introduces genetic diversity, creating larger initial cells through auxospores (Bilcke et al., 2022; Johansson et al., 2019). As a primarily diploid organism during its vegetative phase (Bulankova et al., 2022), *S. marinoi* benefits from the genetic redundancy and potential for variation inherent in diploidy, which may further increases its capacity for resilience and adaptability to changing environments. This interplay allows *S. marinoi* populations to maintain resilience in changing environments, with sexual reproduction likely acting as a buffer against selective pressures and ensuring long-term adaptability. These reproductive strategies, alongside dormancy, may explain the observed genetic shifts in *S. marinoi*, particularly under changing environmental pressures, as they provide mechanisms for both stability and genetic innovation.

In addition to these genetic shifts, Chapter 3 reveals that dormant *S. marinoi* populations preserved for millennia act as genetic reservoirs, maintaining diversity across climatic extremes. These populations

demonstrated phenotypic stability despite genetic divergence, highlighting the robustness of dormancy as a resilience mechanism. Dormancy, through the formation of resting stages, serves as both a resilience strategy and a potential source of vulnerability (Ellegaard and Ribeiro, 2018; Ribeiro et al., 2011).

While dormancy preserves genetic diversity and acts as a historical archive of population dynamics, it may also contribute to maladaptation. Maladaptation, in this context, refers to a situation where traits or genetic variations, instead of improving population fitness, create a mismatch with the local environmental conditions (Negrín Dastis et al., 2019). Dormant cells revived under changing environmental conditions can introduce gene flow from populations less suited to local conditions, potentially compromising population fitness (Gechev et al., 2021). Traits adapted to past conditions may no longer match with the selective pressures of the current environment, leading to ecological mismatch between revived phenotypes and current conditions.

The persistence observed in *S. marinoi* populations over millennia is increasingly challenged by the limits imposed under intensified modern anthropogenic pressures. The adaptive genetic diversity of these populations demonstrates their capacity to endure environmental variability. This is consistent with the principles underlying Hutchinson's (1961) 'paradox of the plankton', which suggests that diversity enables populations to thrive and coexist even under conditions of resource limitation or fluctuation. Genomic insights into *S. marinoi* reveal the mechanisms through which diatoms have historically achieved resilience, though modern stressors present new challenges to this balance. Nevertheless, the risk of maladaptation introduces critical vulnerabilities, as gene flow from dormant populations and the homogenization of genetic diversity may reduce the potential for localized adaptation, especially in ecosystems with strong regional gradients or rapidly changing conditions. These dynamics underscore the tension between resilience and the constraints of adaptability under prolonged or extreme stress, particularly as eutrophication and warming accelerate selective pressures (Steinberg, 2012).

This interplay of resilience and vulnerability underscores the importance of phytoplankton as critical indicators of ecosystem health, revealing multi-factorial influences from nutrient gradients, climate cycles, and anthropogenic impacts. As discussed in Chapter 1, shifts in dominance ratios demonstrate how environmental stressors, including nutrient gradients and climatic cycles, can be recorded in phytoplankton community structures. For example, the process of eutrophication can result in increased nutrient availability, which in turn promotes the formation of algal blooms and alters the composition of the associated communities (Anderson et al., 2002). These findings are consistent with

regional archaeological insights, which demonstrate how land-use practices from the Bronze Age (approx. 4,000-3,500 cal yr BP) to the Viking Age (approx. 1,200-800 cal yr BP) influenced Baltic Sea sediment nutrient loads (Ponomarenko et al., 2024). Archaeological evidence, such as sediment analyses, links historical agricultural and deforestation practices to nutrient influxes, providing a long-term context for modern trends. In particular, historical land-use practices, including agriculture and deforestation, have been identified as contributing factors to increased sedimentation and nutrient influx into the Baltic Sea, with subsequent impacts on phytoplankton dynamics (Ponomarenko et al., 2024). By integrating genomic, archaeological, and ecological data, this research bridges historical baselines with modern trends, providing a nuanced view of ecosystem dynamics.

In summary, the study of *S. marinoi* highlights its role as a model organism for understanding adaptive strategies in phytoplankton, offering critical insights into how these species navigate the interplay of environmental variability and anthropogenic pressures. These findings provide a foundation for broader conservation efforts aimed at preserving marine ecosystems in the face of ongoing environmental change. These results underscore the importance of phytoplankton monitoring for marine ecosystem management in the Baltic Sea and beyond, as evidenced by studies like those of Olofsson et al. (2022), which demonstrate the adaptability of *S. marinoi* in responding under eutrophication conditions, and Hattich et al. (2024), which highlights its capacity to adjust to warming trends.

Limitations, Future Directions, and Vision for Genomic Monitoring in Marine Conservation

This study establishes a multi-dimensional framework for genomic monitoring that advances our understanding of ecosystem robustness and adaptability, particularly in marine environments impacted by human activity. By progressing from single-marker analysis to organelle-enriched DNA techniques, physiological assessments of revived organisms, and spatial genomic analyses, this research provides a comprehensive approach to ecosystem assessment. Each method contributes to understanding phytoplankton/species resilience mechanisms on the example of *S. marinoi* populations, while also demonstrating the genetic and phenotypic stability of these populations in the face of environmental change. These findings collectively demonstrate the capability of genomic monitoring for reconstructing historical ecosystem dynamics and for providing baseline data vital for future conservation efforts.

Although this research demonstrates the broad applicability of genomic monitoring, certain methodological and environmental challenges remain. A notable limitation in environmental DNA (eDNA) research, including sedaDNA, is the variability in DNA preservation due to regional environmental factors (Joseph et al., 2022). DNA quality in sediments is influenced by differences in sedimentation rates, oxygen levels, and temperature, which can lead to regional disparities in data resolution and completeness (Nguyen et al., 2023). This variability may impact the consistency and comparability of genomic data, especially when extending this framework to areas with distinct or less stable environmental conditions. Future studies could address these challenges by creating standardized protocols that account for region-specific preservation factors.

Building on the results, this research identifies several areas for future research that could further refine genomic monitoring frameworks and address the challenges associated with environmental variability and stress. For example analysis of the last 2,000 years at a higher resolution, which allows for the identification of critical ecosystem shifts in response to recent and rapid environmental changes. This period is likely to have experienced the most profound changes within the Baltic Sea, where both anthropogenic and natural pressures have intensified (Chapters 1, 2 & 4, Fig. 5, 10 & 16). A high-resolution analysis over the past two millennia would allow for the identification of subtle genetic and community structure shifts, capturing both pre-shift baselines and post-shift dynamics. Expanding this research to include comparisons with other biomonitoring proxies in regions outside the Baltic Sea would further strengthen the applicability and reliability of this framework, providing a more standardized basis for long-term ecosystem monitoring in diverse marine environments. For instance, *S. marinoi* populations exhibit remarkable adaptability under fluctuating light and nutrient conditions, which can inform broader biomonitoring strategies (Volpe et al., 2024). However, while high-resolution analysis of the last two millennia may reveal shifts in genetic and community structure, it is important to acknowledge the limitations imposed by sedimentation rates and dating accuracy. Low sedimentation rates may not capture rapid changes, blurring pre- and post-shift baselines.

The utility of traditional monitoring can also be significantly enhanced by integrating it with genomic biomonitoring techniques, which was done in Chapter 1. These provide insights that complement conventional methods, including phytoplankton counts and environmental monitoring programs. By further combining genomic data with real-time observations of phytoplankton communities and nutrient dynamics – for example, using automated imaging flow cytometers (Thyssen et al., 2007) – a more comprehensive picture of ecosystem health can emerge. Integrating these molecular techniques into existing monitoring programs could lead to adaptive management strategies informed by both

current and historical data, enabling a more proactive and comprehensive approach to marine ecosystem conservation.

Looking forward, genomic monitoring has the potential to become a pivotal tool in the field of marine conservation. As climate change and anthropogenic pressures reshape ecosystems at accelerating rates, it is increasingly evident that genetic diversity and adaptive monitoring will play an important role in managing and protecting marine biodiversity. Genomic tools offer a robust framework for conservation that goes beyond the limitations of traditional observational methods, providing early indications of ecosystem stress and resilience (Alemzadeh et al., 2014; Jeffery et al., 2022). Integrating genetic insights into conservation strategies allows policymakers to anticipate ecological shifts, safeguard biodiversity, and respond effectively to environmental changes.

To further refine these conservation strategies, experimental research could investigate the impact of environmental stress on genetic diversity across generations, thereby providing crucial insights into the mechanisms underlying population resilience. One possibility for future research could be to test the hypothesis that strong environmental stress increases genetic diversity, which may act as a buffer against change. This hypothesis is consistent with the observed phenotypic convergence (Chapter 3 & 4) and the general high genetic diversity observed. This phenomenon, where populations exhibit similar traits despite genetic differences, may reflect pre-existing adaptive diversity that buffers populations against environmental change. The anchoring effect of dormant resting stages, as discussed by Sundqvist et al. (2018), highlights how genetic reservoirs can enhance diversity, maintaining allelic richness over time and counteracting homogenizing forces. The implementation of controlled experiments across multiple generations, which simulate environmental factors such as temperature fluctuations, nutrient increases, or pollution, could elucidate whether such genetic diversification also manifests in novel phenotypic or adaptive traits. By integrating these findings into genomic monitoring frameworks, we could enhance predictions of how populations respond to environmental change, thereby extending the utility of genomic monitoring for conservation strategies. Such a study could also provide empirical validation for the role of genetic diversity in maintaining phenotypic stability under stress.

Common garden experiments could further test for maladaptation by growing strains of *S. marinoi* from different regions and exposing all strains to simulated region-specific conditions, such as salinity or nutrient concentrations that reflect those of different sub-regions of the Baltic Sea. By comparing performance metrics such as growth rates, photosynthetic efficiency or stress tolerance under these conditions, it could be determined whether local strains consistently outperform non-local strains in

their native environment. Such results would indicate local adaptation, whereas uniform or superior performance of non-local strains could indicate maladaptive traits or a lack of local adaptation. The potential for maladaptation due to environmental variability has been demonstrated in similar studies, where fluctuating environmental conditions significantly influence phenotypic traits in phytoplankton populations (Schaum et al., 2016).

Building on this, experiments could also test which individuals within populations enter dormancy in response to environmental stressors. By exposing populations to simulated nutrient depletion, light reduction or temperature change, it would be possible to determine the proportion of individuals that go dormant and whether this varies between genotypes or regions. Similarly, as Sundqvist et al. (2018) describe, the anchoring effect of dormant resting stages contributes significantly to genetic diversity by counteracting genetic drift and migration, a mechanism broadly applicable to organisms employing dormancy and potentially stabilizing phytoplankton populations over time. Such studies would help determine whether dormancy is a locally adaptive trait or whether populations exhibit maladaptive behaviour under stress. Integrating genetic and phenotypic profiling of these individuals could further identify links between the tendency to enter dormancy and fitness under changing environmental conditions.

In conclusion, this research provides a robust basis for integrating genomic tools into marine conservation. The study reveals the genetic and phenotypic stability of *S. marinoi* populations over millennia, demonstrating the resilience mechanisms inherent in marine ecosystems. At the same time, it highlights the ability to detect significant genetic and community shifts in the last millennium, likely driven by anthropogenic pressures. This underscores the critical role of genomic monitoring in identifying both long-term resilience and recent vulnerabilities in marine populations. As highlighted by recent studies (Jeffery et al., 2022; Van Oppen and Coleman, 2022), genomic approaches are offering deeper insights into biodiversity and adaptive potential, providing essential tools for ecosystem management. This framework, while demonstrated on *S. marinoi*, could be adapted to monitor other important species in diverse marine environments. By doing so, it could provide a universal model for assessing genetic resilience and adaptability in ecosystems worldwide. The approach developed here provides a clear basis for extending these approaches to other species and regions, emphasizing the importance of genetic diversity in maintaining ecosystem health under current and future stressors.

General conclusion

The work presented in this research represents an important contribution to the field of phytoplankton research, offering insights into the resilience and adaptability of populations, with a particular focus on a diatom in the Baltic Sea. By using advanced molecular tools, genomic analyses and resurrection ecology, this research addresses the key question of whether diatom populations are in equilibrium or undergoing fundamental shifts due to anthropogenic pressures. While crucial questions have been addressed, this research also provides a basis for future research, offering a robust framework to advance ecological monitoring and conservation strategies.

This study makes a progress in the field of marine phytoplankton research. The development of molecular indices, in particular the ske/apo index outlined in Chapter 1, offer an innovative tool for integrating modern monitoring frameworks into historical contexts. By establishing a link between shifts in species dominance and ecosystem health, this index facilitates the documentation of long-term dynamics, thereby improving our capacity to assess historical baselines and anticipate future changes. The findings presented in Chapters 2 and 4 demonstrate that *Skeletonema marinoi* populations have exhibited remarkable genomic stability over millennia, underscoring their resilience in the face of natural environmental variability. These genomic findings are of critical importance for understanding long-term stability and provide a basis for evaluating the impacts of modern stressors. Furthermore, the resurrection ecology approach detailed in Chapter 3 emphasises the exceptional resilience of diatoms, which have been observed to remain viable after dormancy periods exceeding 6,800 years. This finding not only extends the known limits of dormancy but also provides unique opportunities to study evolutionary responses of ancient populations. Finally, this research demonstrates that modern allele turnover rates and dominance shifts, linked to anthropogenic pressures, may point to a departure from historical baselines. These findings emphasise the urgent need for targeted conservation strategies to address the challenges of the Anthropocene.

Notwithstanding these advances, several limitations remain that could be addressed by future research. The principal focus of this research was on diatoms, with only limited exploration of dinoflagellates and other taxa. Further research could extend the taxonomic scope in order to gain insight into the interactions and responses of different phytoplankton groups to environmental changes collectively. Furthermore, while this study highlights accelerated allele turnover and dominance shifts, the underlying mechanisms driving these changes remain unresolved. A further objective would be to investigate specific selective pressures, such as warming and eutrophication, and their impacts on genetic diversity and resilience, in order to provide actionable insights. Moreover, the Baltic Sea, given

its status as a highly dynamic and anthropogenically influenced system, constituted the primary focus of this research. Comparative studies across other marine ecosystems would assist in determining whether the observed patterns are unique to the Baltic Sea or reflective of broader trends. Ultimately, while this research presents the potential of molecular indices and genomic tools, integrating these approaches into real-time conservation and management practices represents a crucial subsequent step in the protection of marine ecosystems.

The findings of this study address critical gaps in understanding phytoplankton dynamics and establish a basis for future research and conservation. By establishing a link between historical data and modern monitoring frameworks, this work demonstrates the resilience of phytoplankton populations while acknowledging the challenges posed by the Anthropocene. The integration of sediment archives, molecular tools and genomic insights offers compelling evidence that diatoms such as *S. marinoi* are highly adaptable, providing grounds for anticipating their continued persistence in the face of rapid environmental change. With continued advancements in genomic technologies and increased collaboration across disciplines, the resilience and adaptability of marine ecosystems can be better understood, protected and sustainably managed for future generations.

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Author's contributions

Chapter 1:

Alexandra Schmidt: lead author, conceptualization, data generation, analyses, data visualization, data interpretation, writing - original draft, writing - review and editing

Juliane Romahn: data generation, analyses, writing - review and editing

Elinor Andrén: methodology, writing - review and editing

Anke Kremp: conceptualization, sampling, writing - review and editing, funding acquisition

Jérôme Kaiser: data generation, analyses, data visualization, data interpretation, writing - review and editing

Helge W. Arz: data generation, analyses, data visualization, data interpretation, writing - review and editing

Olaf Dellwig: data generation, analyses, data interpretation, writing - review and editing (supported)

Miklós Bálint: conceptualization, analyses, funding acquisition, writing - review and editing (supported)

Laura S. Epp: conceptualization, sampling, writing - review and editing, supervision, project administration, funding acquisition

Chapter 2:

Alexandra Schmidt: lead author, conceptualization, data generation, analyses, data visualization, data interpretation, writing - original draft, writing - review and editing

Sarah Bolius: analyses (supported), writing - review and editing

Anna Chagas: data generation (supported), analyses (supported), writing - review and editing (supported)

Juliane Romahn: data generation (supported), analyses (supported), writing - review and editing (supported)

Jérôme Kaiser: analyses (supported), writing - review and editing

Helge W. Arz: analyses (supported), writing - review and editing

Miklós Bálint: funding acquisition, writing - review and editing (supported)

Anke Kremp: conceptualization, sampling, writing - review and editing, funding acquisition

Laura S. Epp: conceptualization, sampling, writing - review and editing, supervision, project administration, funding acquisition

Chapter 3:

Sarah Bolius: lead author, conceptualization, resurrection, phenotypic and physiological analyses, data analyses, writing - original draft

Alexandra Schmidt: genetic analyses, data analyses, writing - original draft (supported), writing - review and editing

Jérôme Kaiser: data generation, analyses, data visualization, data interpretation, writing - review and editing

Helge W. Arz: data generation, analyses, data visualization, data interpretation, writing - original draft (supported), writing - review and editing

Olaf Dellwig: data generation, analyses, data visualization, data interpretation, writing - review and editing

Ulf Karsten: data analyses, writing - review and editing

Laura S. Epp: conceptualization, genetic analyses (supported), writing - review and editing

Anke Kremp: conceptualization, writing - original draft (supported), writing - review and editing

Chapter 4:

Alexandra Schmidt: lead author, conceptualization, data generation, analyses, data visualization, data interpretation, writing - original draft, writing - review and editing

Sarah Bolius: resurrection ecology, DNA extraction, phenotypic trait experiments, writing - review and editing

Juliane Romahn: analyses (supported), writing - review and editing

Markus Pfenninger: conceptualization, analyses (supported), writing - review and editing

Miklós Bálint: conceptualization, sequencing set up (supported)

Anke Kremp: conceptualization, funding acquisition, resurrection ecology (supported)

Laura S. Epp: conceptualization, sampling, writing - review and editing, supervision, project administration, funding acquisition

References

Akoglu, E., 2023. Ecological indicators reveal historical regime shifts in the Black Sea ecosystem. PeerJ 11, e15649.

Alemzadeh, E., Haddad, R., Ahmadi, A.-R., 2014. Phytoplanktons and DNA barcoding: Characterization and molecular analysis of phytoplanktons on the Persian Gulf. Iran J Microbiol 6, 296–302.

Alexander, D.H., Novembre, J., Lange, K., 2009. Fast model-based estimation of ancestry in unrelated individuals. Genome research 19, 1655–1664.

Allen, M.R., 2007. Measuring and modeling dispersal of adult zooplankton. Oecologia 153, 135–143.

Anderson, D.M., Glibert, P.M., Burkholder, J.M., 2002. Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. Estuaries 25, 704–726. https://doi.org/10.1007/BF02804901

Andersson, B., Rengefors, K., Kourtchenko, O., Johannesson, K., Berglund, O., Filipsson, H.L., 2023. Cross-contamination risks in sediment-based resurrection studies of phytoplankton. Limnol Oceanogr Letters 8, 376–384. https://doi.org/10.1002/lol2.10291

Andrén, E., Andrén, T., Kunzendorf, H., 2000. Holocene history of the Baltic Sea as a background for assessing records of human impact in the sediments of the Gotland Basin. The Holocene 10, 687–702. https://doi.org/10.1191/09596830094944

Andrén, E., van Wirdum, F., Norbäck Ivarsson, L., Lönn, M., Moros, M., Andrén, T., 2020. Medieval versus recent environmental conditions in the Baltic Proper, what was different a thousand years ago? Palaeogeography, Palaeoclimatology, Palaeoecology 555, 109878. https://doi.org/10.1016/j.palaeo.2020.109878

Andrén, T., Björck, S., Andrén, E., Conley, D., Zillén, L., Anjar, J., 2011. The Development of the Baltic Sea Basin During the Last 130 ka, in: Harff, J., Björck, S., Hoth, P. (Eds.), The Baltic Sea Basin, Central and Eastern European Development Studies (CEEDES). Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 75–97. https://doi.org/10.1007/978-3-642-17220-5_4

Andrés, J., Czechowski, P., Grey, E., Saebi, M., Andres, K., Brown, C., Chawla, N., Corbett, J.J., Brys, R., Cassey, P., Correa, N., Deveney, M.R., Egan, S.P., Fisher, J.P., Vanden Hooff, R., Knapp, C.R., Leong, S.C.Y., Neilson, B.J., Paolucci, E.M., Pfrender, M.E., Pochardt, M.R., Prowse, T.A.A., Rumrill, S.S., Scianni, C., Sylvester, F., Tamburri, M.N., Therriault, T.W., Yeo, D.C.J., Lodge, D.M., 2023. Environment and shipping drive environmental DNA beta-diversity among commercial ports. Molecular Ecology 32, 6696–6709. https://doi.org/10.1111/mec.16888

Andrews, S., 2021. FastQC: A quality control analysis tool for high throughput sequencing data.

Appleby, P.G., 2001. Chronostratigraphic Techniques in Recent Sediments, in: Last, W.M., Smol, J.P. (Eds.), Tracking Environmental Change Using Lake Sediments: Basin Analysis, Coring, and Chronological Techniques, Developments in Paleoenvironmental Research. Springer Netherlands, Dordrecht, pp. 171–203. https://doi.org/10.1007/0-306-47669-X_9

Argyle, P.A., Hinners, J., Walworth, N.G., Collins, S., Levine, N.M., Doblin, M.A., 2021. A High-Throughput Assay for Quantifying Phenotypic Traits of Microalgae. Frontiers in Microbiology 12, 1–14. https://doi.org/10.3389/fmicb.2021.706235

Armbrecht, L., 2022. Sedimentary ancient DNA (sedaDNA) as a new paleo proxy to investigate organismal responses to past environmental changes in Antarctica. PAGES Mag 30, 78–79. https://doi.org/10.22498/pages.30.2.78

Armbrecht, L., Hallegraeff, G., Bolch, C.J.S., Woodward, C., Cooper, A., 2021. Hybridisation capture allows DNA damage analysis of ancient marine eukaryotes. Sci Rep 11, 3220. https://doi.org/10.1038/s41598-021-82578-6

Armbrecht, L., Weber, M.E., Raymo, M.E., Peck, V.L., Williams, T., Warnock, J., Kato, Y., Hernández-Almeida, I., Hoem, F., Reilly, B., Hemming, S., Bailey, I., Martos, Y.M., Gutjahr, M., Percuoco, V., Allen, C., Brachfeld, S., Cardillo, F.G., Du, Z., Fauth, G., Fogwill, C., Garcia, M., Glüder, A., Guitard, M., Hwang, J.-H., Iizuka, M., Kenlee, B., O'Connell, S., Pérez, L.F., Ronge, T.A., Seki, O., Tauxe, L., Tripathi, S., Zheng, X., 2022. Ancient marine sediment DNA reveals diatom transition in Antarctica. Nat Commun 13, 5787. https://doi.org/10.1038/s41467-022-33494-4

Armbrecht, L.H., 2020. The Potential of Sedimentary Ancient DNA to Reconstruct Past Ocean Ecosystems. Oceanography 33, 116–123.

Armbrecht, L.H., Coolen, M.J., Lejzerowicz, F., George, S.C., Negandhi, K., Suzuki, Y., Young, J., Foster, N.R., Armand, L.K., Cooper, A., 2019. Ancient DNA from marine sediments: Precautions and considerations for seafloor coring, sample handling and data generation. Earth-Science Reviews 196, 102887.

Auguie, B., Antonov, A., 2017. gridExtra: Miscellaneous Functions for "Grid" Graphics.

Autin, W.J., Holbrook, J.M., 2012. Is the Anthropocene an issue of stratigraphy or pop culture. gsa Today 22, 60–61.

Bach, L.T., Taucher, J., 2019. CO2 effects on diatoms: a synthesis of more than a decade of ocean acidification experiments with natural communities. Ocean Science 15, 1159–1175. https://doi.org/10.5194/os-15-1159-2019

Baho, D.L., Rizzuto, S., Nizzetto, L., Hessen, D.O., Norberg, J., Skjelbred, B., Jones, K.C., Zhang, H., Leu, E., 2021. Ecological Memory of Historical Contamination Influences the Response of Phytoplankton Communities. Ecosystems 24, 1591–1607. https://doi.org/10.1007/s10021-021-00604-0

Behrenfeld, M.J., O'Malley, R., Boss, E., Karp-Boss, L., Mundt, C., 2021. Phytoplankton biodiversity and the inverted paradox. ISME Communications 1, 52. https://doi.org/10.1038/s43705-021-00056-6

Benoiston, A.-S., Ibarbalz, F.M., Bittner, L., Guidi, L., Jahn, O., Dutkiewicz, S., Bowler, C., 2017. The evolution of diatoms and their biogeochemical functions. Phil. Trans. R. Soc. B 372, 20160397. https://doi.org/10.1098/rstb.2016.0397

Benson, D.A., Clark, K., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Sayers, E.W., 2015. GenBank. Nucleic Acids Res 43, D30–D35. https://doi.org/10.1093/nar/gku1216

Berthold, M., Campbell, D.A., 2021. Restoration, conservation and phytoplankton hysteresis. Conservation Physiology 9, coab062. https://doi.org/10.1093/conphys/coab062

Bi, R., Cao, Z., Ismar-Rebitz, S.M.H., Sommer, U., Zhang, H., Ding, Y., Zhao, M., 2021. Responses of Marine Diatom-Dinoflagellate Competition to Multiple Environmental Drivers: Abundance, Elemental, and Biochemical Aspects. Frontiers in Microbiology 12.

Bilcke, G., Ferrante, M.I., Montresor, M., De Decker, S., De Veylder, L., Vyverman, W., 2022. Life Cycle Regulation, in: Falciatore, A., Mock, T. (Eds.), The Molecular Life of Diatoms. Springer International Publishing, Cham, pp. 205–228. https://doi.org/10.1007/978-3-030-92499-7_8

Björck, S., 2008. The late Quaternary development of the Baltic Sea basin, in: Assessment of Climate Change for the Baltic Sea Basin. Springer, pp. 398–407.

Blaauw, M., Christen, J.A., 2011. Flexible paleoclimate age-depth models using an autoregressive gamma process. Bayesian Anal. 6. https://doi.org/10.1214/11-BA618

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bolius, S., Schmidt, A., Kaiser, J., Arz, H.W., Dellwig, O., Karsten, U., Epp, L.S., Kremp, A., 2024. Back to life after 6,800 years: Resurrection of a diatom from anoxic Baltic Sea sediments. ISME J.

Borics, G., Abonyi, A., Salmaso, N., Ptacnik, R., 2021. Freshwater phytoplankton diversity: models, drivers and implications for ecosystem properties. Hydrobiologia 848, 53–75. https://doi.org/10.1007/s10750-020-04332-9

Borzenkova, I., Zorita, E., Borisova, O., Kalniņa, L., Kisielienė, D., Koff, T., Kuznetsov, D., Lemdahl, G., Sapelko, T., Stančikaitė, M., Subetto, D., 2015. Climate Change During the Holocene (Past 12,000 Years), in: The BACC II Author Team (Ed.), Second Assessment of Climate Change for the Baltic Sea Basin, Regional Climate Studies. Springer International Publishing, Cham, pp. 25–49. https://doi.org/10.1007/978-3-319-16006-1_2

Brand, L.E., 1980. Review of Genetic Variation in Marine Phytoplankton Species and the Ecological Implications. Biological Oceanography. https://doi.org/10.1080/01965581.1988.1074954

Brasell, K.A., Pochon, X., Howarth, J., Pearman, J.K., Zaiko, A., Thompson, L., Vandergoes, M.J., Simon, K.S., Wood, S.A., 2022. Shifts in DNA yield and biological community composition in stored sediment: implications for paleogenomic studies. MBMG 6, e78128. https://doi.org/10.3897/mbmg.6.78128

Breitburg, D., Levin, L.A., Oschlies, A., Grégoire, M., Chavez, F.P., Conley, D.J., Garçon, V., Gilbert, D., Gutiérrez, D., Isensee, K., Jacinto, G.S., Limburg, K.E., Montes, I., Naqvi, S.W.A., Pitcher, G.C., Rabalais, N.N., Roman, M.R., Rose, K.A., Seibel, B.A., Telszewski, M., Yasuhara, M., Zhang, J., 2018. Declining oxygen in the global ocean and coastal waters. Science 359, eaam7240. https://doi.org/10.1126/science.aam7240

Brendonck, L., De Meester, L., 2003. Egg banks in freshwater zooplankton: evolutionary and ecological archives in the sediment. Hydrobiologia 491, 65–84. https://doi.org/10.1023/A:1024454905119

Briddon, C.L., Nicoară, M., Hegedüs, A., Niculea, A., Bellerby, R., Eikrem, W., Crespo, B.G., Dupont, S., Drugă, B., 2023. Acclimation to various temperature and pCO2 levels does not impact the competitive ability of two strains of Skeletonema marinoi in natural communities. Front. Mar. Sci. 10, 1197570. https://doi.org/10.3389/fmars.2023.1197570

Browning, B.L., Tian, X., Zhou, Y., Browning, S.R., 2021. Fast two-stage phasing of large-scale sequence data. The American Journal of Human Genetics 108, 1880–1890. https://doi.org/10.1016/j.ajhg.2021.08.005

Browning, T.J., Al-Hashem, A.A., Hopwood, M.J., Engel, A., Wakefield, E.D., Achterberg, E.P., 2020. Nutrient regulation of late spring phytoplankton blooms in the midlatitude North Atlantic. Limnology & Oceanography 65, 1136–1148. https://doi.org/10.1002/lno.11376

Buchwald, S.Z., Herzschuh, U., Nürnberg, D., Harms, L., Stoof-Leichsenring, K.R., 2024. Plankton community changes during the last 124 000 years in the subarctic Bering Sea derived from sedimentary ancient DNA. The ISME Journal 18, wrad006.

Bulankova, P., Bilcke, G., Vyverman, W., De Veylder, L., 2022. Cellular Hallmarks and Regulation of the Diatom Cell Cycle, in: Falciatore, A., Mock, T. (Eds.), The Molecular Life of Diatoms. Springer International Publishing, Cham, pp. 229–263. https://doi.org/10.1007/978-3-030-92499-7_9

Bunse, C., Bertos-Fortis, M., Sassenhagen, I., Sildever, S., Sjöqvist, C., Godhe, A., Gross, S., Kremp, A., Lips, I., Lundholm, N., Rengefors, K., Sefbom, J., Pinhassi, J., Legrand, C., 2016. Spatio-Temporal Interdependence of Bacteria and Phytoplankton during a Baltic Sea Spring Bloom. Front. Microbiol. 7. https://doi.org/10.3389/fmicb.2016.00517

Büntgen, U., Myglan, V.S., Ljungqvist, F.C., McCormick, M., Di Cosmo, N., Sigl, M., Jungclaus, J., Wagner, S., Krusic, P.J., Esper, J., Kaplan, J.O., De Vaan, M.A.C., Luterbacher, J., Wacker, L., Tegel, W., Kirdyanov, A.V., 2016. Cooling and societal change during the Late Antique

Little Ice Age from 536 to around 660 AD. Nature Geosci 9, 231–236. https://doi.org/10.1038/ngeo2652
Cantalapiedra, C.P., Hernández-Plaza, A., Letunic, I., Bork, P., Huerta-Cepas, J., 2021. eggNOG-mapper v2: functional annotation, orthology assignments, and domain prediction at the metagenomic scale. Molecular biology and evolution 38, 5825–5829.
Canuti, E., Penna, A., 2024. Dynamics of phytoplankton communities in the Baltic Sea: insights from a multi-dimensional analysis of pigment and spectral data—part I, pigment dataset. Front. Mar. Sci. 11, 1425347. https://doi.org/10.3389/fmars.2024.1425347
Capo, E., Giguet-Covex, C., Rouillard, A., Nota, K., Heintzman, P.D., Vuillemin, A., Ariztegui, D., Arnaud, F., Belle, S., Bertilsson, S., 2021. Lake sedimentary DNA research on past terrestrial and aquatic biodiversity: Overview and recommendations. Quaternary 4, 6.
Carstensen, J., Andersen, J.H., Gustafsson, B.G., Conley, D.J., 2014a. Deoxygenation of the Baltic Sea during the last century. Proc. Natl. Acad. Sci. U.S.A. 111, 5628–5633. https://doi.org/10.1073/pnas.1323156111
Carstensen, J., Conley, D.J., Bonsdorff, E., Gustafsson, B.G., Hietanen, S., Janas, U., Jilbert, T., Maximov, A., Norkko, A., Norkko, J., Reed, D.C., Slomp, C.P., Timmermann, K., Voss, M., 2014b. Hypoxia in the Baltic Sea: Biogeochemical Cycles, Benthic Fauna, and Management. AMBIO 43, 26–36. https://doi.org/10.1007/s13280-013-0474-7
Casabianca, S., Capellacci, S., Ricci, F., Andreoni, F., Russo, T., Scardi, M., Penna, A., 2020. Structure and environmental drivers of phytoplanktonic resting stage assemblages in the central Mediterranean Sea. Marine Ecology Progress Series 639, 73–89.
Cheng, L., von Schuckmann, K., Abraham, J.P., Trenberth, K.E., Mann, M.E., Zanna, L., England, M.H., Zika, J.D., Fasullo, J.T., Yu, Y., Pan, Y., Zhu, J., Newsom, E.R., Bronselaer, B., Lin, X., 2022. Past and future ocean warming. Nat Rev Earth Environ 3, 776–794. https://doi.org/10.1038/s43017-022-00345-1
Chiswell, S.M., Calil, P.H.R., Boyd, P.W., 2015. Spring blooms and annual cycles of phytoplankton: a unified perspective. Journal of Plankton Research 37, 500–508. https://doi.org/10.1093/plankt/fbv021
Cingolani, P., Platts, A., Wang, L.L., Coon, M., Nguyen, T., Wang, L., Land, S.J., Lu, X., Ruden, D.M., 2012. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w^{1118} ; iso-2; iso-3. Fly 6, 80–92. https://doi.org/10.4161/fly.19695
Clark, L.V., Schreier, A.D., 2017. Resolving microsatellite genotype ambiguity in populations of allopolyploid and diploidized autopolyploid organisms using negative correlations between allelic variables. Molecular Ecology Resources 17, 1090–1103. https://doi.org/10.1111/1755-0998.12639
Collins, S., Rost, B., Rynearson, T.A., 2014. Evolutionary potential of marine phytoplankton under ocean acidification. Evol Appl 7, 140–155. https://doi.org/10.1111/eva.12120
Conde-Porcuna, J.M., Ramos-Rodríguez, E., Pérez-Martínez, C., 2014. In situ production of empty ephippia and resting eggs by an obligate parthenogenetic Daphnia population. Journal of Plankton Research 36, 157–169.
Conley, D.J., Björck, S., Bonsdorff, E., Carstensen, J., Destouni, G., Gustafsson, B.G., Hietanen, S., Kortekaas, M., Kuosa, H., Markus Meier, H.E., Müller-Karulis, B., Nordberg, K., Norkko, A., Nürnberg, G., Pitkänen, H., Rabalais, N.N., Rosenberg, R., Savchuk, O.P., Slomp, C.P., Voss, M., Wulff, F., Zillén, L., 2009. Hypoxia-Related Processes in the Baltic Sea. Environ. Sci. Technol. 43, 3412–3420. https://doi.org/10.1021/es802762a
Conley, D.J., Humborg, C., Smedberg, E., Rahm, L., Papush, L., Danielsson, Å., Clarke, A., Pastuszak, M., Aigars, J., Ciuffa, D., Mörth, C.-M., 2008. Past, present and future state of the biogeochemical Si cycle in the Baltic Sea. Journal of Marine Systems 73, 338–346. https://doi.org/10.1016/j.jmarsys.2007.10.016

Costello, M.J., Basher, Z., McLeod, L., Asaad, I., Claus, S., Vandepitte, L., Yasuhara, M., Gislason, H., Edwards, M., Appeltans, W., Enevoldsen, H., Edgar, G.J., Miloslavich, P., De Monte, S., Pinto, I.S., Obura, D., Bates, A.E., 2017. Methods for the Study of Marine Biodiversity, in: Walters, M., Scholes, R.J. (Eds.), The GEO Handbook on Biodiversity Observation Networks. Springer International Publishing, Cham, pp. 129–163. https://doi.org/10.1007/978-3-319-27288-7_6

Covarrubias-Pazaran, G., Diaz-Garcia, L., Schlautman, B., Salazar, W., Zalapa, J., 2016. Fragman: an R package for fragment analysis. BMC Genet 17, 62. https://doi.org/10.1186/s12863-016-0365-6

Craveiro, S.C., Daugbjerg, N., Moestrup, Ø., Calado, A.J., 2017. Studies on Peridinium aciculiferum and Peridinium malmogiense (=Scrippsiella hangoei): comparison with Chimonodinium lomnickii and description of Apocalathium gen. nov. (Dinophyceae). Phycologia 56, 21–35. https://doi.org/10.2216/16-20.1

Crump, S.E., 2021. Sedimentary ancient DNA as a tool in paleoecology. Nat Rev Earth Environ 2, 229–229. https://doi.org/10.1038/s43017-021-00158-8

Crutzen, P.J., Stoermer, E.F., 2000. Global change newsletter. The Anthropocene 41, 17–18.

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R., 1000 Genomes Project Analysis Group, 2011. The variant call format and VCFtools. Bioinformatics 27, 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

Danecek, P., Bonfield, J.K., Liddle, J., Marshall, J., Ohan, V., Pollard, M.O., Whitwham, A., Keane, T., McCarthy, S.A., Davies, R.M., Li, H., 2021. Twelve years of SAMtools and BCFtools. GigaScience 10, giab008. https://doi.org/10.1093/gigascience/giab008

Datta, M.G., 2023. Pollution in Marine Ecosystem: Impact and Prevention, in: Soni, R., Suyal, D.C., Morales-Oyervides, L., Fouillaud, M. (Eds.), Current Status of Marine Water Microbiology. Springer Nature, Singapore, pp. 169–191. https://doi.org/10.1007/978-981-99-5022-5_8

Delebecq, G., Schmidt, S., Ehrhold, A., Latimier, M., Siano, R., 2020. Revival of Ancient Marine Dinoflagellates Using Molecular Biostimulation. Journal of Phycology 56, 1077–1089. https://doi.org/10.1111/jpy.13010

Dellwig, O., Schnetger, B., Meyer, D., Pollehne, F., Häusler, K., Arz, H.W., 2018. Impact of the major Baltic inflow in 2014 on manganese cycling in the Gotland Deep (Baltic Sea). Frontiers in Marine Science 5, 248.

Devlin, M., Brodie, J., 2023. Nutrients and Eutrophication, in: Reichelt-Brushett, A. (Ed.), Marine Pollution – Monitoring, Management and Mitigation, Springer Textbooks in Earth Sciences, Geography and Environment. Springer Nature Switzerland, Cham, pp. 75–100. https://doi.org/10.1007/978-3-031-10127-4_4

Diaz, R.J., Rosenberg, R., 2008. Spreading Dead Zones and Consequences for Marine Ecosystems. Science 321, 926–929. https://doi.org/10.1126/science.1156401

Dingle, T.C., Sedlak, R.H., Cook, L., Jerome, K.R., 2013. Tolerance of Droplet-Digital PCR vs Real-Time Quantitative PCR to Inhibitory Substances. Clinical Chemistry 59, 1670–1672. https://doi.org/10.1373/clinchem.2013.211045

Dixon, P., 2003. VEGAN, a package of R functions for community ecology. Journal of Vegetation Science 14, 927–930. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x

Doi, H., Uchii, K., Takahara, T., Matsuhashi, S., Yamanaka, H., Minamoto, T., 2015. Use of Droplet Digital PCR for Estimation of Fish Abundance and Biomass in Environmental DNA Surveys. PLOS ONE 10, e0122763. https://doi.org/10.1371/journal.pone.0122763

Dokulil, M.T., Qian, K., 2021. Photosynthesis, carbon acquisition and primary productivity of phytoplankton: a review dedicated to Colin Reynolds. Hydrobiologia 848, 77–94. https://doi.org/10.1007/s10750-020-04321-y

Dokulil, M.T., Teubner, K., 2024. Long-term adjustment of phytoplankton structure to environmental traits at timescales during lifetime development and over generations. Hydrobiologia 851, 823–847. https://doi.org/10.1007/s10750-023-05365-6
Dollinger, P., 1970. The German Hansa, translated by Ault, DS, Steinberg.
Dong, K., Kvile, K., Stenseth, N., Stige, L., 2021. Associations between timing and magnitude of spring blooms and zooplankton dynamics in the southwestern Barents Sea. Mar. Ecol. Prog. Ser. 668, 57–72. https://doi.org/10.3354/meps13740
Dunnington, D.W., Libera, N., Kurek, J., Spooner, I.S., Gagnon, G.A., 2022. **tidypaleo** : Visualizing Paleoenvironmental Archives Using **ggplot2**. J. Stat. Soft. 101. https://doi.org/10.18637/jss.v101.i07
Ehrlich, H., Witkowski, A., 2015. Biomineralization in Diatoms: The Organic Templates, in: Hamm, C. (Ed.), Evolution of Lightweight Structures, Biologically-Inspired Systems. Springer Netherlands, Dordrecht, pp. 39–58. https://doi.org/10.1007/978-94-017-9398-8_3
Ellegaard, M., Clokie, M.R.J., Czypionka, T., Frisch, D., Godhe, A., Kremp, A., Letarov, A., McGenity, T.J., Ribeiro, S., John Anderson, N., 2020. Dead or alive: sediment DNA archives as tools for tracking aquatic evolution and adaptation. Commun Biol 3, 1–11. https://doi.org/10.1038/s42003-020-0899-z
Ellegaard, M., Moestrup, Ø., Joest Andersen, T., Lundholm, N., 2016. Long-term survival of haptophyte and prasinophyte resting stages in marine sediment. European Journal of Phycology 51, 328–337. https://doi.org/10.1080/09670262.2016.1161243
Ellegaard, M., Ribeiro, S., 2018. The long-term persistence of phytoplankton resting stages in aquatic 'seed banks.' Biological Reviews 93, 166–183. https://doi.org/10.1111/brv.12338
Ellis, E.C., 2011. Anthropogenic transformation of the terrestrial biosphere. Phil. Trans. R. Soc. A. 369, 1010–1035. https://doi.org/10.1098/rsta.2010.0331
Emeis, K.-C., Struck, U., Blanz, T., Kohly, A., Voβ, M., 2003. Salinity changes in the central Baltic Sea (NW Europe) over the last 10000 years. The Holocene 13, 411–421. https://doi.org/10.1191/0959683603hl634rp
Eom, S.H., Jeong, H.J., Ok, J.H., Park, S.A., Kang, H.C., You, J.H., 2024. Combined effects of hypoxia and starvation on the survival and growth rates of autotrophic, mixotrophic, and heterotrophic dinoflagellates. Mar Biol 171, 42. https://doi.org/10.1007/s00227-023-04363-5
Epp, L.S., Kruse, S., Kath, N.J., Stoof-Leichsenring, K.R., Tiedemann, R., Pestryakova, L.A., Herzschuh, U., 2018. Temporal and spatial patterns of mitochondrial haplotype and species distributions in Siberian larches inferred from ancient environmental DNA and modeling. Sci Rep 8, 17436. https://doi.org/10.1038/s41598-018-35550-w
Epp, L.S., Zimmermann, H.H., Stoof-Leichsenring, K.R., 2019. Sampling and Extraction of Ancient DNA from Sediments, in: Shapiro, B., Barlow, A., Heintzman, P.D., Hofreiter, M., Paijmans, J.L.A., Soares, A.E.R. (Eds.), Ancient DNA, Methods in Molecular Biology. Springer New York, New York, NY, pp. 31–44. https://doi.org/10.1007/978-1-4939-9176-1_5
European Commission, 2008. Directive 2008/56/EC of the European Parliament and of the Council. Journal). Council Decision of.
Ewels, P., Magnusson, M., Lundin, S., Käller, M., 2016. MultiQC: summarize analysis results for multiple tools and samples in a single report. Bioinformatics 32, 3047–3048. https://doi.org/10.1093/bioinformatics/btw354
Falkowski, P.G., Barber, R.T., Smetacek, V., 1998. Biogeochemical Controls and Feedbacks on Ocean Primary Production. Science 281, 200–206. https://doi.org/10.1126/science.281.5374.200

Ferreira, V., Bini, L.M., González Sagrario, M. de los Á., Kovalenko, K.E., Naselli-Flores, L., Padial, A.A., Padisák, J., 2023. Aquatic ecosystem services: an overview of the Special Issue. Hydrobiologia 850, 2473–2483. https://doi.org/10.1007/s10750-023-05235-1

Ficetola, G.F., Coissac, E., Zundel, S., Riaz, T., Shehzad, W., Bessière, J., Taberlet, P., Pompanon, F., 2010. An In silico approach for the evaluation of DNA barcodes. BMC Genomics 11, 434. https://doi.org/10.1186/1471-2164-11-434

Florenzano, A., Clò, E., Jacob, J., 2023. Pollen and Molecular Biomarkers from Sedimentary Archives in the Central Po Plain (N Italy): Assessing Their Potential to Deepen Changes in Natural and Agricultural Systems. Sustainability 15, 10408. https://doi.org/10.3390/su151310408

Flück, B., Mathon, L., Manel, S., Valentini, A., Dejean, T., Albouy, C., Mouillot, D., Thuiller, W., Murienne, J., Brosse, S., Pellissier, L., 2022. Applying convolutional neural networks to speed up environmental DNA annotation in a highly diverse ecosystem. Sci Rep 12, 10247. https://doi.org/10.1038/s41598-022-13412-w

Fontaneto, D., 2019. Long-distance passive dispersal in microscopic aquatic animals. Mov Ecol 7, 10. https://doi.org/10.1186/s40462-019-0155-7

Foucher, A., Chaboche, P.-A., Sabatier, P., Evrard, O., 2021. A worldwide meta-analysis (1977–2020) of sediment core dating using fallout radionuclides including ¹³⁷Cs and ²¹⁰Pb_{xs} Earth Syst. Sci. Data 13, 4951–4966. https://doi.org/10.5194/essd-13-4951-2021

Frisch, D., Morton, P.K., Chowdhury, P.R., Culver, B.W., Colbourne, J.K., Weider, L.J., Jeyasingh, P.D., 2014. A millennial-scale chronicle of evolutionary responses to cultural eutrophication in *Daphnia*. Ecology Letters 17, 360–368. https://doi.org/10.1111/ele.12237

Gansauge, M.-T., Aximu-Petri, A., Nagel, S., Meyer, M., 2020. Manual and automated preparation of single-stranded DNA libraries for the sequencing of DNA from ancient biological remains and other sources of highly degraded DNA. Nat Protoc 15, 2279–2300. https://doi.org/10.1038/s41596-020-0338-0

Gardoki, J., Morellón, M., Leira, M., Ezquerra, F.J., Remondo, J., Tinner, W., Canales, M.L., Van Der Horst, A., Morales-Molino, C., 2023. Abrupt diatom responses to recent climate and land use changes in the Cantabrian Mountains (NW Spain). J Paleolimnol 69, 213–230. https://doi.org/10.1007/s10933-022-00269-2

Gechev, T., Lyall, R., Petrov, V., Bartels, D., 2021. Systems biology of resurrection plants. Cell. Mol. Life Sci. 78, 6365–6394. https://doi.org/10.1007/s00018-021-03913-8

Gielings, R., Fais, M., Fontaneto, D., Creer, S., Costa, F.O., Renema, W., Macher, J.-N., 2021. DNA Metabarcoding Methods for the Study of Marine Benthic Meiofauna: A Review. Front. Mar. Sci. 8, 730063. https://doi.org/10.3389/fmars.2021.730063

Godhe, A., Härnström, K., 2010. Linking the planktonic and benthic habitat: genetic structure of the marine diatom Skeletonema marinoi. Molecular Ecology 19, 4478–4490. https://doi.org/10.1111/j.1365-294X.2010.04841.x

Godhe, A., Rynearson, T., 2017. The role of intraspecific variation in the ecological and evolutionary success of diatoms in changing environments. Phil. Trans. R. Soc. B 372, 20160399. https://doi.org/10.1098/rstb.2016.0399

Goulletquer, P., Gros, P., Boeuf, G., Weber, J., 2014. The Impacts of Human Activities on Marine Biodiversity, in: Biodiversity in the Marine Environment. Springer Netherlands, Dordrecht, pp. 15–20. https://doi.org/10.1007/978-94-017-8566-2_2

Graff Van Creveld, S., Rosenwasser, S., Levin, Y., Vardi, A., 2016. Chronic iron limitation confers transient resistance to oxidative stress in marine diatoms. Plant Physiol. pp.00840.2016. https://doi.org/10.1104/pp.16.00840

Grant, K.M., Rohling, E.J., Bar-Matthews, M., Ayalon, A., Medina-Elizalde, M., Ramsey, C.B., Satow, C., Roberts, A.P., 2012. Rapid coupling between ice volume and polar temperature over the past 150,000 years. Nature 491, 744–747.

Gros-Balthazard, M., Flowers, J.M., Hazzouri, K.M., Ferrand, S., Aberlenc, F., Sallon, S., Purugganan, M.D., 2021. The genomes of ancient date palms germinated from 2,000 y old seeds. Proc. Natl. Acad. Sci. U.S.A. 118, e2025337118. https://doi.org/10.1073/pnas.2025337118

Guardiola, M., Uriz, M.J., Taberlet, P., Coissac, E., Wangensteen, O.S., Turon, X., 2015. Deep-Sea, Deep-Sequencing: Metabarcoding Extracellular DNA from Sediments of Marine Canyons. PLoS ONE 10, e0139633. https://doi.org/10.1371/journal.pone.0139633

Guillard, R.R.L., 1975. Culture of Phytoplankton for Feeding Marine Invertebrates, in: Smith, W.L., Chanley, M.H. (Eds.), Culture of Marine Invertebrate Animals. Springer US, Boston, MA, pp. 29–60. https://doi.org/10.1007/978-1-4615-8714-9_3

Gustafsson, B.G., Schenk, F., Blenckner, T., Eilola, K., Meier, H.E.M., Müller-Karulis, B., Neumann, T., Ruoho-Airola, T., Savchuk, O.P., Zorita, E., 2012. Reconstructing the Development of Baltic Sea Eutrophication 1850–2006. AMBIO 41, 534–548. https://doi.org/10.1007/s13280-012-0318-x

Gustafsson, B.G., Westman, P., 2002. On the causes for salinity variations in the Baltic Sea during the last 8500 years. Paleoceanography 17. https://doi.org/10.1029/2000PA000572

Häder, D.-P., Gao, K., 2015. Interactions of anthropogenic stress factors on marine phytoplankton. Front. Environ. Sci. 3. https://doi.org/10.3389/fenvs.2015.00014

Hald, M.M., Styring, A., Mortensen, M.F., Maltas, T., Vidas, D., Henriksen, P.S., Pihl, A., Jensen, P.M., Christensen, L.B., Hansen, J., Dollar, S., Egeberg, T., Lundø, M.B., Haue, N., Hertz, E., Iversen, R.B., Jørgensen, T., Kristensen, I.K., Klassen, L., Møller, N.A., Pedersen, V.J., Ravn, M., Vestergaard, K., Jessen, M.D., 2024. Farming during turbulent times: Agriculture, food crops, and manuring practices in Bronze Age to Viking Age Denmark. Journal of Archaeological Science: Reports 58, 104736. https://doi.org/10.1016/j.jasrep.2024.104736

Hällfors, G., 2004. Checklist of Baltic Sea Phytoplankton Species Baltic Sea Environment Proceedings No. 95. Helsinki Commission, Baltic Marine Environment Protection Commission.

Haraguchi, L., Kraft, K., Ylöstalo, P., Kielosto, S., Hällfors, H., Tamminen, T., Seppälä, J., 2023. Trait response of three Baltic Sea spring dinoflagellates to temperature, salinity, and light gradients. Frontiers in Marine Science 10.

Harff, J., Endler, R., Emelyanov, E., Kotov, S., Leipe, T., Moros, M., Olea, R., Tomczak, M., Witkowski, A., 2011. Late Quaternary Climate Variations Reflected in Baltic Sea Sediments, in: Harff, J., Björck, S., Hoth, P. (Eds.), The Baltic Sea Basin, Central and Eastern European Development Studies (CEEDES). Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 99–132. https://doi.org/10.1007/978-3-642-17220-5_5

Härnström, K., Ellegaard, M., Andersen, T.J., Godhe, A., 2011. Hundred years of genetic structure in a sediment revived diatom population. Proc. Natl. Acad. Sci. U.S.A. 108, 4252–4257. https://doi.org/10.1073/pnas.1013528108

Hattich, G.S.I., Jokinen, S., Sildever, S., Gareis, M., Heikkinen, J., Junghardt, N., Segovia, M., Machado, M., Sjöqvist, C., 2024. Temperature optima of a natural diatom population increases as global warming proceeds. Nature Climate Change 1–8.

Häusler, K., Dellwig, O., Schnetger, B., Feldens, P., Leipe, T., Moros, M., Pollehne, F., Schönke, M., Wegwerth, A., Arz, H.W., 2018. Massive Mn carbonate formation in the Landsort Deep (Baltic Sea): Hydrographic conditions, temporal succession, and Mn budget calculations. Marine Geology 395, 260–270. https://doi.org/10.1016/j.margeo.2017.10.010

Hays, G.C., Richardson, A.J., Robinson, C., 2005. Climate change and marine plankton. Trends in ecology & evolution 20, 337–344.
HELCOM, 2024. HELCOM Map and data service [WWW Document]. URL https://maps.helcom.fi/website/mapservice/index.html (accessed 9.26.24).
HELCOM, 2023. Diatom/Dinoflagellate index [WWW Document]. HELCOM indicators. URL https://indicators.helcom.fi/indicator/diatom-dinoflagellate-index/ (accessed 9.11.23).
HELCOM, 2021. HELCOM Thematic assessment of Eutrophication 2016-2021. Baltic Sea Environment Proceedings No.192.
HELCOM, B., 2007. The HELCOM Baltic Sea Action Plan. Helsinki Commission for the Protection of the Baltic Marine Environment Helsinki. Finland. Baltic Sea Environ. Proc. 111.
Hendry, G.A.F., Thompson, K., Moss, C.J., Edwards, E., Thorpe, P.C., 1994. Seed persistence: a correlation between seed longevity in the soil and ortho-dihydroxyphenol concentration. Functional Ecology 658–664.
Hillebrand, H., Dürselen, C.-D., Kirschtel, D., Pollingher, U., Zohary, T., 1999. Biovolume calculation for pelagic and benthic microalgae. Journal of Phycology 35, 403–424. https://doi.org/10.1046/j.1529-8817.1999.3520403.x
Hinners, J., Kremp, A., Hense, I., 2017. Evolution in temperature-dependent phytoplankton traits revealed from a sediment archive: do reaction norms tell the whole story? Proc. R. Soc. B. 284, 20171888. https://doi.org/10.1098/rspb.2017.1888
Hjerne, O., Hajdu, S., Larsson, U., Downing, A.S., Winder, M., 2019. Climate Driven Changes in Timing, Composition and Magnitude of the Baltic Sea Phytoplankton Spring Bloom. Frontiers in Marine Science 6.
Hochfeld, I., Ward, B.A., Kremp, A., Romahn, J., Schmidt, A., Bálint, M., Becks, L., Kaiser, J., Arz, H.W., Bolius, S., Epp, L.S., Pfenninger, M., Klausmeier, C.A., Litchman, E., Hinners, J., 2024. Ideas and perspectives: How sediment archives can improve model projections of marine ecosystem change. https://doi.org/10.5194/egusphere-2024-3297
Holman, L.E., Wang, Y., Sawafuji, R., Epp, L.S., Bohmann, K., Pedersen, M.W., 2023. Perspectives and Future Developments Within Sedimentary DNA Research, in: Capo, E., Barouillet, C., Smol, J.P. (Eds.), Tracking Environmental Change Using Lake Sediments, Developments in Paleoenvironmental Research. Springer International Publishing, Cham, pp. 393–416. https://doi.org/10.1007/978-3-031-43799-1_13
Howarth, R.W., Chan, F., Swaney, D.P., Marino, R.M., Hayn, M., 2021. Role of external inputs of nutrients to aquatic ecosystems in determining prevalence of nitrogen vs. phosphorus limitation of net primary productivity. Biogeochemistry 154, 293–306. https://doi.org/10.1007/s10533-021-00765-z
Huisman, J., Weissing, F.J., 1999. Biodiversity of plankton by species oscillations and chaos. Nature 402, 407–410.
Hutchins, D.A., Tagliabue, A., 2024. Feedbacks between phytoplankton and nutrient cycles in a warming ocean. Nat. Geosci. 17, 495–502. https://doi.org/10.1038/s41561-024-01454-w
Hutchinson, G.E., 1961. The Paradox of the Plankton. The American Naturalist 95, 137–145. https://doi.org/10.1086/282171
ICES, 2022. ICES DataPortal, Dome Phytoplankton [WWW Document]. URL https://data.ices.dk (accessed 12.19.23).
Ito, T., Minobe, S., Long, M.C., Deutsch, C., 2017. Upper ocean O_2 trends: 1958–2015. Geophysical Research Letters 44, 4214–4223. https://doi.org/10.1002/2017GL073613
IUGS-ICS, 2024. The Anthopocene: IUGS-ICS Statement.
Jaanus, A., Hajdu, S., Kaitala, S., Andersson, A., Kaljurand, K., Ledaine, I., Lips, I., Olenina, I., 2006. Distribution Patterns of Isomorphic Cold-Water Dinoflagellates (Scrippsiella/Woloszynskia Complex) Causing 'red tides' in the Baltic Sea. Hydrobiologia 554, 137–146. https://doi.org/10.1007/s10750-005-1014-7

Jacob, M., Thomas, P.K., Giebel, H.-A., Billerbeck, S., Simon, M., Striebel, M., Dlugosch, L., 2024. Cross-domain diversity effects: linking diatom species richness, intraspecific richness, and biomass production to host-associated bacterial diversity. ISME Communications 4, ycae046. https://doi.org/10.1093/ismeco/ycae046
Jeffery, N.W., Lehnert, S.J., Kess, T., Layton, K.K.S., Wringe, B.F., Stanley, R.R.E., 2022. Application of Omics Tools in Designing and Monitoring Marine Protected Areas For a Sustainable Blue Economy. Front. Genet. 13. https://doi.org/10.3389/fgene.2022.886494
Jing, X., Zhen, Y., Mi, T., Yu, Z., Wang, Y., Wang, X., 2024. Transcriptome response of diatom Skeletonema marinoi to lower temperature. Mar Biol 171, 115. https://doi.org/10.1007/s00227-024-04434-1
Johansson, O.N., Töpel, M., Pinder, M.I.M., Kourtchenko, O., Blomberg, A., Godhe, A., Clarke, A.K., 2019. Skeletonema marinoi as a new genetic model for marine chain-forming diatoms. Sci Rep 9, 5391. https://doi.org/10.1038/s41598-019-41085-5
Jónsson, H., Ginolhac, A., Schubert, M., Johnson, P.L.F., Orlando, L., 2013. mapDamage2.0: fast approximate Bayesian estimates of ancient DNA damage parameters. Bioinformatics 29, 1682–1684. https://doi.org/10.1093/bioinformatics/btt193
Joseph, C., Faiq, M.E., Li, Z., Chen, G., 2022. Persistence and degradation dynamics of eDNA affected by environmental factors in aquatic ecosystems. Hydrobiologia 849, 4119–4133. https://doi.org/10.1007/s10750-022-04959-w
Juggins, S., 2015. Rioja: analysis of quaternary science data.
Kabel, K., Moros, M., Porsche, C., Neumann, T., Adolphi, F., Andersen, T.J., Siegel, H., Gerth, M., Leipe, T., Jansen, E., 2012. Impact of climate change on the Baltic Sea ecosystem over the past 1,000 years. Nature Climate Change 2, 871–874.
Kaiser, J., Abel, S., Arz, H.W., Cundy, A.B., Dellwig, O., Gaca, P., Gerdts, G., Hajdas, I., Labrenz, M., Milton, J.A., Moros, M., Primpke, S., Roberts, S.L., Rose, N.L., Turner, S.D., Voss, M., Ivar do Sul, J.A., 2023. The East Gotland Basin (Baltic Sea) as a candidate Global boundary Stratotype Section and Point for the Anthropocene series. The Anthropocene Review 10, 25–48. https://doi.org/10.1177/20530196221132709
Kang, Y., Moon, C.-H., Kim, H.-J., Yoon, Y.H., Kang, C.-K., 2021. Water Quality Improvement Shifts the Dominant Phytoplankton Group From Cryptophytes to Diatoms in a Coastal Ecosystem. Frontiers in Marine Science 8.
Karnauskas, M., Schirripa, M.J., Craig, J.K., Cook, G.S., Kelble, C.R., Agar, J.J., Black, B.A., Enfield, D.B., Lindo-Atichati, D., Muhling, B.A., Purcell, K.M., Richards, P.M., Wang, C., 2015. Evidence of climate-driven ecosystem reorganization in the Gulf of Mexico. Global Change Biology 21, 2554–2568. https://doi.org/10.1111/gcb.12894
Karsten, U., Lütz, C., Holzinger, A., 2010. Ecophysiological performance of the aeroterrestrial green alga Klebsormidium crenulatum (charophyceae, streptophyta) isolated from an alpine soil crust with an emphasis on desiccation stress. Journal of Phycology 46, 1187–1197. https://doi.org/10.1111/j.1529-8817.2010.00921.x
Kassambara, A., 2023. ggpubr: “ggplot2” Based Publication Ready Plots.
Kerfoot, W.C., Robbins, J.A., Weider, L.J., 1999. A new approach to historical reconstruction: Combining descriptive and experimental paleolimnology. Limnology & Oceanography 44, 1232–1247. https://doi.org/10.4319/lo.1999.44.5.1232
Kirch, P.V., 2005. Archaeology and global change: The Holocene record. Annu. Rev. Environ. Resour. 30, 409–440. https://doi.org/10.1146/annurev.energy.29.102403.140700
Kirk, J.T., 1994. Light and photosynthesis in aquatic ecosystems. Cambridge university press.
Klais, R., Tamminen, T., Kremp, A., Spilling, K., Olli, K., 2011. Decadal-Scale Changes of Dinoflagellates and Diatoms in the Anomalous Baltic Sea Spring Bloom. PLoS ONE 6, e21567. https://doi.org/10.1371/journal.pone.0021567

Knudsen, S.W., Ebert, R.B., Hesselsøe, M., Kuntke, F., Hassingboe, J., Mortensen, P.B., Thomsen, P.F., Sigsgaard, E.E., Hansen, B.K., Nielsen, E.E., Møller, P.R., 2019. Species-specific detection and quantification of environmental DNA from marine fishes in the Baltic Sea. Journal of Experimental Marine Biology and Ecology 510, 31–45. https://doi.org/10.1016/j.jembe.2018.09.004

Kontny, B., 2023. Maritime contacts across the Baltic Sea during the Roman and Migration Periods (1st-7th centuries AD) in the light of archaeological sources from the central-European perspective.

Korpinen, S., Laamanen, M., Andersen, J.H., Asplund, L., Berger, U., Bignert, A., Boalt, E., Broeg, K., Brzozowska, A., Cato, I., 2010. Hazardous substances in the Baltic Sea: An integrated thematic assessment of hazardous substances in the Baltic Sea.

Kremp, A., Hinners, J., Klais, R., Leppänen, A.-P., Kallio, A., 2018. Patterns of vertical cyst distribution and survival in 100-year-old sediment archives of three spring dinoflagellate species from the Northern Baltic Sea. European Journal of Phycology 53, 135–145. https://doi.org/10.1080/09670262.2017.1386330

Kremp, A., Rengefors, K., Montresorc, M., 2009. Species specific encystment patterns in three Baltic cold-water dinoflagellates: The role of multiple cues in resting cyst formation. Limnology & Oceanography 54, 1125–1138. https://doi.org/10.4319/lo.2009.54.4.1125

Krumhardt, K.M., Long, M.C., Sylvester, Z.T., Petrik, C.M., 2022. Climate drivers of Southern Ocean phytoplankton community composition and potential impacts on higher trophic levels. Front. Mar. Sci. 9, 916140. https://doi.org/10.3389/fmars.2022.916140

Lal, D., Krishnaswami, S., 1998. carbon-14 datingCarbon-14 dating and other applications in earth sciences, in: Geochemistry. Springer Netherlands, Dordrecht, pp. 59–65. https://doi.org/10.1007/1-4020-4496-8_43

Lammers, Y., Clarke, C.L., Erséus, C., Brown, A.G., Edwards, M.E., Gielly, L., Haflidason, H., Mangerud, J., Rota, E., Svendsen, J.I., Alsos, I.G., 2019. Clitellate worms (Annelida) in lateglacial and Holocene sedimentary DNA records from the Polar Urals and northern Norway. Boreas 48, 317–329. https://doi.org/10.1111/bor.12363

Lammers, Y., Heintzman, P.D., Alsos, I.G., 2021. Environmental palaeogenomic reconstruction of an Ice Age algal population. Commun Biol 4, 220. https://doi.org/10.1038/s42003-021-01710-4

Laso-Jadart, R., O'Malley, M., Sykulski, A.M., Ambroise, C., Madoui, M.-A., 2023. Holistic view of the seascape dynamics and environment impact on macro-scale genetic connectivity of marine plankton populations. BMC Ecol Evo 23, 46. https://doi.org/10.1186/s12862-023-02160-8

Legrand, B., Miras, Y., Beauger, A., Dussauze, M., Latour, D., 2019. Akinetes and ancient DNA reveal toxic cyanobacterial recurrences and their potential for resurrection in a 6700-year-old core from a eutrophic lake. Science of the Total Environment 687, 1369–1380.

Lehmann, A., Myrberg, K., Post, P., Chubarenko, I., Dailidiene, I., Hinrichsen, H.-H., Hüssy, K., Liblik, T., Meier, H.E.M., Lips, U., Bukanova, T., 2022. Salinity dynamics of the Baltic Sea. Earth System Dynamics 13, 373–392. https://doi.org/10.5194/esd-13-373-2022

Leino, M.W., Edqvist, J., 2010. Germination of 151-year old Acacia spp. seeds. Genetic Resources and Crop Evolution 57, 741–746.

Lennon, J.T., den Hollander, F., Wilke-Berenguer, M., Blath, J., 2021. Principles of seed banks and the emergence of complexity from dormancy. Nature Communications 12, 4807.

Leppäranta, M., 2023. History and Future of Snow and Sea Ice in the Baltic Sea, in: Oxford Research Encyclopedia of Climate Science. https://doi.org/10.1093/acrefore/9780190228620.013.891

Lewis, S.L., Maslin, M.A., 2015. Defining the anthropocene. Nature 519, 171–180.

Li, H., Durbin, R., 2009. Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics 25, 1754–1760. https://doi.org/10.1093/bioinformatics/btp324
Li, H., Zhang, H., Chang, F., Liu, Q., Zhang, Y., Liu, F., Zhang, X., 2023. Sedimentary DNA for tracking the long-term changes in biodiversity. Environ Sci Pollut Res 30, 17039–17050. https://doi.org/10.1007/s11356-023-25130-5
Lin, M., Qiao, J., Hou, X., Dellwig, O., Steier, P., Hain, K., Golser, R., Zhu, L., 2021. 70-Year Anthropogenic Uranium Imprints of Nuclear Activities in Baltic Sea Sediments. Environ. Sci. Technol. 55, 8918–8927. https://doi.org/10.1021/acs.est.1c02136
Listmann, L., Kerl, F., Martens, N., Schaum, C.-E., 2021. Differences in Carbon Acquisition Could Explain Adaptive Responses in a Baltic Sea Pico-Phytoplankton. Front. Mar. Sci. 8, 740763. https://doi.org/10.3389/fmars.2021.740763
Litchman, E., Edwards, K.F., Klausmeier, C.A., Thomas, M.K., 2012. Phytoplankton niches, traits and eco-evolutionary responses to global environmental change. Marine Ecology Progress Series 470, 235–248.
Logares, R., Rengefors, K., Kremp, A., Shalchian-Tabrizi, K., Boltovskoy, A., Tengs, T., Shurtleff, A., Klaveness, D., 2007. Phenotypically Different Microalgal Morphospecies with Identical Ribosomal DNA: A Case of Rapid Adaptive Evolution? Microb Ecol 53, 549–561. https://doi.org/10.1007/s00248-006-9088-y
Lundholm, N., Ribeiro, S., Andersen, T.J., Koch, T., Godhe, A., Ekelund, F., Ellegaard, M., 2011. Buried alive – germination of up to a century-old marine protist resting stages. Phycologia 50, 629–640. https://doi.org/10.2216/11-16.1
Machado, K.B., Bini, L.M., Melo, A.S., Andrade, A.T.D., Almeida, M.F.D., Carvalho, P., Teresa, F.B., Roque, F.D.O., Bortolini, J.C., Padial, A.A., Vieira, L.C.G., Dala-Corte, R.B., Siqueira, T., Juen, L., Dias, M.S., Gama Júnior, W.A., Martins, R.T., Nabout, J.C., 2023. Functional and taxonomic diversities are better early indicators of eutrophication than composition of freshwater phytoplankton. Hydrobiologia 850, 1393–1411. https://doi.org/10.1007/s10750-022-04954-1
Mack, L., Attila, J., Aylagas, E., Beermann, A., Borja, A., Hering, D., Kahlert, M., Leese, F., Lenz, R., Lehtiniemi, M., Liess, A., Lips, U., Mattila, O.-P., Meissner, K., Pyhälahti, T., Setälä, O., Strehse, J.S., Uusitalo, L., Willstrand Wranne, A., Birk, S., 2020. A Synthesis of Marine Monitoring Methods With the Potential to Enhance the Status Assessment of the Baltic Sea. Frontiers in Marine Science 7.
Mägi, M., 2018. In Austrvegr: The Role of the Eastern Baltic in Viking Age Communication across the Baltic Sea. Brill; Leiden., The Northern World.
Malone, T.C., Newton, A., 2020. The Globalization of Cultural Eutrophication in the Coastal Ocean: Causes and Consequences. Frontiers in Marine Science 7.
Manni, M., Berkeley, M.R., Seppey, M., Simao, F.A., Zdobnov, E.M., 2021. BUSCO update: novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. https://doi.org/10.48550/ARXIV.2106.11799
Massicotte, P., South, A., 2024. rnaturalearth: world map data from natural earth. R package version 1.0. 1.9000.
McClenachan, L., Rick, T., Thurstan, R., Trant, A., Alagona, P., Alleway, H., Armstrong, C., Bliege Bird, R., Rubio-Cisneros, N., Clavero, M., Colonese, A., Cramer, K., Davis, A., Drew, J., Early-Capistrán, M., Gil-Romera, G., Grace, M., Hatch, M., Higgs, E., Hoffman, K., Jackson, J., Jerardino, A., LeFebvre, M., Lotze, H., Mohammed, R., Morueta-Holme, N., Munteanu, C., Mychajliw, A., Newsom, B., O'Dea, A., Pauly, D., Szabó, P., Torres, J., Waldman, J., West, C., Xu, L., Yasuoka, H., Zu Ermgassen, P., Van Houtan, K., 2024. Global research priorities for historical ecology to inform conservation. Endang. Species. Res. 54, 285–310. https://doi.org/10.3354/esr01338

McInnes, A.S., Laczka, O.F., Baker, K.G., Larsson, M.E., Robinson, C.M., Clark, J.S., Laiolo, L., Alvarez, M., Laverock, B., Kremer, C.T., van Sebille, E., Doblin, M.A., 2019. Live cell analysis at sea reveals divergent thermal performance between photosynthetic ocean microbial eukaryote populations. ISME Journal 13, 1374–1378. https://doi.org/10.1038/s41396-019-0355-6

McLauchlan, K.K., Williams, J.J., Craine, J.M., Jeffers, E.S., 2013. Changes in global nitrogen cycling during the Holocene epoch. Nature 495, 352–355.

McNeill, J.R., Engelke, P., 2014. The Great Acceleration: An Environmental History of the Anthropocene since 1945.

McQuoid, M.R., Godhe, A., Nordberg, K., 2002. Viability of phytoplankton resting stages in the sediments of a coastal Swedish fjord. European Journal of Phycology 37, 191–201. https://doi.org/10.1017/S0967026202003670

Medwed, C., Karsten, U., Romahn, J., Kaiser, J., Dellwig, O., Arz, H., Kremp, A., 2024. Archives of cyanobacterial traits: insights from resurrected Nodularia spumigena from Baltic Sea sediments reveal a shift in temperature optima. ISME Communications ycae140.

Mejbel, H.S., Dodsworth, W., Baud, A., Gregory-Eaves, I., Pick, F.R., 2021. Comparing Quantitative Methods for Analyzing Sediment DNA Records of Cyanobacteria in Experimental and Reference Lakes. Front. Microbiol. 12, 669910. https://doi.org/10.3389/fmicb.2021.669910

Mejbel, H.S., Dodsworth, W., Pick, F.R., 2022. Effects of temperature and oxygen on cyanobacterial DNA preservation in sediments: A comparison study of major taxa. Environmental DNA 4, 717–731. https://doi.org/10.1002/edn3.289

Mena, C., Long, M., Lorand, O., Malestroit, P., Rabiller, E., Maguer, J.-F., L'helguen, S., Regaudie De Gioux, A., 2024. Impact of light and nutrient availability on the phagotrophic activity of harmful bloom-forming dinoflagellates. Journal of Plankton Research fbae038. https://doi.org/10.1093/plankt/fbae038

Mestre, M., Höfer, J., 2021. The microbial conveyor belt: connecting the globe through dispersion and dormancy. Trends in Microbiology 29, 482–492.

Meyers, P.A., 2003. Applications of organic geochemistry to paleolimnological reconstructions: a summary of examples from the Laurentian Great Lakes. Organic geochemistry 34, 261–289.

Micheli, F., Halpern, B.S., Walbridge, S., Ciriaco, S., Ferretti, F., Fraschetti, S., Lewison, R., Nykjaer, L., Rosenberg, A.A., 2013. Cumulative Human Impacts on Mediterranean and Black Sea Marine Ecosystems: Assessing Current Pressures and Opportunities. PLoS ONE 8, e79889. https://doi.org/10.1371/journal.pone.0079889

Mistry, J., Chuguransky, S., Williams, L., Qureshi, M., Salazar, G.A., Sonnhammer, E.L.L., Tosatto, S.C.E., Paladin, L., Raj, S., Richardson, L.J., Finn, R.D., Bateman, A., 2021. Pfam: The protein families database in 2021. Nucleic Acids Research 49, D412–D419. https://doi.org/10.1093/nar/gkaa913

Moros, M., Andersen, T.J., Schulz-Bull, D., Häusler, K., Bunke, D., Snowball, I., Kotilainen, A., Zillén, L., Jensen, J.B., Kabel, K., Hand, I., Leipe, T., Lougheed, B.C., Wagner, B., Arz, H.W., 2017. Towards an event stratigraphy for Baltic Sea sediments deposited since AD 1900: approaches and challenges. Boreas 46, 129–142. https://doi.org/10.1111/bor.12193

Moros, M., Kotilainen, A.T., Snowball, I., Neumann, T., Perner, K., Meier, H.E.M., Leipe, T., Zillén, L., Sinninghe Damsté, J.S., Schneider, R., 2020. Is 'deep-water formation' in the Baltic Sea a key to understanding seabed dynamics and ventilation changes over the past 7,000 years? Quaternary International 550, 55–65. https://doi.org/10.1016/j.quaint.2020.03.031

Moullec, F., Asselot, R., Auch, D., Blöcker, A.M., Börner, G., Färber, L., Ofelio, C., Petzold, J., Santelia, M.E., Schwermer, H., Sguotti, C., Steidle, L., Tams, V., Pellerin, F., 2021.

Identifying and addressing the anthropogenic drivers of global change in the North Sea: a systematic map protocol. Environmental Evidence 10, 19. https://doi.org/10.1186/s13750-021-00234-y
Murchie, E.H., Lawson, T., 2013. Chlorophyll fluorescence analysis: A guide to good practice and understanding some new applications. Journal of Experimental Botany 64, 3983–3998. https://doi.org/10.1093/jxb/ert208
Murchie, T.J., Kuch, M., Duggan, A.T., Ledger, M.L., Roche, K., Klunk, J., Karpinski, E., Hackenberger, D., Sadoway, T., MacPhee, R., 2021. Optimizing extraction and targeted capture of ancient environmental DNA for reconstructing past environments using the PalaeoChip Arctic-1.0 bait-set. Quaternary Research 99, 305–328.
Murray, C.J., Müller-Karulis, B., Carstensen, J., Conley, D.J., Gustafsson, B.G., Andersen, J.H., 2019. Past, Present and Future Eutrophication Status of the Baltic Sea. Frontiers in Marine Science 6.
Musielak, M.M., Karp-Boss, L., Jumars, P.A., Fauci, L.J., 2009. Nutrient transport and acquisition by diatom chains in a moving fluid. Journal of Fluid Mechanics 638, 401–421.
Myrberg, K., Soomere, T., 2013. The Gulf of Finland, Its Hydrography and Circulation Dynamics, in: Soomere, T., Quak, E. (Eds.), Preventive Methods for Coastal Protection: Towards the Use of Ocean Dynamics for Pollution Control. Springer International Publishing, Heidelberg, pp. 181–222. https://doi.org/10.1007/978-3-319-00440-2_6
Nakov, T., Beaulieu, J.M., Alverson, A.J., 2019. Diatoms diversify and turn over faster in freshwater than marine environments*. Evolution 73, 2497–2511. https://doi.org/10.1111/evo.13832
Nakweya, G., 2023. Southern Ocean phytoplankton changes affect global climate regulation. Nat Africa d44148-023-00242–9. https://doi.org/10.1038/d44148-023-00242-9
Negrín Dastis, J.O., Milne, R., Guichard, F., Derry, A.M., 2019. Phenotype–environment mismatch in metapopulations—Implications for the maintenance of maladaptation at the regional scale. Evolutionary Applications 12, 1475–1486. https://doi.org/10.1111/eva.12833
Nguyen, N.-L., Devendra, D., Szymańska, N., Greco, M., Angeles, I.B., Weiner, A.K.M., Ray, J.L., Cordier, T., De Schepper, S., Pawłowski, J., Pawłowska, J., 2023. Sedimentary ancient DNA: a new paleogenomic tool for reconstructing the history of marine ecosystems. Front. Mar. Sci. 10, 1185435. https://doi.org/10.3389/fmars.2023.1185435
Niedźwiedź, T., Glaser, R., Hansson, D., Helama, S., Klimenko, V., Łupikasza, E., Małarzewski, Ł., Nordli, Ø., Przybylak, R., Riemann, D., Solomina, O., 2015. The Historical Time Frame (Past 1000 Years), in: The Bacc Ii Author Team (Ed.), Second Assessment of Climate Change for the Baltic Sea Basin, Regional Climate Studies. Springer International Publishing, Cham, pp. 51–65. https://doi.org/10.1007/978-3-319-16006-1_3
NRCM, Bio-Rad, 2020. A quality control revolution for droplet digital PCR. Nature.
Obst, M., Exter, K., Allcock, A.L., Arvanitidis, C., Axberg, A., Bustamante, M., Cancio, I., Carreira-Flores, D., Chatzinikolaou, E., Chatzigeorgiou, G., Chrismas, N., Clark, M.S., Comtet, T., Dailianis, T., Davies, N., Deneudt, K., De Cerio, O.D., Fortič, A., Gerovasileiou, V., Hablützel, P.I., Keklikoglou, K., Kotoulas, G., Lasota, R., Leite, B.R., Loisel, S., Lévêque, L., Levy, L., Malachowicz, M., Mavrič, B., Meyer, C., Mortelmans, J., Norkko, J., Pade, N., Power, A.M., Ramšak, A., Reiss, H., Solbakken, J., Staehr, P.A., Sundberg, P., Thyrring, J., Troncoso, J.S., Viard, F., Wenne, R., Yperifanou, E.I., Zbawicka, M., Pavloudi, C., 2020. A Marine Biodiversity Observation Network for Genetic Monitoring of Hard-Bottom Communities (ARMS-MBON). Front. Mar. Sci. 7, 572680. https://doi.org/10.3389/fmars.2020.572680
Ocean economy and developing countries - OECD [WWW Document], 2023. URL https://www.oecd.org/ocean/topics/developing-countries-and-the-ocean-economy/ (accessed 1.16.24).

Ojaveer, H., Jaanus, A., MacKenzie, B.R., Martin, G., Olenin, S., Radziejewska, T., Telesh, I., Zettler, M.L., Zaiko, A., 2010. Status of Biodiversity in the Baltic Sea. PLoS ONE 5, e12467. https://doi.org/10.1371/journal.pone.0012467

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., 2020. vegan: Community Ecology Package. R package version 2.5-6. 2019. Community Ecol 8, 732–740.

Olofsson, M., Almén, A.-K., Jaatinen, K., Scheinin, M., 2022. Temporal escape–adaptation to eutrophication by *Skeletonema marinoi*. FEMS Microbiology Letters 369, fnac011. https://doi.org/10.1093/femsle/fnac011

Ooms, J., cre, details, J.M. (Author of libxlsxwriter (see A. and C. files for details)) writexl author, 2023. writexl: Export Data Frames to Excel "xlsx" Format.

Orsi, W.D., Coolen, M.J.L., Wuchter, C., He, L., More, K.D., Irigoien, X., Chust, G., Johnson, C., Hemingway, J.D., Lee, M., Galy, V., Giosan, L., 2017. Climate oscillations reflected within the microbiome of Arabian Sea sediments. Sci Rep 7, 6040. https://doi.org/10.1038/s41598-017-05590-9

Otero, J., Álvarez-Salgado, X.A., Bode, A., 2020. Phytoplankton Diversity Effect on Ecosystem Functioning in a Coastal Upwelling System. Front. Mar. Sci. 7. https://doi.org/10.3389/fmars.2020.592255

Özdoğan, K.T., Gelabert, P., Hammers, N., Altınışık, N.E., de Groot, A., Plets, G., 2024. Archaeology meets environmental genomics: implementing sedaDNA in the study of the human past. Archaeol Anthropol Sci 16, 108. https://doi.org/10.1007/s12520-024-01999-2

Pandion, K., Arunachalam, K.D., Ayyamperumal, R., Chang, S.W., Chung, W.J., Rajagopal, R., Kalavathi, F., Iwai, C.B., Gayathiri, E., Ravindran, B., 2022. Environmental and anthropogenic impact on conservation and sustainability of marine fish diversity. Environ Sci Pollut Res. https://doi.org/10.1007/s11356-022-21260-4

Panja, P., Kar, T., Jana, D.K., 2024. Impacts of global warming on phytoplankton–zooplankton dynamics: a modelling study. Environ Dev Sustain. https://doi.org/10.1007/s10668-023-04430-3

Pawlowski, J., Apothéloz-Perret-Gentil, L., Altermatt, F., 2020. Environmental DNA: What's behind the term? Clarifying the terminology and recommendations for its future use in biomonitoring. Molecular Ecology 29, 4258–4264. https://doi.org/10.1111/mec.15643

Pedersen, M.W., Overballe-Petersen, S., Ermini, L., Sarkissian, C.D., Haile, J., Hellstrom, M., Spens, J., Thomsen, P.F., Bohmann, K., Cappellini, E., Schnell, I.B., Wales, N.A., Carøe, C., Campos, P.F., Schmidt, A.M.Z., Gilbert, M.T.P., Hansen, A.J., Orlando, L., Willerslev, E., 2015. Ancient and modern environmental DNA. Phil. Trans. R. Soc. B 370, 20130383. https://doi.org/10.1098/rstb.2013.0383

Pedersen, M.W., Ruter, A., Schweger, C., Friebe, H., Staff, R.A., Kjeldsen, K.K., Mendoza, M.L.Z., Beaudoin, A.B., Zutter, C., Larsen, N.K., Potter, B.A., Nielsen, R., Rainville, R.A., Orlando, L., Meltzer, D.J., Kjær, K.H., Willerslev, E., 2016. Postglacial viability and colonization in North America's ice-free corridor. Nature 537, 45–49. https://doi.org/10.1038/nature19085

Pham, B., McConnaughay, K., 2013. Environmental Selection and Convergent Evolution of Plant Phenotypes, in: Monson, R.K. (Ed.), Ecology and the Environment. Springer New York, New York, NY, pp. 1–20. https://doi.org/10.1007/978-1-4614-7612-2_16-1

Ponomarenko, E., Pugacheva, T., Kuleshova, L., 2024. Palaeoecological Conditions in the South-Eastern and Western Baltic Sea during the Last Millennium. Quaternary 7, 44. https://doi.org/10.3390/quat7040044

Prelle, L.R., Graiff, A., Gründling-Pfaff, S., Sommer, V., Kuriyama, K., Karsten, U., 2019. Photosynthesis and respiration of Baltic Sea benthic diatoms to changing environmental

conditions and growth responses of selected species as affected by an adjacent peatland (Hütelmoor). Frontiers in microbiology 10, 1500.
Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., De Bakker, P.I., Daly, M.J., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. The American journal of human genetics 81, 559–575.
Rabalais, N.N., Cai, W.-J., Carstensen, J., Conley, D.J., Fry, B., Hu, X., Quinones-Rivera, Z., Rosenberg, R., Slomp, C.P., Turner, R.E., 2014. Eutrophication-driven deoxygenation in the coastal ocean. Oceanography 27, 172–183.
Rački, N., Dreo, T., Gutierrez-Aguirre, I., Blejec, A., Ravnikar, M., 2014. Reverse transcriptase droplet digital PCR shows high resilience to PCR inhibitors from plant, soil and water samples. Plant Methods 10, 42. https://doi.org/10.1186/s13007-014-0042-6
Rapport, D.J., 1989. What constitutes ecosystem health? Perspectives in biology and medicine 33, 120–132.
Reckermann, M., Omstedt, A., Soomere, T., Aigars, J., Akhtar, N., Bełdowska, M., Bełdowski, J., Cronin, T., Czub, M., Eero, M., Hyytiäinen, K.P., Jalkanen, J.-P., Kiessling, A., Kjellström, E., Kuliński, K., Larsén, X.G., McCrackin, M., Meier, H.E.M., Oberbeckmann, S., Parnell, K., Pons-Seres De Brauwer, C., Poska, A., Saarinen, J., Szymczycha, B., Undeman, E., Wörman, A., Zorita, E., 2022. Human impacts and their interactions in the Baltic Sea region. Earth Syst. Dynam. 13, 1–80. https://doi.org/10.5194/esd-13-1-2022
Rengefors, K., Karlsson, I., Hansson, L., 1998. Algal cyst dormancy: a temporal escape from herbivory. Proceedings of the Royal Society of London. Series B: Biological Sciences 265, 1353–1358. https://doi.org/10.1098/rspb.1998.0441
Rengefors, K., Kremp, A., Reusch, T.B.H., Wood, A.M., 2017. Genetic diversity and evolution in eukaryotic phytoplankton: revelations from population genetic studies. J. Plankton Res. plankt;fbw098v1. https://doi.org/10.1093/plankt/fbw098
Reusch, T.B.H., Boyd, P.W., 2013. Experimental Evolution meets Marine Phytoplankton. Evolution 67, 1849–1859. https://doi.org/10.1111/evo.12035
Reusch, T.B.H., Dierking, J., Andersson, H.C., Bonsdorff, E., Carstensen, J., Casini, M., Czajkowski, M., Hasler, B., Hinsby, K., Hyytiäinen, K., Johannesson, K., Jomaa, S., Jormalainen, V., Kuosa, H., Kurland, S., Laikre, L., MacKenzie, B.R., Margonski, P., Melzner, F., Oesterwind, D., Ojaveer, H., Refsgaard, J.C., Sandström, A., Schwarz, G., Tonderski, K., Winder, M., Zandersen, M., 2018. The Baltic Sea as a time machine for the future coastal ocean. Sci. Adv. 4, eaar8195. https://doi.org/10.1126/sciadv.aar8195
Ribeiro, S., Berge, T., Lundholm, N., Andersen, T.J., Abrantes, F., Ellegaard, M., 2011. Phytoplankton growth after a century of dormancy illuminates past resilience to catastrophic darkness. Nature communications 2, 311.
Ribeiro, S., Berge, T., Lundholm, N., Ellegaard, M., 2013. Hundred years of environmental change and phytoplankton ecophysiological variability archived in coastal sediments. PLoS One 8, e61184.
Rillig, M.C., Van Der Heijden, M.G.A., Berdugo, M., Liu, Y.-R., Riedo, J., Sanz-Lazaro, C., Moreno-Jiménez, E., Romero, F., Tedersoo, L., Delgado-Baquerizo, M., 2023. Increasing the number of stressors reduces soil ecosystem services worldwide. Nat. Clim. Chang. 13, 478–483. https://doi.org/10.1038/s41558-023-01627-2
Rosentau, A., Bennike, O., Uścinowicz, S., Miotk-Szpiganowicz, G., 2017. The Baltic Sea Basin, in: Flemming, N.C., Harff, J., Moura, D., Burgess, A., Bailey, G.N. (Eds.), Submerged Landscapes of the European Continental Shelf. Wiley, pp. 103–133. https://doi.org/10.1002/9781118927823.ch5
Rynearson, T.A., Bishop, I.W., Collins, S., 2022. The Population Genetics and Evolutionary Potential of Diatoms, in: Falciatore, A., Mock, T. (Eds.), The Molecular Life of Diatoms.

Springer International Publishing, Cham, pp. 29–57. https://doi.org/10.1007/978-3-030-92499-7_2
Rytkönen, J., Siitonen, L., Riipi, T., Sassi, J., Sukselainen, J., 2002. Statistical analyses of the Baltic maritime traffic, VTT Research Report. VTT Technical Research Centre of Finland.
Salimi, P.A., Creed, J.C., Esch, M.M., Fenner, D., Jaafar, Z., Levesque, J.C., Montgomery, A.D., Alidoost Salimi, M., Edward, J.K.P., Raj, K.D., Sweet, M., 2021. A review of the diversity and impact of invasive non-native species in tropical marine ecosystems. Mar Biodivers Rec 14, 11. https://doi.org/10.1186/s41200-021-00206-8
Sallon, S., Cherif, E., Chabrillange, N., Solowey, E., Gros-Balthazard, M., Ivorra, S., Terral, J.-F., Egli, M., Aberlenc, F., 2020. Origins and insights into the historic Judean date palm based on genetic analysis of germinated ancient seeds and morphometric studies. Sci. Adv. 6, eaax0384. https://doi.org/10.1126/sciadv.aax0384
Sallon, S., Solowey, E., Cohen, Y., Korchinsky, R., Egli, M., Woodhatch, I., Simchoni, O., Kislev, M., 2008. Germination, Genetics, and Growth of an Ancient Date Seed. Science 320, 1464–1464. https://doi.org/10.1126/science.1153600
Salmaso, N., Tolotti, M., 2021. Phytoplankton and anthropogenic changes in pelagic environments. Hydrobiologia 848, 251–284. https://doi.org/10.1007/s10750-020-04323-w
Samant, B., 2024. Precambrian Microfossils: Indicators of Early Life and Environments on the Earth, in: Samant, B., Thakre, D. (Eds.), Applications of Palynology in Stratigraphy and Climate Studies, Society of Earth Scientists Series. Springer International Publishing, Cham, pp. 1–12. https://doi.org/10.1007/978-3-031-51877-5_1
Sanyal, A., Larsson, J., Van Wirdum, F., Andrén, T., Moros, M., Lönn, M., Andrén, E., 2022. Not dead yet: Diatom resting spores can survive in nature for several millennia. American J of Botany 109, 67–82. https://doi.org/10.1002/ajb2.1780
Saravanan, V., Godhe, A., 2010. Genetic heterogeneity and physiological variation among seasonally separated clones of *Skeletonema marinoi* (Bacillariophyceae) in the Gullmar Fjord, Sweden. European Journal of Phycology 45, 177–190. https://doi.org/10.1080/09670260903445146
Sarno, D., Kooistra, W.H.C.F., Medlin, L.K., Percopo, I., Zingone, A., 2005. Diversity in the Genus Skeletonema (bacillariophyceae). Ii. an Assessment of the Taxonomy of S. Costatum-Like Species with the Description of Four New Species1. Journal of Phycology 41, 151–176. https://doi.org/10.1111/j.1529-8817.2005.04067.x
Saunders, K.M., Taffs, K.H., 2009. Palaeoecology: A tool to improve the management of Australian estuaries. Journal of Environmental Management 90, 2730–2736. https://doi.org/10.1016/j.jenvman.2009.03.001
Savchuk, O.P., 2018. Large-Scale Nutrient Dynamics in the Baltic Sea, 1970–2016. Front. Mar. Sci. 5, 95. https://doi.org/10.3389/fmars.2018.00095
Schaum, C.-E., Rost, B., Collins, S., 2016. Environmental stability affects phenotypic evolution in a globally distributed marine picoplankton. ISME J 10, 75–84. https://doi.org/10.1038/ismej.2015.102
Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. Appl Environ Microbiol 75, 7537–7541. https://doi.org/10.1128/AEM.01541-09
Schmidt, A., Romahn, J., Andrén, E., Kremp, A., Kaiser, J., Arz, H.W., Dellwig, O., Bálint, M., Epp, L.S., 2024. Decoding the Baltic Sea's past and present: A simple molecular index for ecosystem assessment. Ecological Indicators 166, 112494. https://doi.org/10.1016/j.ecolind.2024.112494

Schouten, S., Hopmans, E.C., Sinninghe Damsté, J.S., 2013. The organic geochemistry of glycerol dialkyl glycerol tetraether lipids: A review. Organic Geochemistry 54, 19–61. https://doi.org/10.1016/j.orggeochem.2012.09.006
Schulte, L., Bernhardt, N., Stoof-Leichsenring, K., Zimmermann, H.H., Pestryakova, L.A., Epp, L.S., Herzschuh, U., 2021. Hybridization capture of larch (Larix Mill.) chloroplast genomes from sedimentary ancient DNA reveals past changes of Siberian forest. Mol Ecol Resour 21, 801–815. https://doi.org/10.1111/1755-0998.13311
Seeber, P.A., Batke, L., Dvornikov, Y., Schmidt, A., Wang, Y., Stoof-Leichsenring, K., Moon, K., Vohr, S.H., Shapiro, B., Epp, L.S., 2024. Mitochondrial genomes of Pleistocene megafauna retrieved from recent sediment layers of two Siberian lakes. eLife 12, RP89992. https://doi.org/10.7554/eLife.89992
Seppä, H., Bjune, A.E., Telford, R.J., Birks, H.J.B., Veski, S., 2009. Last nine-thousand years of temperature variability in Northern Europe. Clim. Past 5, 523–535. https://doi.org/10.5194/cp-5-523-2009
Seppä, H., Hammarlund, D., Antonsson, K., 2005. Low-frequency and high-frequency changes in temperature and effective humidity during the Holocene in south-central Sweden: implications for atmospheric and oceanic forcings of climate. Clim Dyn 25, 285–297. https://doi.org/10.1007/s00382-005-0024-5
Sexton, J.P., Hangartner, S.B., Hoffmann, A.A., 2014. Genetic isolation by environment or distance: which pattern of gene flow is most common? Evolution 68, 1–15.
Sharma, A., Kumar, V., Shahzad, B., Ramakrishnan, M., Singh Sidhu, G.P., Bali, A.S., Handa, N., Kapoor, D., Yadav, P., Khanna, K., 2020. Photosynthetic response of plants under different abiotic stresses: a review. Journal of Plant Growth Regulation 39, 509–531.
Shen, C., Testa, J.M., Li, M., Cai, W., 2020. Understanding Anthropogenic Impacts on pH and Aragonite Saturation State in Chesapeake Bay: Insights From a 30-Year Model Study. JGR Biogeosciences 125, e2019JG005620. https://doi.org/10.1029/2019JG005620
Shen-Miller, J., Mudgett, M.B., Schopf, J.W., Clarke, S., Berger, R., 1995. Exceptional seed longevity and robust growth: ancient Sacred Lotus from China. American J of Botany 82, 1367–1380. https://doi.org/10.1002/j.1537-2197.1995.tb12673.x
Siano, R., Lassudrie, M., Cuzin, P., Briant, N., Loizeau, V., Schmidt, S., Ehrhold, A., Mertens, K.N., Lambert, C., Quintric, L., Noël, C., Latimier, M., Quéré, J., Durand, P., Penaud, A., 2021. Sediment archives reveal irreversible shifts in plankton communities after World War II and agricultural pollution. Current Biology 31, 2682-2689.e7. https://doi.org/10.1016/j.cub.2021.03.079
Siauciunaite, R., Foulkes, N.S., Calabrò, V., Vallone, D., 2019. Evolution Shapes the Gene Expression Response to Oxidative Stress. IJMS 20, 3040. https://doi.org/10.3390/ijms20123040
Sjöqvist, C., 2022. Evolution of Phytoplankton as Estimated from Genetic Diversity. JMSE 10, 456. https://doi.org/10.3390/jmse10040456
Sjöqvist, C.O., Kremp, A., 2016. Genetic diversity affects ecological performance and stress response of marine diatom populations. The ISME Journal 10, 2755–2766. https://doi.org/10.1038/ismej.2016.44
Skeffington, A.W., Gentzel, M., Ohara, A., Milentyev, A., Heintze, C., Böttcher, L., Görlich, S., Shevchenko, A., Poulsen, N., Kröger, N., 2022. Shedding light on silica biomineralization by comparative analysis of the silica-associated proteomes from three diatom species. The Plant Journal 110, 1700–1716. https://doi.org/10.1111/tpj.15765
Slatkin, M., 1987. Gene Flow and the Geographic Structure of Natural Populations. Science 236, 787–792. https://doi.org/10.1126/science.3576198

Smol, J.P., 2010. The power of the past: using sediments to track the effects of multiple stressors on lake ecosystems. Freshwater Biology 55, 43–59. https://doi.org/10.1111/j.1365-2427.2009.02373.x
Smol, J.P., 2009. Pollution of lakes and rivers: a paleoenvironmental perspective. John Wiley & Sons.
Smol, J.P., Stoermer, E.F., 2010. The diatoms: applications for the environmental and earth sciences. Cambridge University Press.
Snoeijs-Leijonmalm, P., Andrén, E., 2017. Why is the Baltic Sea so special to live in?, in: Snoeijs-Leijonmalm, P., Schubert, H., Radziejewska, T. (Eds.), Biological Oceanography of the Baltic Sea. Springer Netherlands, Dordrecht, pp. 23–84. https://doi.org/10.1007/978-94-007-0668-2_2
Snoeijs-Leijonmalm, P., Schubert, H., Radziejewska, T. (Eds.), 2017. Biological oceanography of the Baltic Sea. Springer, Dordrecht.
South, A., Michael, S., Massicotte, P., 2024. rnaturalearthdata: World Vector Map Data from Natural Earth Used in "rnaturalearth."
Souza Araujo, G., Pacheco, D., Cotas, J., William Alves Da Silva, J., Saboya, J., Teixeira Moreira, R., Pereira, L., 2022. Plankton: Environmental and Economic Importance for a Sustainable Future, in: Pereira, L., Marta Gonçalves, A. (Eds.), Plankton Communities. IntechOpen. https://doi.org/10.5772/intechopen.100433
Spear, S.F., Groves, J.D., Williams, L.A., Waits, L.P., 2015. Using environmental DNA methods to improve detectability in a hellbender (Cryptobranchus alleganiensis) monitoring program. Biological Conservation, Special Issue: Environmental DNA: A powerful new tool for biological conservation 183, 38–45. https://doi.org/10.1016/j.biocon.2014.11.016
Spilling, K., Olli, K., Lehtoranta, J., Kremp, A., Tedesco, L., Tamelander, T., Klais, R., Peltonen, H., Tamminen, T., 2018. Shifting Diatom—Dinoflagellate Dominance During Spring Bloom in the Baltic Sea and its Potential Effects on Biogeochemical Cycling. Frontiers in Marine Science 5.
Stanke, M., Keller, O., Gunduz, I., Hayes, A., Waack, S., Morgenstern, B., 2006. AUGUSTUS: ab initio prediction of alternative transcripts. Nucleic acids research 34, W435–W439.
Steffen, W., Broadgate, W., Deutsch, L., Gaffney, O., Ludwig, C., 2015. The trajectory of the Anthropocene: The Great Acceleration. The Anthropocene Review 2, 81–98. https://doi.org/10.1177/2053019614564785
Steffen, W., Grinevald, J., Crutzen, P., McNeill, J., 2011. The Anthropocene: conceptual and historical perspectives. Phil. Trans. R. Soc. A. 369, 842–867. https://doi.org/10.1098/rsta.2010.0327
Steinberg, C.E.W., 2012. Environmental Stresses: Ecological Driving Force and Key Player in Evolution, in: Stress Ecology. Springer Netherlands, Dordrecht, pp. 369–386. https://doi.org/10.1007/978-94-007-2072-5_15
Stenow, R., Olofsson, M., Robertson, E.K., Kourtchenko, O., Whitehouse, M.J., Ploug, H., Godhe, A., 2020. Resting Stages of *Skeletonema marinoi* Assimilate Nitrogen From the Ambient Environment Under Dark, Anoxic Conditions. Journal of Phycology 56, 699–708. https://doi.org/10.1111/jpy.12975
Stenow, R., Robertson, E.K., Kourtchenko, O., Whitehouse, M.J., Pinder, M.I.M., Benvenuto, G., Töpel, M., Godhe, A., Ploug, H., 2024. Resting cells of *Skeletonema marinoi* assimilate organic compounds and respire by dissimilatory nitrate reduction to ammonium in dark, anoxic conditions. Environmental Microbiology 26, e16625. https://doi.org/10.1111/1462-2920.16625
Stockner, J.G., Lund, J.W.G., 1970. Live alage in postglacial lake depostits. Limnology & Oceanography 15, 41–58. https://doi.org/10.4319/lo.1970.15.1.0041

Storie, J., Suškevičs, M., Nevzati, F., Külvik, M., Kuhn, T., Burkhard, B., Vikström, S., Lehtoranta, V., Riikonen, S., Oinonen, S., 2021. Evidence on the impact of Baltic Sea ecosystems on human health and well-being: a systematic map. Environmental Evidence 10, 30. https://doi.org/10.1186/s13750-021-00244-w

Summons, R.E., Welander, P.V., Gold, D.A., 2022. Lipid biomarkers: molecular tools for illuminating the history of microbial life. Nature Reviews Microbiology 20, 174–185.

Sundqvist, L., Godhe, A., Jonsson, P.R., Sefbom, J., 2018. The anchoring effect—long-term dormancy and genetic population structure. ISME J 12, 2929–2941. https://doi.org/10.1038/s41396-018-0216-8

Taberlet, P., Bonin, A., Zinger, L., Coissac, E., 2018. Environmental DNA: for biodiversity research and monitoring. Oxford University Press, Oxford New York.

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., Willerslev, E., 2012. Towards next-generation biodiversity assessment using DNA metabarcoding. Molecular Ecology 21, 2045–2050. https://doi.org/10.1111/j.1365-294X.2012.05470.x

Taiyun, 2023. taiyun/corrplot.

Takahara, T., Minamoto, T., Yamanaka, H., Doi, H., Kawabata, Z., 2012. Estimation of Fish Biomass Using Environmental DNA. PLOS ONE 7, e35868. https://doi.org/10.1371/journal.pone.0035868

Thakur, N.K., Ojha, A., 2020. Complex plankton dynamics induced by adaptation and defense. Model. Earth Syst. Environ. 6, 907–916. https://doi.org/10.1007/s40808-020-00727-8

Thorpe, A.C., Mackay, E.B., Goodall, T., Bendle, J.A., Thackeray, S.J., Maberly, S.C., Read, D.S., 2024. Evaluating the use of lake sedimentary DNA in palaeolimnology: A comparison with long-term microscopy-based monitoring of the phytoplankton community. Molecular Ecology Resources 24, e13903. https://doi.org/10.1111/1755-0998.13903

Thyssen, M., Tarran, G.A., Zubkov, M.V., Holland, R.J., Gregori, G., Burkill, P.H., Denis, M., 2007. The emergence of automated high-frequency flow cytometry: revealing temporal and spatial phytoplankton variability. Journal of Plankton Research 30, 333–343. https://doi.org/10.1093/plankt/fbn005

Tockner, K., 2021. Freshwaters: Global Distribution, Biodiversity, Ecosystem Services, and Human Pressures, in: Bogardi, J.J., Gupta, J., Nandalal, K.D.W., Salamé, L., Van Nooijen, R.R.P., Kumar, N., Tingsanchali, T., Bhaduri, A., Kolechkina, A.G. (Eds.), Handbook of Water Resources Management: Discourses, Concepts and Examples. Springer International Publishing, Cham, pp. 489–501. https://doi.org/10.1007/978-3-030-60147-8_16

Tokarev, Y., Shulman, G., 2007. Biodiversity in the Black Sea: effects of climate and anthropogenic factors, in: Relini, G., Ryland, J. (Eds.), Biodiversity in Enclosed Seas and Artificial Marine Habitats, Developments in Hydrobiology. Springer Netherlands, Dordrecht, pp. 23–33. https://doi.org/10.1007/978-1-4020-6156-1_2

Torti, A., Lever, M.A., Jørgensen, B.B., 2015. Origin, dynamics, and implications of extracellular DNA pools in marine sediments. Marine genomics 24, 185–196.

Tréguer, P., Bowler, C., Moriceau, B., Dutkiewicz, S., Gehlen, M., Aumont, O., Bittner, L., Dugdale, R., Finkel, Z., Iudicone, D., 2018. Influence of diatom diversity on the ocean biological carbon pump. Nature Geoscience 11, 27–37.

Treitel, C., 2015. Artificial or Biological? Nature, Fertilizer, and the German Origins of Organic Agriculture, in: Phillips, D., Kingsland, S. (Eds.), New Perspectives on the History of Life Sciences and Agriculture, Archimedes. Springer International Publishing, Cham, pp. 183–203. https://doi.org/10.1007/978-3-319-12185-7_10

Trombetta, T., Vidussi, F., Mas, S., Parin, D., Simier, M., Mostajir, B., 2019. Water temperature drives phytoplankton blooms in coastal waters. PLOS ONE 14, e0214933. https://doi.org/10.1371/journal.pone.0214933

Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P.F., Bellemain, E., Besnard, A., Coissac, E., Boyer, F., Gaboriaud, C., Jean, P., Poulet, N., Roset, N., Copp, G.H., Geniez, P., Pont, D., Argillier, C., Baudoin, J., Peroux, T., Crivelli, A.J., Olivier, A., Acqueberge, M., Le Brun, M., Møller, P.R., Willerslev, E., Dejean, T., 2016. Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. Molecular Ecology 25, 929–942. https://doi.org/10.1111/mec.13428

Van De Putte, A.P., Griffiths, H.J., Brooks, C., Bricher, P., Sweetlove, M., Halfter, S., Raymond, B., 2021. From Data to Marine Ecosystem Assessments of the Southern Ocean: Achievements, Challenges, and Lessons for the Future. Front. Mar. Sci. 8, 637063. https://doi.org/10.3389/fmars.2021.637063

Van Oppen, M.J.H., Coleman, M.A., 2022. Advancing the protection of marine life through genomics. PLoS Biol 20, e3001801. https://doi.org/10.1371/journal.pbio.3001801

van Wirdum, F., Andrén, E., Wienholz, D., Kotthoff, U., Moros, M., Fanget, A.-S., Seidenkrantz, M.-S., Andrén, T., 2019. Middle to Late Holocene Variations in Salinity and Primary Productivity in the Central Baltic Sea: A Multiproxy Study From the Landsort Deep. Frontiers in Marine Science 6.

Vaulot, D., Geisen, S., Mahé, F., Bass, D., 2022. pr2-primers: An 18S rRNA primer database for protists. Molecular Ecology Resources 22, 168–179. https://doi.org/10.1111/1755-0998.13465

Vavrek, M.C., McCgraw, J.B., Bennington, C.C., 1991. Ecological genetic variation in seed banks. III. Phenotypic and genetic differences between young and old seed populations of Carex bigelowii. The Journal of Ecology 645–662.

Viitasalo, M., Bonsdorff, E., 2022. Global climate change and the Baltic Sea ecosystem: direct and indirect effects on species, communities and ecosystem functioning. Earth Syst. Dynam. 13, 711–747. https://doi.org/10.5194/esd-13-711-2022

Vitousek, P.M., Mooney, H.A., Lubchenco, J., Melillo, J.M., 1997. Human Domination of Earth's Ecosystems. Science 277, 494–499. https://doi.org/10.1126/science.277.5325.494

Volpe, C., Nymark, M., Andersen, T., Winge, P., Lavaud, J., Vadstein, O., 2024. *Skeletonema marinoi* ecotypes show specific habitat-related responses to fluctuating light supporting high potential for growth under photobioreactor light regime. New Phytologist 243, 145–161. https://doi.org/10.1111/nph.19788

Waga, H., Eicken, H., Hirawake, T., Fukamachi, Y., 2021. Variability in spring phytoplankton blooms associated with ice retreat timing in the Pacific Arctic from 2003–2019. PLoS ONE 16, e0261418. https://doi.org/10.1371/journal.pone.0261418

Wainman, L.M., Sathyanarayana, S.H., Lefferts, J.A., 2024. Applications of Digital Polymerase Chain Reaction (dPCR) in Molecular and Clinical Testing. The Journal of Applied Laboratory Medicine 9, 124–137. https://doi.org/10.1093/jalm/jfad103

Wakeham, S.G., Canuel, E.A., 2006. Degradation and Preservation of Organic Matter in Marine Sediments, in: Volkman, J.K. (Ed.), Marine Organic Matter: Biomarkers, Isotopes and DNA, The Handbook of Environmental Chemistry. Springer-Verlag, Berlin/Heidelberg, pp. 295–321. https://doi.org/10.1007/698_2_009

Waldvogel, A.-M., Wieser, A., Schell, T., Patel, S., Schmidt, H., Hankeln, T., Feldmeyer, B., Pfenninger, M., 2018. The genomic footprint of climate adaptation in Chironomus riparius. Molecular Ecology 27, 1439–1456. https://doi.org/10.1111/mec.14543

Walsby, A.E., 1997. Numerical integration of phytoplankton photosynthesis through time and depth in a water column. The New Phytologist 136, 189–209.

Wang, H., Liu, F., Wang, M., Bettarel, Y., Eissler, Y., Chen, F., Kan, J., 2024. Planktonic eukaryotes in the Chesapeake Bay: contrasting responses of abundant and rare taxa to estuarine gradients. Microbiol Spectr 12, e04048-23. https://doi.org/10.1128/spectrum.04048-23

Wang, Q., Cheng, F., Xue, J., Xiao, N., Wu, H., 2020. Bacterial community composition and diversity in the ballast water of container ships arriving at Yangshan Port, Shanghai, China. Mar Pollut Bull 160, 111640. https://doi.org/10.1016/j.marpolbul.2020.111640
Wang, X., Jiang, S., Wu, Y., Wang, Y., 2022. Synchronous Shifts in Nutrients and Organic Carbon Responses Over the Diatom-to-Dinoflagellate Succession. Front. Mar. Sci. 9, 845372. https://doi.org/10.3389/fmars.2022.845372
Wang, Y., Wessels, M., Pedersen, M.W., Epp, L.S., 2023. Spatial distribution of sedimentary DNA is taxon-specific and linked to local occurrence at intra-lake scale. Communications Earth & Environment 4, 172.
Wanner, H., Beer, J., Bütikofer, J., Crowley, T.J., Cubasch, U., Flückiger, J., Goosse, H., Grosjean, M., Joos, F., Kaplan, J.O., 2008. Mid-to Late Holocene climate change: an overview. Quaternary Science Reviews 27, 1791–1828.
Wanner, H., Mercolli, L., Grosjean, M., Ritz, S.P., 2015. Holocene climate variability and change; a data-based review. JGS 172, 254–263. https://doi.org/10.1144/jgs2013-101
Ward, D., Melbourne-Thomas, J., Pecl, G.T., Evans, K., Green, M., McCormack, P.C., Novaglio, C., Trebilco, R., Bax, N., Brasier, M.J., Cavan, E.L., Edgar, G., Hunt, H.L., Jansen, J., Jones, R., Lea, M.-A., Makomere, R., Mull, C., Semmens, J.M., Shaw, J., Tinch, D., van Steveninck, T.J., Layton, C., 2022. Safeguarding marine life: conservation of biodiversity and ecosystems. Rev Fish Biol Fisheries 32, 65–100. https://doi.org/10.1007/s11160-022-09700-3
Warden, L., Moros, M., Neumann, T., Shennan, S., Timpson, A., Manning, K., Sollai, M., Wacker, L., Perner, K., Häusler, K., 2017. Climate induced human demographic and cultural change in northern Europe during the mid-Holocene, Sci. Rep., 7, 15251.
Wasmund, N., 2017. The Diatom/Dinoflagellate Index as an Indicator of Ecosystem Changes in the Baltic Sea. 2. Historical Data for Use in Determination of Good Environmental Status. Front. Mar. Sci. 4, 153. https://doi.org/10.3389/fmars.2017.00153
Wasmund, N., Dutz, J., Pollehne, F., Siegel, H., Zettler, M.L., 2016. Biological assessment of the Baltic Sea 2015. Meereswiss. Ber 102.
Wasmund, N., Kownacka, J., Göbel, J., Jaanus, A., Johansen, M., Jurgensone, I., Lehtinen, S., Powilleit, M., 2017. The Diatom/Dinoflagellate Index as an Indicator of Ecosystem Changes in the Baltic Sea 1. Principle and Handling Instruction. Front. Mar. Sci. 4. https://doi.org/10.3389/fmars.2017.00022
Wasmund, N., Uhlig, S., 2003. Phytoplankton trends in the Baltic Sea. ICES Journal of Marine Science 60, 177–186. https://doi.org/10.1016/S1054-3139(02)00280-1
Weckström, K., Lewis, J.P., Andrén, E., Ellegaard, M., Rasmussen, P., Ryves, D.B., Telford, R., 2017. Palaeoenvironmental History of the Baltic Sea: One of the Largest Brackish-Water Ecosystems in the World, in: Weckström, K., Saunders, K.M., Gell, P.A., Skilbeck, C.G. (Eds.), Applications of Paleoenvironmental Techniques in Estuarine Studies. Springer Netherlands, Dordrecht, pp. 615–662. https://doi.org/10.1007/978-94-024-0990-1_24
Wickham, H., 2016. ggplot2: elegant graphics for data analysis, Second edition. ed, Use R! Springer, Cham.
Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., Yutani, H., 2019. Welcome to the Tidyverse. JOSS 4, 1686. https://doi.org/10.21105/joss.01686
Wickham, H., Bryan, J., Posit, attribution), P. (Copyright holder of all R. code and all C. code without explicit copyright, code), M.K. (Author of included R., code), K.V. (Author of included libxls, code), C.L. (Author of included libxls, code), B.C. (Author of included

libxls, code), D.H. (Author of included libxls, code), E.M. (Author of included libxls, 2023. readxl: Read Excel Files.
Wickham, H., Seidel, D., RStudio, 2022. scales: Scale Functions for Visualization.
Wilke, C.O., 2020. cowplot: Streamlined Plot Theme and Plot Annotations for "ggplot2."
Wingard, G.L., Bernhardt, C.E., Wachnicka, A.H., 2017. The Role of Paleoecology in Restoration and Resource Management—The Past As a Guide to Future Decision-Making: Review and Example from the Greater Everglades Ecosystem, U.S.A. Front. Ecol. Evol. 5. https://doi.org/10.3389/fevo.2017.00011
Wood, S.M., Kremp, A., Savela, H., Akter, S., Vartti, V.-P., Saarni, S., Suikkanen, S., 2021. Cyanobacterial akinete distribution, viability, and cyanotoxin records in sediment archives from the Northern Baltic Sea. Frontiers in Microbiology 12, 681881.
Wood, S.N., 2011. Fast Stable Restricted Maximum Likelihood and Marginal Likelihood Estimation of Semiparametric Generalized Linear Models. Journal of the Royal Statistical Society Series B: Statistical Methodology 73, 3–36. https://doi.org/10.1111/j.1467-9868.2010.00749.x
Worm, B., Barbier, E.B., Beaumont, N., Duffy, J.E., Folke, C., Halpern, B.S., Jackson, J.B.C., Lotze, H.K., Micheli, F., Palumbi, S.R., Sala, E., Selkoe, K.A., Stachowicz, J.J., Watson, R., 2006. Impacts of Biodiversity Loss on Ocean Ecosystem Services. Science 314, 787–790. https://doi.org/10.1126/science.1132294
Yu, G., 2023. ggimage: Use Image in "ggplot2."
Yu, J., Ran, Z., Zhang, J., Wei, L., Ma, W., 2022. Genome-Wide Insights Into the Organelle Translocation of Photosynthetic NDH-1 Genes During Evolution. Front. Microbiol. 13, 956578. https://doi.org/10.3389/fmicb.2022.956578
Yu, R.-S., Singh, S., 2023. Microplastic Pollution: Threats and Impacts on Global Marine Ecosystems. Sustainability 15, 13252. https://doi.org/10.3390/su151713252
Zalasiewicz, J., Williams, M., Smith, A., Barry, T.L., Coe, A.L., Bown, P.R., Brenchley, P., Cantrill, D., Gale, A., Gibbard, P., 2008. Are we now living in the Anthropocene? Gsa Today 18, 4.
Zhang, X., Yu, K., Li, M., Jiang, H., Gao, W., Zhao, J., Li, K., 2024. Diatom-dinoflagellate succession in the Bohai Sea: The role of N/P ratios and dissolved organic nitrogen components. Water Research 251, 121150. https://doi.org/10.1016/j.watres.2024.121150
Zhang, Y., Lin, X., Shi, X., Lin, L., Luo, H., Li, L., Lin, S., 2019. Metatranscriptomic Signatures Associated With Phytoplankton Regime Shift From Diatom Dominance to a Dinoflagellate Bloom. Frontiers in Microbiology 10.
Zhou, L., Wu, S., Gu, W., Wang, L., Wang, J., Gao, S., Wang, G., 2021. Photosynthesis acclimation under severely fluctuating light conditions allows faster growth of diatoms compared with dinoflagellates. BMC Plant Biol 21, 164. https://doi.org/10.1186/s12870-021-02902-0
Ziegler, M., Jilbert, T., De Lange, G.J., Lourens, L.J., Reichart, G., 2008. Bromine counts from XRF scanning as an estimate of the marine organic carbon content of sediment cores. Geochem Geophys Geosyst 9, 2007GC001932. https://doi.org/10.1029/2007GC001932
Zillén, L., Conley, D.J., Andrén, T., Andrén, E., Björck, S., 2008. Past occurrences of hypoxia in the Baltic Sea and the role of climate variability, environmental change and human impact. Earth-Science Reviews 91, 77–92. https://doi.org/10.1016/j.earscirev.2008.10.001
Zimmermann, H.H., Stoof-Leichsenring, K.R., Dinkel, V., Harms, L., Schulte, L., Hütt, M.-T., Nürnberg, D., Tiedemann, R., Herzschuh, U., 2023. Marine ecosystem shifts with deglacial sea-ice loss inferred from ancient DNA shotgun sequencing. Nat Commun 14, 1650. https://doi.org/10.1038/s41467-023-36845-x

“Living systems are never in equilibrium. They are inherently unstable. They may seem stable, but they’re not. Everything is moving and changing. In a sense, everything is on the edge of collapse.”

Ian Malcolm

(*Jurassic Park*)

Appendix 1 to § 6 General Provisions of Doctoral Regulations

Affirmation in Lieu of Oath

1. The doctoral thesis on the topic

Environmental Genomics

is entirely my own work.

2. ☒ I have used only the sources and aids indicated, and have not received assistance from unauthorized third parties. In particular, texts or ideas that were obtained from other works have been identified as such.
3. ☐ My work does not copy or paraphrase any text passages or other content generated by an AI tool.

   ☒ If I have used text-generating AI tools as **aids**, I understand that I am solely responsible for the accuracy of the content of the AI-generated text passages and other AI-generated content, as well as for referencing other people's wording and ideas in accordance with the principles of good scientific practice. I have used AI tools as described below.

I employed AI tools for grammatical and linguistic proofreading and to enhance my scripts. This resulted in improved script performance, reproducibility, and reliability. I made all final content and interpretation decisions. Written text and analyses were my original ideas. I used AI for cross-checking and proofing.

(If necessary, please use additional insertion sheets!)

4. ☒ The work or parts of the work ~~have already been~~/have never been [1] submitted to an institute of higher education in Germany or abroad as part of an examination or qualification procedure.

Title of the work:

Unravelling the Past and Present: Marine Phytoplankton Population Dynamics in the face of the Anthropocene

Institution of higher education; year:

Universität Konstanz, 2024

Type of examination or qualifications procedure:

5. ☒ I hereby affirm that the above statements are true and correct.
6. ☒ I am aware of the significance of the affirmation in lieu of oath and the consequences under the penal code of giving an incorrect or incomplete affirmation in lieu of oath.

I hereby affirm that I have stated the truth to the best of my knowledge and have concealed nothing.

Konstanz, 29.11.2024 A. Schmidt

place and date **(signature)**

[1] **Delete as appropriate. If the work has already been submitted, complete the fields indicating the title of the submitted work, the institute of higher education, the year in which it was submitted, and the type of examination or qualification procedure**