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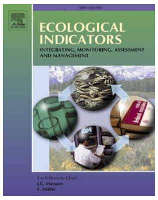

Original Articles

Decoding the Baltic Sea's past and present: A simple molecular index for ecosystem assessment

Alexandra Schmidt [a,b,*], Juliane Romahn [c,d,e], Elinor Andrén [f], Anke Kremp [g], Jérôme Kaiser [g], Helge W. Arz [g], Olaf Dellwig [g], Miklós Bálint [c,d,e], Laura S. Epp [a,*]

[a] *Environmental Genomics, Department of Biology, University of Konstanz, Constance, Germany*
[b] *International Max Planck Research School Quantitative Behaviour Ecology & Evolution, Constance, Germany*
[c] *Senckenberg Biodiversity and Climate Research Centre, Frankfurt am Main, Germany*
[d] *Loewe Center for Translational Biodiversity Genomics (LOEWE-TBG), Frankfurt am Main, Germany*
[e] *Institute for Insect Biotechnology, Justus Liebig University, Giessen, Germany*
[f] *Department of Natural Science, Technology and Environmental Studies, Södertörn University, Stockholm, Sweden*
[g] *Leibniz Institute for Baltic Sea Research Warnemünde, Rostock, Germany*

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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 paleoecological 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

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* Corresponding authors at: University of Konstanz, Mainaustr. 252, 78464 Constance, Germany.
E-mail addresses: Alexandra.schmidt@uni-konstanz.de (A. Schmidt), Laura.epp@uni-konstanz.de (L.S. Epp).

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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 paleoecological 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).

Paleoecological 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 paleoecological 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, paleoecological archives, like sediment core slices, integrate information from longer periods. Living organisms in current ecosystems can generally be identified to species level visually, while paleoecological 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), millenia (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 favoring pelagic organisms and indicates eutrophication due to silicate limitation (Wasmund et al., 2017; Conley et al., 2008). 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. Analyzing 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 dynamics 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, 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., 2014). 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 unfavorable 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

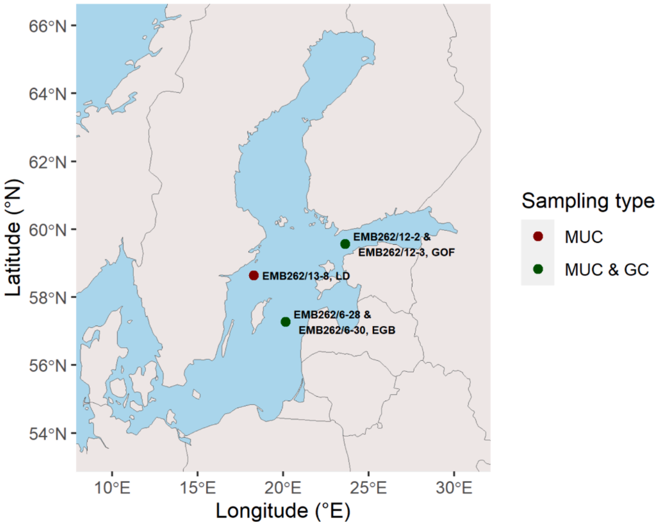

Fig. 1. Location of the coring sites in the central Baltic Sea. Gulf of Finland (GOF; cores EMB262/12-2MUC and EMB262/12-3GC), Landsort Deep (LD; core EMB262/13-8MUC) and Eastern Gotland Basin (EGB; cores EMB262/6-28MUC and EMB262/6-30GC).

future trends.

Five sediment cores were retrieved (Fig. 1) in April 2021, during expedition EMB262 in April 2021 onboard the research vessel Elisabeth Mann Borgese from three different locations (Fig. 1): 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: 436 m 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 1980 s. 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).

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 20 µL of proteinase K (20 mg/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 min instead of the recommended 2 min before conducting the elution. The spin columns were incubated twice with 75 µl of elution buffer for 5 min 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 30 s, 45 °C for 30 s, 68 °C for 45 s 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 the 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 lower 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 (900 nM/250 nM); 4) 1.1 v 20x Target-Primers/Probes HEX (900 nM/250 nM); 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^{TM} thermal cycler using the following program: 10 min at 95 °C, 40 cycles of 30 s at 94 °C, 30 s at 50 °C and 30 s 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^{TM} Analysis Pro software (v. 1.0.596) were used to analyze 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 (~1980 s). Importantly, only the years for which 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 and Seidel, 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:

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

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

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

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, 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 1980 s, 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

Fig. 2 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.

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 segmented regression analysis (Fig. 3, A).

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. 3 B). 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 1990 s. The Dia/Dino-index shows that the index was around 0.6 in the 1980 s, 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.

Overall, the two biomonitoring indices display a weak but significant

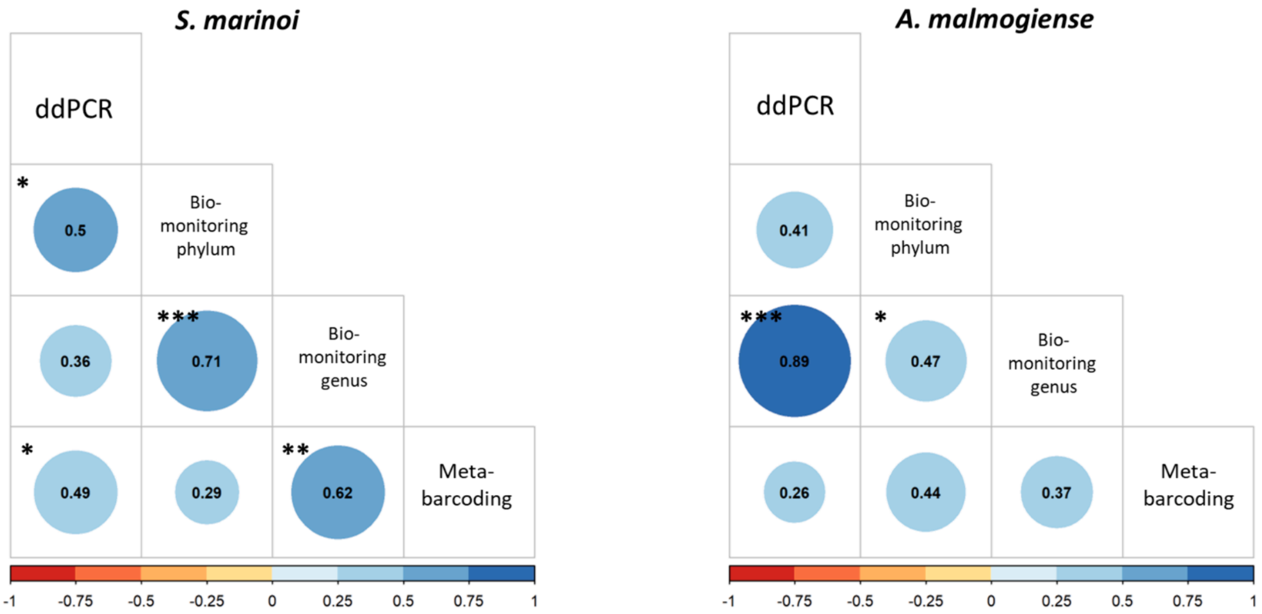

Fig. 2. Pearson's correlation between the four different datasets for *A. malmogiense* and *S. marinoi*: 1) ddPCR copies, 2) Biomonitoring phylum, 3) Biomonitoring species, 4) Metabarcoding phylum. Correlation coefficients from −1 (red) to 1 (blue) and their significance. *: $p < 0.05$, **: $p < 0.01$,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

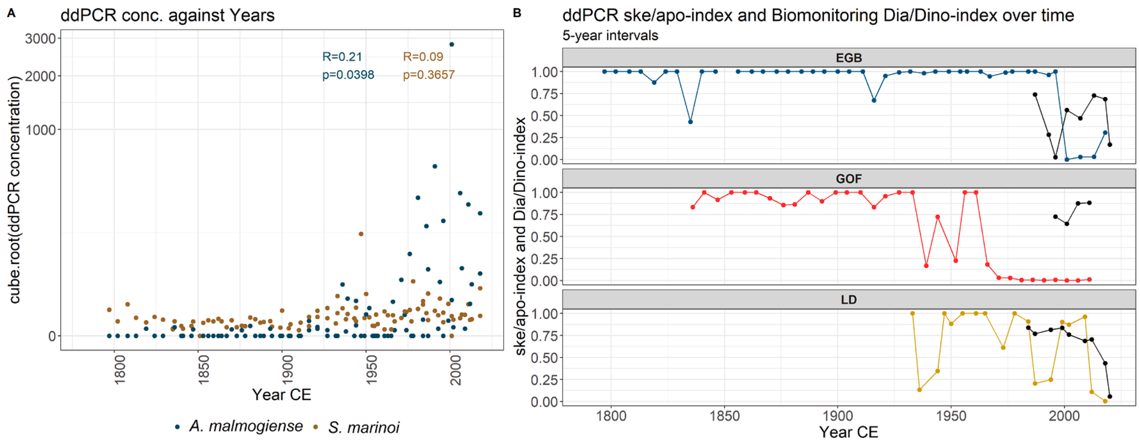

Fig. 3. A) Pearson's correlation between time and ddPCR concentration (measured in copies/μL, cube root transformed) for *A. malmogiense* (blue) and *S. marinoi* (brown). B) ddPCR ske/apo-index over time in five year intervals at all the locations (1798–2018) (colored) and biomonitoring Dia/Dino-index over time in five year intervals at all three locations (1984–2021) (black).

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 Fig. 4, 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.

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

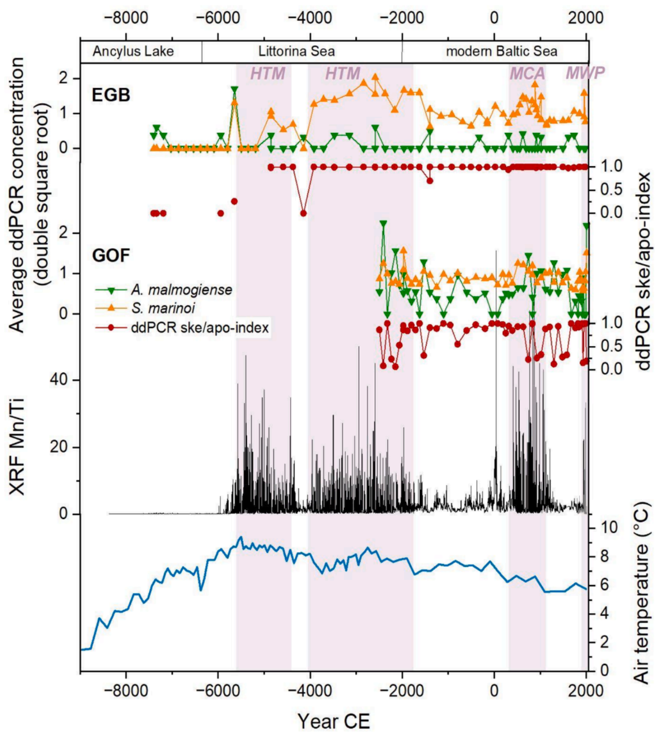

Fig. 4. Temporal development of the double square root transformed ddPCR concentration (expressed in copies/μL) at two locations: the Eastern Gotland Basin (EGB) and the Gulf of Finland (GOF). The ddPCR ske/apo-index over time is highlighted in red. The air temperature is shown in blue and based on data 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.

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 centered 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 paleoecological 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. 4). 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. 3). The instability of the index in the GOF, as well as the historical drops in the relatively stable EGB record (Fig. 4) 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. 3). 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, modeling 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 analyzing 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 and temperature. 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. 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 paleoecological 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 et al. 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.

CRediT authorship contribution statement

Alexandra Schmidt: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Juliane Romahn:** Writing – review & editing, Visualization, Investigation, Formal analysis, Data curation. **Elinor Andrén:** Writing – review & editing, Conceptualization. **Anke Kremp:** Writing – review & editing, Funding acquisition, Data curation, Conceptualization. **Jérôme Kaiser:** Writing – review & editing, Visualization, Investigation, Data curation. **Helge W. Arz:** . **Olaf Dellwig:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Miklós Bálint:** Writing – review & editing, Funding acquisition, Formal analysis, Conceptualization. **Laura S. Epp:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

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).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2024.112494.

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