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EVALUATIONS OF EXISTING METHODS

Extending monitoring with sediment archive approaches: Comparison of biomonitoring, metabarcoding, and biomarkers to assess past phytoplankton dynamics

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

[1]Institute of Insect Biotechnology, Justus Liebig University Giessen, Giessen, Germany; [2]Biodiversity and Climate Research Centre, Senckenberg Biodiversity and Climate Research Centre, Frankfurt am Main, Germany; [3]Functional Environmental Genomics, LOEWE Centre for Translational Biodiversity Genomics, Frankfurt am Main, Germany; [4]Limnological Institute, University of Constance, Constance, Germany; [5]Department of Marine Geology, Leibniz Institute for Baltic Sea Research Warnemünde, Rostock, Germany; [6]Department of Biological Oceanograph, Leibniz Institute for Baltic Sea Research Warnemünde, Rostock, Germany

Abstract

Sedimentary archives can provide valuable insights into the study of anthropogenic impacts on marine and limnic ecosystems over centennial and millennial timescales, potentially extending the temporal breadth of observation-based biomonitoring. Sedimentary archives allow for the tracking of biodiversity changes over long time periods, potentially including periods before human-induced changes. However, evaluations of biodiversity reconstructions using sedimentary approaches through comparisons with existing observation-based biomonitoring data are limited. Here we compared sedimentary ancient DNA metabarcoding and several biomarkers with >50 years of phytoplankton biomonitoring data from the Baltic Sea. Our findings indicated that both sedimentary ancient DNA metabarcoding and biomarkers reveal historical trends in phytoplankton communities. Sedimentary ancient DNA data was strongly correlated with biomonitoring data, while biomarkers showed weaker correlations, particularly for dinoflagellates. In addition, the sedimentary ancient DNA data indicated the past prevalence of ecological communities with no present-day analogs, highlighting the challenges of using modern observational data to infer historical biodiversity trends. The study underscores the importance of validating sedimentary approaches against observation-based data and calls for further research to improve the taxonomic resolution of metabarcoding and the specificity of biomarkers. These advancements could significantly enhance our ability to reconstruct historical biodiversity trends and inform future conservation strategies.

*Correspondence: juliane.romahn@stud.uni-giessen.de

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Data Availability Statement: The sequencing data can be accessed at European Nucleotide Archive at EMBL's European Bioinformatics Institute (EMBL-EBI) under the accession number PRJEB85715. The R scripts for comparison statistics are accessible at GitHub (https://github.com/jromahn/BalticSeaProxyComparison). Data and metadata are accessible via FigShare: Supporting Information Table S1—detailed sediment horizon metadata table (https://doi.org/10.6084/m9.figshare.28457471.v1); Supporting Information Tables S2–S4, S6, and S7 (https://doi.org/10.6084/m9.figshare.28457498.v3); Supporting Information Table S5—biomarker data table (https://doi.org/10.6084/m9.figshare.28457492.v2); uncleaned community matrix (https://doi.org/10.6084/m9.figshare.28457474.v1); ASV sequences (https://doi.org/10.6084/m9.figshare.28457495.v1); pr2 mothur taxonomy table (https://doi.org/10.6084/m9.figshare.28457483.v2); and cleaned community matrix (https://doi.org/10.6084/m9.figshare.28457489.v2).

The terms "global change" and "Anthropocene" have become prominent in everyday discourses (Head et al. 2022; Kaiser et al. 2023; Williams et al. 2023). Both terms point to human induced changes of the natural environment which include significant impacts on biodiversity. Temporal data, particularly long time series, are essential for understanding the influence of those changes on ecosystems and biodiversity (García-Barón et al. 2021; Jackson 2001; Wauchope et al. 2021). However, it is challenging to identify the extent and nature of these changes, especially without good knowledge of the conditions preceding the changes. Unfortunately, data recording often begins only after changes have become apparent. This limits our ability to grasp the full scope of impacts and formulate effective management strategies.

The conventional time series data are observation-based records, obtained by qualitative and quantitative surveys of the organisms of interest usually done by identification and counting of specimens. The collection of such monitoring data is often highly time-intensive and needs considerable taxonomic expertise (Meyer et al. 2021). As an alternative, environmental DNA is becoming a more and more prominent approach for tracking and monitoring ecosystem change and biodiversity (Bálint et al. 2018). Nevertheless, classical environmental DNA-based biodiversity assessment faces the same problem as conventional observation-based records with regard to the starting point: changes can only be studied from the time when monitoring programs were implemented in the second half of the 20th century. Thus, most records suffer from a lack of information regarding biodiversity in the absence of anthropogenic stressors.

When marine or lake sediments accumulate, they can incorporate and preserve remains of past organisms for long time periods, turning them into repositories of past ecosystems (Bálint et al. 2018; Nguyen et al. 2023). While relatively few organism groups leave behind fossilized parts, they all contribute to molecular remains which are preserved in sediments (Killops and Killops 2013). These remains are increasingly used to investigate past ecosystems in terms of environmental settings and past cohabiting communities (McClymont et al. 2023; Nguyen et al. 2023; Summons et al. 2022). Such molecular organic compounds, or biomarkers, are compounds that characterize biotic sources and that retain their source information after burial in sediments (Bianchi and Canuel 2011). They are commonly used in paleoenvironmental studies to reconstruct past climates and biomasses of past taxonomic groups (Eglinton and Eglinton 2008; Gaines et al. 2009; Marino et al. 2022). However, it is complex to determine their exact biotic source(s) and only some biomarkers are organism-specific (Holtvoeth et al. 2019; Zhang et al. 2011). In contrast, another established resource is sedimentary ancient DNA (sedaDNA). It allows, like extant environmental DNA, more detailed taxonomic resolution, but is considered less stable than biomarkers (Killops and Killops 2013).

Integrating recent monitoring data with sedimentary archives is an advancing field of research for developing assessment techniques. There are several important considerations when comparing sedimentary organismic remains with direct biological observation approaches (Thorpe et al. 2024). First, direct observations present a snapshot of biodiversity at a specific time. In contrast, sedimentary remains are an accumulation of information where one cm can represent the average community composition of many years (Bálint et al. 2018). Second, comparing taxonomy is challenging considering incomplete reference databases for DNA, continuously developing taxonomic classification, and cryptic species complexes which are often missed by morphology, but readily detected by DNA (Jaanus et al. 2006; Kremp et al. 2005; Sundström et al. 2009). Finally, each of these approaches has their distinct observation biases including differences in taxonomic expertise among observers in morphological identification (Douda et al. 2023; Straile et al. 2013), primer bias in metabarcoding (Polz and Cavanaugh 1998), or origin uncertainty in biomarkers (Holtvoeth et al. 2019). So far, only a few studies exist investigating to what extent sedimentary approaches are suitable as abundance proxies (Boere et al. 2009; Coolen et al. 2009; Thorpe et al. 2024), even if multiproxy approaches become more common (Nwosu et al. 2021; Pawłowska et al. 2020; Pieńkowski et al. 2024; Villanueva and Coolen 2022).

Here, we compare and evaluate the sedimentary approaches of sedaDNA metabarcoding and biomarkers with observation-based HELCOM monitoring time series of the Baltic Sea. In the Baltic Sea, a systematic phytoplankton monitoring program was established in the 1970s, and a > 50 year time series is available for comparison (Reusch et al. 2018). Therefore, we do not focus on a single location in the Baltic Sea, but rather investigate general trends in the Eastern Gotland Basin, Gulf of Finland, and Landsort Deep. First, we evaluate how well both sedimentary approaches detect recent monitored changes in the phytoplankton communities in general and in dinoflagellate communities in particular. Second, we extend the monitoring data with the sedimentary approaches to track how phytoplankton trends changed with increasing anthropogenic impact. Additionally, we examine trends of major eukaryotic groups from the metabarcoding data. Our comparison focuses on the last 100 years, a time period when massive environmental changes occurred in the Baltic Sea, including eutrophication and deoxygenation processes (Reusch et al. 2018; Zillén et al. 2008). Our results show that validation of sedimentary approaches is necessary, and more research is required to integrate their results with extant monitoring programs.

Materials and procedures

Study area and sediment sampling

The Baltic Sea is a semi-enclosed postglacial intracontinental brackish sea in Northern Europe (Fig. 1A; Reusch et al. 2018; Rosentau et al. 2021; Zillén et al. 2008). It is connected to the

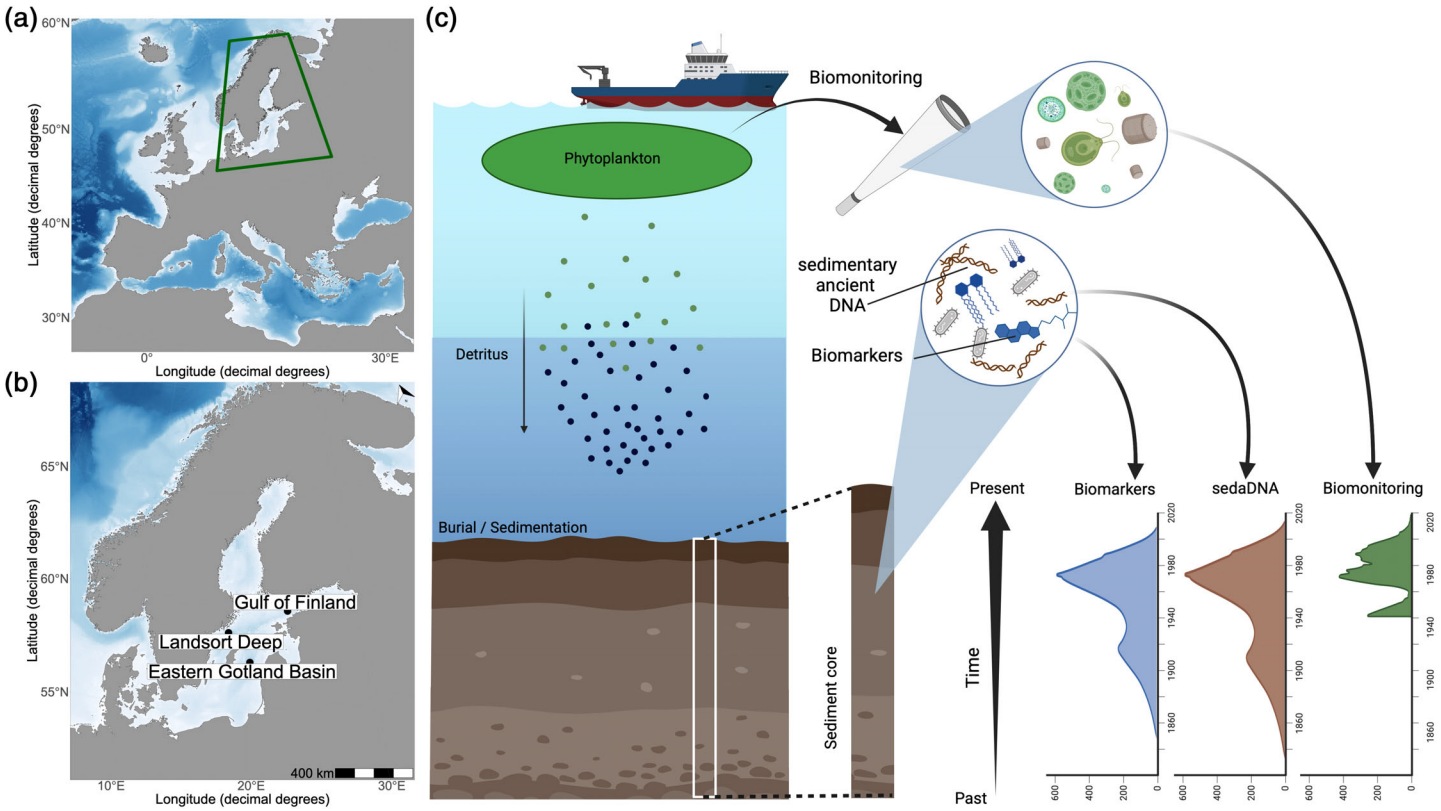

Fig. 1. (**a**) Map of Europe with the Baltic Sea area highlighted in green. (**b**) Map of the Baltic Sea with sediment core locations highlighted. (**c**) Conceptual illustration of the present study aiming to compare different approaches to validate and assess past abundance changes of phytoplankton in the Baltic Sea. Created in BioRender. https://BioRender.com/u15w501.

Atlantic Ocean by a small connection through the North Sea, which results in a strong salinity gradient from south to north. Due to these characteristics, the Baltic Sea is already subject to multiple stressors such as pollution, warming, hypoxia, and eutrophication (Reusch et al. 2018; Zillén et al. 2008).

Short sediment cores were retrieved from three different sites in the Baltic Sea during expedition EMB262 on board the R/V *Elisabeth Mann Borgese* in April 2021 (Fig. 1B). A multicorer keeping the interface between the bottom water and the surface sediment undisturbed was used for sampling. The sediment cores were located in the Gulf of Finland (59°34.443′N, 23°36.461′E), the Eastern Gotland Basin (57°17.004′N, 20°07.244′E) and the Landsort Deep (58°38.391′N, 18°15.997′E). Subsampling took place in a clean dedicated on-board laboratory. The sediment cores from the Eastern Gotland Basin (EMB262/6-28) and the Gulf of Finland (EMB262/12-2) were sampled every cm, while the core from Landsort Deep (EMB262/13-8) was sampled every second cm. Sediment samples were immediately frozen until further processing. The age models of the short sediment cores were published in Schmidt et al. (2024) and Kaiser et al. (2023). The core EMB201/7-4 from the Eastern Gotland Basin (57°16.980′N, 020°07.228′E) recovered at virtually the same location as core EMB262/6-28, was analyzed for biomarkers.

Metabarcoding

Molecular lab work

DNA was extracted in a clean room designated for ancient DNA extraction. The DNA extraction of approximately 0.5 g of wet sediment involved protocol adaptations of the DNeasy PowerSoil Pro kits (Qiagen) as described in Romahn et al. (2024). Extracts were stored at − 20°C.

Polymerase chain reaction (PCR) conditions were optimized with quantative polymerase chain reaction (qPCR), including tests of dilution series for signs of inhibition. Metabarcoding PCR reactions were automated using a Biomek i7 workstation (Beckman Counter) in a molecular prePCR laboratory observing multiple measures to avoid contamination: strict access rules, frequent surface decontamination with DNA-ExitusPlus (Kisker) and bleach, and protective equipment (full body suits, face masks, shoe covers, etc.). In total, 110 samples with four PCR replicates were amplified with the Euka02 primer (F: 5′-TTTGTCTGSTTAATTSCG-3′, 5′-CACAGACCTGTTATTGC-3′; Guardiola et al. 2015) using AmpliTaq Gold™ Mastermix (Thermo Fisher). We followed a single-PCR approach in which every sample had a unique nucleotide index combination of both primer pairs of 11 bp. This allowed for three base mismatches for index identification (Bohmann et al. 2022; Taberlet et al. 2018; Supporting Information Table S1). The PCR

was performed with a 1 : 2 dilution using 6 μL water, 11 μL mastermix, 2 primer pair mixture (5 μM), and 3 μL sample. The PCR positive control (containing DNA extracts of 24 species) was diluted to 1 : 25 (Supporting Information Table S2). The metabarcoding PCRs were run with 22 extraction negative controls, 52 PCR negative controls, 96 multiplexing negative controls, and 6 PCR positive controls. The multiplexing negative controls are no-template no-mastermix control samples to control for index jumps. Extraction negative controls and PCR positive controls had 4 replicates as well. The cycling program included an initial denaturation step at 94°C for 5 min, 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. After amplification, PCR products were pooled and 1200 μL of the pool was purified using the MinElute PCR Purification Kit (Qiagen). Library preparation and paired-end sequencing (NovaSeq 6000, 2 × 150 bp) was performed at Fasteris SA. The pool was sequenced twice to increase the sequencing depth (0.10 Mio reads per sample and 0.78 Mio reads per sample).

Bioinformatic steps

Bioinformatic processing involved ObiTools4 (version 4.0.4) for read merging, demultiplexing, dereplication, and prefiltering. Reads with an alignment score higher than 0.8 and an overlap of minimum of 10 nucleotides were merged and kept. Only head sequences were kept that had no variants of other sequences with a count greater than 5% of their own counts (Flück et al. 2022). Additionally, we discarded reads with a length shorter than 80 bp and longer than 300 bp and a count below 10. We taxonomically assigned the final amplicon sequence variant (ASV) with mothur (version 1.40.4) and a bootstrap confidence threshold of 80% to the pr2 SSU reference database (version 4.14.0; Vaulot et al., 2022). Eukaryotic unassigned ASVs were blasted (version 2.14.1+) against the NCBI nucleotide database (downloaded 7 May 2024; Camacho et al. 2009) and the last common ancestor of each ASV was determined with MEGAN6 (version 6.25.9) using the default parameters (Huson et al. 2016).

We further cleaned sequence data along considerations presented by (Taberlet et al. 2018) in R (version 4.1.3). This included the removal of low-frequency ASVs based on read count frequency (less than 85 total reads). Additionally, PCR replicates with less than 100,000 reads were considered failed, and these replicates were omitted from further analyses. The maximum read number of each ASV in any negative control (extraction, PCR, or multiplexing negatives) was calculated and subtracted from each sample replicate. Finally, samples with only two replicates left were discarded. The success of sequence processing was controlled by assessing community composition in replicates using nonmetric multidimensional scaling with the "vegan" package (seed number 25; Supporting Information S1, Table S3; Capo et al. 2021; Foulquier et al. 2023; Lopes et al. 2020). Nonmetric multidimensional scaling confirmed that remaining replicates of the same sample were highly similar (Supporting Information S1). Finally, the read number of every ASV was normalized to 500 μg sediment. Afterwards, the reads of the replicates were averaged for every ASV and every sample.

Biomonitoring

The phytoplankton monitoring data retrieved from ICES on 18 October 2022, underwent a refinement process (ICES Data Portal 2022). Monitoring data were assigned to a location if they were within 5% deviation in decimal degrees of the core coordinates (Supporting Information Table S4). Species validation and taxonomy queries were performed using the "wm_record" function from the "worrms" package (version 0.4.3; Chamberlain 2020). Years with missing data for April, May, or June were excluded. The count data were aggregated per species, with counts from individual monitoring stations averaged monthly. For the general analysis of single phytoplanktonic taxa and phytoplankton in general, the sum of the count data for each phytoplankton taxon per month served as the basis for calculating an annual biomass proxy aggregated over a 3-month period.

Total organic carbon

Total organic carbon was calculated by the subtraction of total inorganic carbon from total carbon values. Total carbon was analyzed by means of a Euro EA (EuroVector) elemental analyzer. Total inorganic carbon was determined by means of a total inorganic carbon module connected with a Multi EA 4000 (Analytik Jena) elemental analyzer, involving the acidic removal of carbonates from sediment samples and the analysis of the CO_2 released in a carrier gas stream (Leipe et al. 2011).

Biomarkers

Cores EMB201/7-4 (Kaiser et al. 2023) and EMB262/6-28 were analyzed for biomarkers. The sediment samples have been processed as described in (Kaiser and Arz 2016) and (Kaiser and Lerch 2022). Briefly, sediments (0.5–1 g) were extracted with a dichloromethane (DCM)/MeOH (9 : 1) mixture using an Accelerated Solvent Extraction device (Dionex™ ASE™ 350; Thermo Scientific). After the addition of internal standards (squalane and 5α-androstan-3β-ol), the extracts were separated into four fractions by microscale flash column chromatography using activated silica gel and hexane, hexane/DCM (1 : 1), DCM, and DCM/MeOH (1 : 1) as eluting solvents. The DCM/MeOH fractions containing the sterols were derivatized using 10 μL of bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane and 10 μL of pyridine heated at 60°C for an hour. The analysis of the hexane fractions containing hydrocarbons was performed with a gas chromatograph equipped with a flame ionization detector (Thermo Scientific TraceUltra GC). The target compounds were identified and quantified with internal and external standards. The sterol analysis was performed with a gas chromatograph (Agilent Technologies 7890B GC)

coupled to a mass spectrometer detector (Agilent Technologies 5977B Mass Selective Detector). Compound identification was achieved by chromatographic (retention index) and mass spectral comparison with the National Institute of Standards and Technology mass spectral library. For semi-quantitative estimation using 5α-androstan-3β-ol as the internal standard, dinosterol (4α,23,24-trimethyl-5α-cholest-22E-en-3β-ol) and dinostanol (4α,23,24-trimethyl-5α-cholestan-3β-ol) were extracted from the full scan mode (m/z 50–550) using m/z 359 and 75, respectively, and 5α-androstan-3β-ol using m/z 333. To correct for potential effects of lipid preservation, degradation, and/or dilution by terrestrial inputs, the data were normalized to total organic carbon (Supporting Information Fig. S1 and Table S1, S5).

Comparison of the different approaches

To validate both sediment approaches, we compared both separately with the late spring/ early summer biomonitoring data.

To ensure comparability when investigating general eukaryotic community patterns, we curated the taxonomy of several phytoplankton groups, including the combination of several taxonomic groups. The division Rhodophyta, the class Phaeophyceae, and Ulvophyceae were defined as macroalgae; the class Zygnemophyceae was excluded from the taxon Streptophyta. The dinoflagellate class Syndiniales (according to the WoRMs database) was removed from the metabarcoding dataset. They are considered as parasites and are lacking in the monitoring data due to their small size. For comparability, pr2 taxonomy was transformed to the taxonomy of the WoRMS database. Therefore, the division Chlorophyta and division Prasinodermophyta was defined as Chlorophyta, the classes MOCH-3, MOCH-5, Eustigmatophyceae, Coscinodiscophyceae, Xanthophyceae, Bolidophyceae, Chrysophyceae, Chrysomeridophyceae, Chrysomerophyceae, and Dictyochophyceae as Ochrophyta, class Zygnemophyceae of Streptophyta as Charophyta, and the division Cryptophyta and division Kathablepharidacea as Cryptophyta.

We excluded diatoms from the analysis for metabarcoding and biomonitoring. Although the Baltic Sea has a long-term monitoring dataset, we required data from several months of the same year for comparability to the sedimentary archives. The most continuously sampled months were April to June (Fig. S2). Diatoms are most abundant in winter and early spring, depending on the taxonomic group (Hjerne et al. 2019), and consequently they are rare in the late spring/ early summer biomonitoring dataset. Therefore, the diatoms were discarded from the monitoring and metabarcoding datasets for the comparison.

For visualization purposes and to ensure consistency in the statistics, we defined a relative abundance proxy for every approach. The abundance proxy in metabarcoding (P_{meta}) is reflected by the relative read abundance in that sample (Thorpe et al. 2024). It was calculated by the total read count of the taxon of interest divided by the maximum total reads of that location (n) for every sample (t) multiplied by 100:

$$P_{meta} = (\text{taxon read number}_{n,t} / \text{total read number}_{n,t}) \times 100 \quad (1)$$

The relative abundance proxy of monitoring (P_{moni}) reflected the relative count number of the maximum value of each location. It was calculated by dividing the cell count in a sample of the taxonomic group by the maximum cell count of the taxonomic group at the location (n) of every time point (t) multiplied by 100:

$$P_{moni} = (\text{taxon cell count}_{n,t} / \max[\text{total cell count}_{n}]) \times 100 \quad (2)$$

The relative abundance proxy of each biomarker (P_{biom}) reflected the relative biomarker amount of the maximum value. It was calculated by the biomarker content (c) of that sample (t) divided by the maximum biomarker content at a location (n) multiplied by 100:

$$P_{biom} = (c_{n,t} / \max[c_{n}]) \times 100 \quad (3)$$

The relative abundance proxy values were calculated for each sample of each location and for both phytoplankton and dinoflagellates.

To account for dating errors, information accumulation in the sediments, and missing years, we fitted generalized additive models (GAMs) to the abundance proxy data, as recommended by Simpson (2018). To do this, we fitted GAMs with generalized cross-validation smoothness selection of the mgcv R package (version 1.9.1; Wood and Wood 2015) with the random seed number 12345 (Simpson 2018; Thorpe et al. 2023). For each location, the organism, and approach, smooth as a random effect was estimated with maximum likelihood and restricted maximum likelihood. Additionally, thin plate regression (tr), cubic regression (cr), and Gaussian process spline (gp) were used as the basis functions. Smooth was evaluated based on 300 points. As possible k values, 15, 20, 25, 30,35, 40, 45, and 50 were tested, considering that the maximum k has to be smaller or equal to the number of events per approach and location. The final k, basis function, and spline for modeling were chosen based on the following two criteria: k index is larger than 0.8, p-value is larger than 0.05. Then, for every location, approach, and organism, the k with the highest k-index was chosen (Supporting Information Table S6). Annual GAM predictions of P_{moni} were correlated based on Spearman correlation with GAM predictions of P_{meta} and P_{biom} in the time period 1983–2018 in the Landsort Deep, 1983–2016 in the Eastern Gotland Basin, and 1998–2016 in the Gulf of Finland. Additionally, the annual GAM predictions of P_{meta} were correlated with annual GAM predictions of P_{biom} for the period 1797–2016 in the Eastern Gotland Basin and for the period 1836–2016 in the Gulf of Finland.

For visualization of the abundance trends of the various taxonomic groups, sedaDNA metabarcoding values were summarized into average 5-year intervals by calculating mean abundance proxy values of the communities between April and June.

Assessment

Phytoplankton communities recorded by biomonitoring

An increase in phytoplankton counts was observed at all three locations (Fig. 2C). The increase started slowly in the Eastern Gotland Basin around 2004 and became steep in 2012. In the Gulf of Finland, counts of phytoplankton increased in the late 1990s, reached their maximum in 2004, and started decreasing until 2019, when an increase in counts was observed again. Phytoplankton counts increased at Landsort Deep since the mid-2000s (Fig. 2C). The prominence of the major phytoplankton groups varied across the three locations. In the Eastern Gotland Basin, Cryptophyta and Haptophyta were the most abundant taxa. In the Gulf of Finland, Chlorophyta, Cryptophyta, Dinoflagellata, Haptophyta, and Ochrophyta were all prominent. In the Landsort Deep, Chlorophyta, Haptophyta, and Ochrophyta were the prominent phytoplankton taxa until 2003. After 2003, Haptophyta became the dominant phytoplankton taxon. Single-year peaks in high count numbers could be observed in the Gulf of Finland in 2004 and in the Landsort Deep in 1996 (Fig. 2C). Dinoflagellate abundance followed similar trends as the general phytoplankton abundance in the Eastern Gotland Basin and Landsort Deep (Fig. 2C; Supporting Information Fig. S3). Their abundance increased in the Eastern Gotland Basin since 2005 and decreased in the Gulf of Finland after a peak in 2004. The dinoflagellate abundance was steady in Landsort Deep since monitoring started in 1980.

Phytoplankton and dinoflagellate abundance recorded by biomarkers

The biomarkers indicated a general increase in phytoplankton abundance since 1950 (Fig. 3). In the Eastern Gotland Basin, phytoplankton abundance increased slowly until 1975 and had two major peaks in the 1980s and early 2000s. In contrast, a steep increase in phytoplankton occurred only since 1985, with a peak around 2000 and a decreasing trend until 2010 in the Gulf of Finland. The dinoflagellate biomarkers show different trends in both locations. In the Eastern Gotland Basin, dinoflagellate biomarkers have been fluctuating and increasing since the 1930s, suggesting a steady increase in dinoflagellate abundance. In the Gulf of Finland, the dinoflagellate biomarkers indicated an increase in dinoflagellate abundance since mid-2000, contrary to the phytoplankton biomarkers. Overall, both biomarkers indicated a difference in abundance between the Eastern Gotland Basin and the Gulf of Finland.

Eukaryotic communities recorded by metabarcoding

We received 465,123,663 (R1) and 465,123,663 (R2) after multiplexing the unmerged sequences. After bioinformatic processing and prefiltering, these were classified into 388,953 ASVs, represented by 422,298,703 reads. A total of 36,409 ASVs and 279,749,200 reads remained after final cleaning.

Eukaryotic community composition exhibited notable variation over time in the Landsort Deep, the Eastern Gotland Basin, and the Gulf of Finland (Fig. 2A). The relative abundances (estimated as the abundance proxy P_{meta}) of taxonomic groups also showed considerable changes in this period. Throughout the entire study period, phytoplankton, fungi, Rhizaria, and other protists such as Syndiniales (parasitic dinoflagellates) were consistently prominent at all three locations (Supporting Information Fig. S4). Fungi were relatively more abundant in the Eastern Gotland Basin, with a decreasing trend observed towards the present. The Gulf of Finland had the highest proportion of Streptophyta when it was abundant between 1800 and 2005, followed by a decreasing trend in the relative abundance (Fig. 2A). Noteworthy are the presence and abundance trends of Opalozoa and Metazoa. The metazoans were abundant in the Eastern Gotland Basin until the 1930s, decreased then, and remained low. In contrast, the relative abundance of metazoan increased in the Gulf of Finland since the 1930s and persisted prominently. The relative abundance of metazoan increased in Landsort Deep in the 1990s, but remained a minor component of the eukaryotic communities. Opalozoa abundance increased in the Eastern Gotland Basin in the 1880s, peaked when the metazoan abundance proxy dropped (1930s), and has declined since. Similar observations were made in the Landsort Deep. In contrast, Opalozoa exhibited low abundance in the Gulf of Finland throughout the study period. Strikingly, a large proportion of reads could not be assigned taxonomically at all three locations (Fig. 2A; Supporting Information Fig. S5). This proportion fluctuated over time, with peak occurrences of not assignable reads observed in the Eastern Gotland Basin around 1845 and 1980, in the Gulf of Finland in 1905 and 1955, and in Landsort Deep between 1955 and 1960.

Phytoplankton communities showed fluctuating abundances over time (Fig. 2B). The phytoplankton abundance has increased in the Eastern Gotland Basin since 1975, in the Gulf of Finland since 1955, and in Landsort Deep since 1985. Dinoflagellates dominated the phytoplankton communities at all three locations (Fig. 2B; Supporting Information Fig. S6). Low abundance proxy values of Ochrophyta were observed at all three locations. Chlorophytes were present in larger proportions in the Eastern Gotland Basin until 1970 and in smaller proportions at the Gulf of Finland and Landsort Deep during the complete record time (Fig. 2B; Supporting Information Fig. S4). Overall, our findings reveal numerous shifts in community composition over the past two centuries. These shifts included changes not only in the abundance proxy but also in

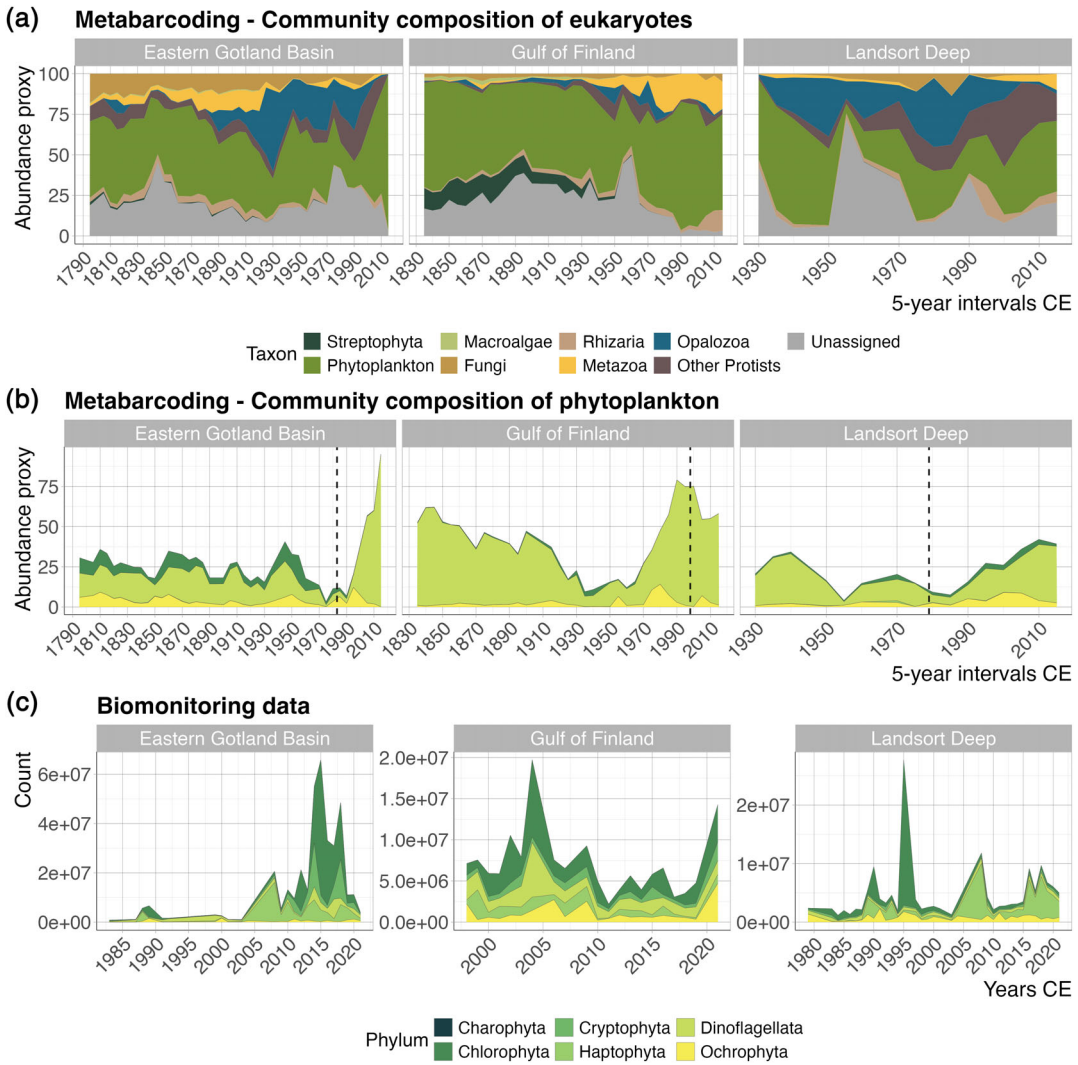

Fig. 2. Community composition of eukaryotes (**a**) detected by metabarcoding and phytoplankton communities detected by metabarcoding (**b**) and biomonitoring (**c**). The abundance proxy represents relative read numbers. Biomonitoring represents April–June communities. Lines highlight the start of monitoring at the specific location. Samples were summarized into 5-year intervals in (**a**). For phytoplankton analysis, the diatom communities were excluded in (**b**) and (**c**).

the occurrence of major taxonomic groups and taxonomically not assignable sequence variants.

Comparison of phytoplankton dynamics over time

All three approaches showed similar, although not always statistically significantly correlating phytoplankton dynamics (Fig. 3; Table 1; Supporting Information Table S7). The GAM-predicted abundance trends correlated strongly (> 0.65 rho) between metabarcoding and biomonitoring for all three locations (Table 1). Similarly, the GAM-predicted abundance trends of the biomarkers and the biomonitoring correlated strongly at both locations (> 0.6 rho). The abundance trends between metabarcoding and biomarkers were not significantly correlated over the last 150 years. In the Eastern Gotland Basin, phytoplankton P_{biom} trends increased more than 20 years earlier than P_{meta} trends. In the Gulf of Finland, P_{biom} increased slowly since the 1950s and strongly in the 1980s, whereas P_{meta} indicated a steady increase. In contrast to the metabarcoding results, the biomarkers indicated that phytoplankton were present at very low abundances before the 1950s. Overall, all approaches showed clear increases in phytoplankton abundance trends, but these trends were correlated only in the metabarcoding—biomonitoring and biomonitoring—biomarker comparisons.

Comparison of dinoflagellate dynamics over time

Focusing on dinoflagellate dynamics, biomonitoring and metabarcoding trends were highly correlated (> 0.75 rho) and showed similar trends at all three locations (Table 1). In the Eastern Gotland Basin and Landsort Deep, both

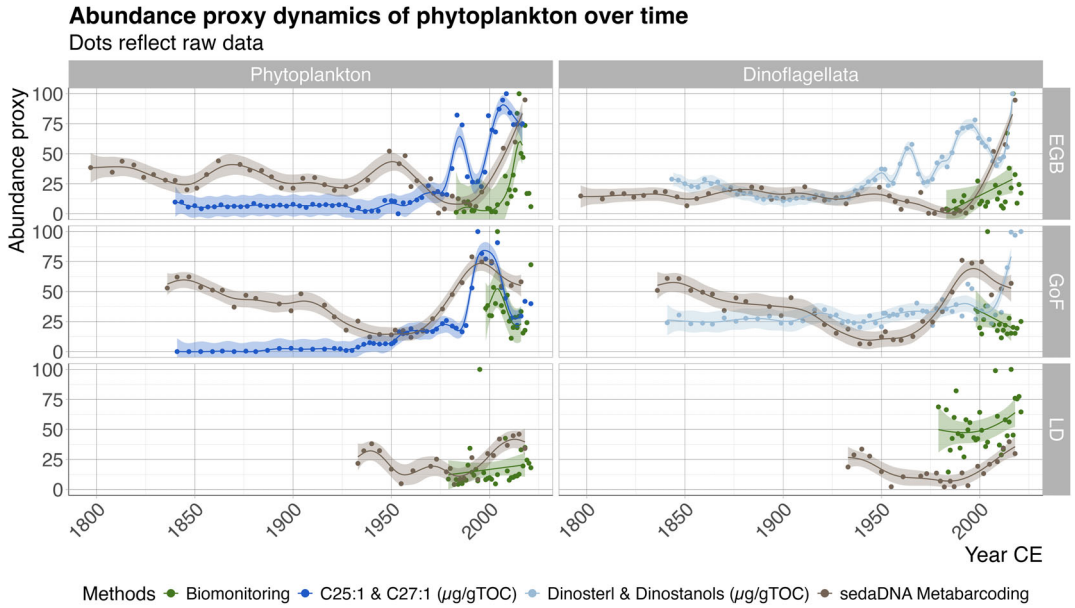

Fig. 3. Community dynamics of dinoflagellates and other phytoplankton in the different basins during the last 220 years. Colors represent the different approaches applied. The dots reflect available raw data of the various approaches and the lines refer to the predicted abundance proxies of a generalized additive model. Biomonitoring represents April–June communities. EGB, Eastern Gotland Basin; GoF, Gulf of Finland; LD, Landsort Deep.

approaches indicated a general increase in dinoflagellate abundance, but the increase was slow in Landsort Deep. Both approaches indicated a decrease in dinoflagellates since the 2000s in the Gulf of Finland. In contrast, biomarkers lacked correlation with the biomonitoring in the Eastern Gotland Basin but correlated strongly negatively with biomonitoring in the Gulf of Finland. There, the biomarkers indicated a strong increase in dinoflagellates since the 2000s, which led to the negative correlation with the biomonitoring data. Biomarkers and metabarcoding are only weakly positively correlated in the Gulf of Finland and weakly negatively correlated in the Eastern Gotland Basin over the last 150 to 200 years (Table 1). Overall, only metabarcoding and not biomarkers reflect dinoflagellate trends observed by biomonitoring.

Discussion

In this study, we investigated the dynamics of phytoplankton communities in the Baltic Sea over the past ~ 200 years. To this end, we employed a combination of sedaDNA metabarcoding, biomarkers, and traditional observation-based biomonitoring data. To gain insight into the historical shifts in biodiversity and the influence of human activities on these aquatic ecosystems, we compared and validated these

Table 1. Spearman correlation values of generalized additive model-predicted annual abundance proxies. Biomonitoring represents April–June communities. Monitoring refers to the monitoring periods of 1983–2016 in the Eastern Gotland Basin, 1998–2016 in the Gulf of Finland, and 1979–2018 in the Landsort Deep. Archive refers to the time intervals covered by the sediment cores, 1797–2016 in the Eastern Gotland Basin and 1836–2016 in the Gulf of Finland. Numbers reflect rho and asterisks reflect *p*-values (***≤ 0.001; **< 0.01; *≤ 0.05).

Organism	Timespan	Approach 1	Approach 2	Eastern Gotland Basin	Gulf of Finland	Landsort Deep
Phytoplankton	Monitoring	Metabarcoding	Biomonitoring	0.71***	0.69**	0.95***
	Monitoring	Phytoplankton biomarkers (n-$C_{25:1}$ + n-$C_{27:1}$ alkene)	Biomonitoring	0.61***	0.7**	NA
	Archive	Phytoplankton biomarkers (n-$C_{25:1}$ + n-$C_{27:1}$ alkene)	Metabarcoding	0.19*	0.04	NA
Dinoflagellates	Monitoring	Metabarcoding	Biomonitoring	1***	1***	0.78***
	Monitoring	Dinoflagellate biomarkers (dinosterol & dinostanol)	Biomonitoring	0.07	0.82***	NA
	Archive	Dinoflagellate biomarkers (dinosterol and dinostanol)	Metabarcoding	0.25***	0.23**	NA

disparate approaches. Our findings illustrate both concordance and discordance between the approaches. These results underscore the challenges inherent in the interpretation of historical biodiversity data and the use of multiple proxies to achieve a comprehensive understanding of past ecological changes.

Similar patterns between archive approaches and monitoring

Although there is a general congruence between the data obtained from metabarcoding and that obtained from biomonitoring, our study reveals both similarities and discrepancies in the dynamics of the phytoplankton community across different locations. Focusing on phytoplankton community dynamics, we found similar patterns between metabarcoding and monitoring data for all three locations. For dinoflagellates, P_{meta} correlated strongly with P_{moni} in the Gulf of Finland and the Eastern Gotland Basin. Our findings confirm previous reports, which show the congruence of metabarcoding and biomonitoring data for specific phytoplankton groups. For example, Thorpe et al. (2024) found a positive correlation for chlorophytes, dinoflagellates, ochrophytes, and bacillariophytes between relative read abundance data of sedaDNA and monitoring counts spanning 65 years. However, most aquatic eDNA studies to date focus more on the relationship between relative read abundance and biovolume, and less on count data. This trend is due to stronger correlations of read abundance with biovolume than with count observations (Andersson et al. 2023; Gran-Stadniczeñko et al. 2019; Santi et al. 2021). Such comparisons make sense since biovolume might better approximate DNA read counts, and larger cells with higher biovolume tend to have more 18S rRNA copies (Godhe et al. 2008; Santi et al. 2021). Both biovolume and count data are standard in actual biomonitoring, but older datasets often include only count/abundance data (Wasmund and Uhlig 2003). For this reason, we have used count data rather than biovolume in this study. Nevertheless, our results support that metabarcoding can well reflect phytoplankton count data despite variance in 18S copies. We also found positive correlations between biomonitoring and biomarker datasets of phytoplankton. The n-$C_{25:1}$ and n-$C_{27:1}$ alkene data reflected total phytoplankton counts in the Eastern Gotland Basin and the Gulf of Finland. This correlation supports previous reports on correlating biomarker and biomonitoring records of historical trends (Kaiser et al. 2023). Overall, the general similarity of patterns recorded by molecular organismal remains (metabarcoding, biomarkers) and direct observations (biomonitoring) is striking. It suggests that sedimentary archives and direct observations can be combined to inform about historical biodiversity change well beyond the start of modern records. However, some disagreements related to the different approaches are also evident.

Differences between biomarkers and monitoring

Despite similarities in phytoplankton dynamics, our study reveals substantial discrepancies between dinoflagellate biomarkers and biomonitoring data. A correlation between the dinoflagellate biomarkers and biomonitoring is missing for the Eastern Gotland Basin. In contrast, the correlation is strongly negative between both approaches for the Gulf of Finland. This is noteworthy because we used dinosterol and dinostanol as biomarkers to specifically measure Dinophyceae biomass (Sachs et al. 2021; Tao et al. 2022). Reasons for the strong negative correlation could be variance in biomarker production. Several studies highlighted that the production of dinosterol and dinostanol varies between diverse dinoflagellate species, and some even lack these methyl-sterols (Amo et al. 2010; Mansour et al. 1999; Volkman, 2006). Although it needs further testing, this explanation might be further supported by observed shifts in dinoflagellate community composition in the biomonitoring data (Fig. 3). The community may have changed from species producing lower amounts of dinosterol and dinostanol to species producing higher quantities. The opposite was observed by Boere et al. (2009): in an Antarctic fjord, the community composition shifted to a non-dinosterol producing dinoflagellate, resulting in a generally low dinosterol concentration in the sediments (Boere et al. 2009). For this reason, it is questionable whether the two biomarkers tested here are really suitable as proxies of Dinophyceae biomass. We do not discuss dinosterol and dinostanol results further, as biomonitoring observations and sedaDNA data did not confirm trends marked by these dinoflagellate biomarkers.

Historic eukaryotic communities as recorded by metabarcoding

By analyzing the metabarcoding data, we observe several changes in community dynamics of the major eukaryotic taxa in the Baltic Sea over the last $\sim$ 200 years. Streptophyta were observed in high abundance in the Gulf of Finland until the 1950s, while metazoan were most abundant in the Eastern Gotland Basin between 1850 and 1925. Of particular interest is the prominence of the opalozoan MArine STramenopiles group 12. MArine STramenopiles group 12 is known for its diverse ecological preferences, ranging from anoxic to oxic habitats, and can live as plankton or in sediments (Massana et al. 2014). Of little interest in modern studies, MArine STramenopiles group 12 seems to be a relevant group in the past. Siano et al. (2021) identified them via sedaDNA metabarcoding in historical communities in the coastal area of Brest, France, during the Middle Ages and the XIX century. In our study, they were prominent in the Eastern Gotland Basin from 1880 to the early 2000s and in Landsort Deep from the 1930s to the early 2000s. The high relative read abundance of taxonomic groups which are unusual components of the present-day communities suggests that eukaryotic communities with no modern analogs were common in the past. This is supported by the large past prevalence of unassignable reads. Unassigned sequences were approximately four times more abundant at some sites and past events than in the present, highlighting the lack of knowledge of these organisms. Similar observations of communities with no

modern analogs are common in plants or mammal palaeoecology. Examples of such communities are the presence of now-extinct megafauna during the last glaciations and the rapid tree succession and loss of arctic-alpine plant taxa after the glaciation (Giesecke et al. 2017; Stivrins et al. 2016; Ukkonen et al. 2011; Ukkonen et al. 2008). These examples come from the more distant past, when climatic conditions were very different compared to today. Possible reasons for the prevalence of non-analog planktonic communities observed by us in the recent past might be linked to the start of the industrial revolution and increase in agricultural production around 1850 (Zillén et al. 2008), the increased use of artificial fertilizers in the 1920s (Hoffmann et al. 2000; Morell 2001; Treitel 2015) and increased salinity and intensified agriculture in the era after World War 2 in the 1950s (Matthäus and Franck 1992; Moros et al. 2023; Savage et al. 2010). Similar impacts of recent anthropogenic events were detected in a multi-proxy study of a sediment core from the Black Sea, showing major shifts in planktonic communities likely related to the onset of deforestation about 200 years ago (Giosan et al. 2012). Our results suggest that massive changes in community composition can create communities with no modern analogs very rapidly, even over a few years. These missing analogs limit our ability to conclude about biodiversity changes in the recent past relying only on modern community data. They also highlight the difficulties of using modern observations of biodiversity, such as recent time series and ongoing biomonitoring, to extrapolate and understand changes beyond the temporal scope of these datasets.

Reconstructing phytoplankton trends in the past

In the Eastern Gotland Basin, sedaDNA metabarcoding indicates low relative phytoplankton abundance until monitoring began (Figs. 2A, 3). Single peaks occurred around 1820, 1860/1870, and 1950, followed by a decline in the 1980s. The phytoplankton biomarker shows low and stable phytoplankton abundance until 1950 and a steady increase peaking in the 1980s. The peaks in sedaDNA metabarcoding in the 19th century and after the Second World War overlap with agricultural and industrial intensification as mentioned above. Other studies observed high eutrophication in the 1980s, possibly linked to high phytoplankton biomass, followed by a recovery in the 2000s (Savage et al. 2010; Wasmund 2017; Schmidt et al. 2024). The results align with the peak in the 1980s observed by the biomarker. The reason for the observed differences between biomarkers and sedaDNA metabarcoding is not evident, especially as they both align partially with historical events. Similar discrepancies in dinoflagellate community composition based on biomarkers, sedaDNA, and dinocysts were also observed by Boere et al. (2009) in Antarctic fjord sediments, probably related to preservation and variance in biomarker production within species. Such discrepancies between different approaches are frequently observed in multi-proxy paleoecological studies, for example, when comparing pollen and sedaDNA data (Liu et al. 2020; Parducci et al. 2019). Despite the differences, the complementary nature of the information obtained has made multi-proxy studies, including pollen as well as DNA, common for studying paleovegetation. In our study, differences could be caused due to variations in taphonomy or also recording different communities (Boere et al. 2009). Savage et al. (2010) noticed that the phytoplankton community mostly consisted of diatoms in the late 1980s and cores from the Eastern Gotland Basin have a diatom layer covering 1988–1990 (Moros et al. 2017). The eutrophication peak in the 1980s may not be reflected in the sedaDNA metabarcoding data, probably due to the exclusion of diatoms. Also, almost 50% of all ASVs could only be assigned to eukaryotes, and further taxonomic information is lacking in the 1970/1980. Nevertheless, sedaDNA metabarcoding reflects the monitoring results better in general.

In the Gulf of Finland, sedaDNA metabarcoding indicates high phytoplankton abundance, which decreases until the 1950s and then rises again. In contrast, phytoplankton biomarkers show low phytoplankton abundance until 1920, a slight increase afterwards, and a strong increase since the 1980s. Kremp et al. (2018) report an increase in cyst counts of only three dinoflagellate spring species since the 1930s and an increased abundance since the late 1950s in the Gulf of Finland. These results are consistent with the biomarker data. Nevertheless, that study is based only on three species of one taxonomic group, whereas sedaDNA metabarcoding and the biomarkers are driven by potentially hundreds of taxa. The metabarcoding data align with industrial and agricultural development intensifying in the 19th century and may indicate earlier eutrophication or at least human impact. Strong human impact on terrestrial and aquatic ecosystems is recorded for the Baltic countries by several studies (Andrén et al. 1999; Zillén et al. 2008). However, the abundance trends should be interpreted carefully until it is confirmed by independent proxies. We could not determine the reason for the observed differences between biomarkers and sedaDNA metabarcoding. After 1950, both sediment approaches are consistent with eutrophication during the Great Acceleration and with the decrease in nutrients after wastewater treatment started in the 1990s (Head et al. 2022; Reusch et al. 2018).

In the Landsort Deep, besides the observation-based data only metabarcoding of sedaDNA data is available. These data show low phytoplankton abundances and a decreasing trend since 1930, with the lowest point in the 1970s, followed by a slight increase. The Landsort Deep observation is consistent with the monitoring data and the results of Schmidt et al. (2024). Schmidt et al. (2024) showed a recovery of the ecosystem in the 1970s, but a poorer state of the ecosystem at the Landsort Deep before and afterwards.

Limitations and outlook

Within our study, we found several discrepancies, especially between the two sediment approaches, but also with

respect to the monitoring method. The discrepancies in the recorded abundance trend could be caused by different factors. First, the taphonomy of organismic remains is known to be influenced by several factors, like oxygen, temperature, mineral composition, and water depth (Freeman et al. 2023; Killops and Killops 2013; Mejbel et al. 2022). Even if biomarkers are considered to be more stable than DNA (Killops and Killops 2013), variations in preservation among different biomarkers are known (Sinninghe Damsté et al. 2002; Hoefs et al. 2002). Additionally, total organic carbon is considered to be better preserved in laminated than in homogenous sediments (Häusler et al. 2017; Sollai et al. 2017). This is likely to be the case for biomarkers as well as for DNA. This is of particular interest as the stratification in the Baltic Sea changed dramatically in 1950, along with the preservation conditions of organic remains (Moros et al. 2023) and most discrepancies between both sedimentary approaches existed before 1950. To better understand taphonomy and preservation of molecular remains, future studies should integrate water column samples and surface sediments. This would allow for understanding changes in the status and composition of organism remains and biomolecules in the water column. Especially for biomarkers, not only a few, but a larger variety of molecules should be included to consider the preservation of the different chemical structures. Second, metabarcoding in general, and not only for sedaDNA, is prone to primer bias and incomplete reference databases. Primer bias refers to differences in amplification of the DNA sequences caused by primer annealing differences (Polz and Cavanaugh 1998). For example, comparing the metabarcoding and biomonitoring data in the Eastern Gotland Basin and the Landsort Deep, we can notice a low amplification of Haptophyta. This phytoplankton group is known to be problematic for amplification by primer pairs targeting the V4 and V7 regions of the 18S rRNA gene (Bradley et al. 2016; Choi and Park 2020; Kezlya et al. 2023). Despite potential primer-specific PCR bias, haptophytes also have a low 18S rRNA copy gene number, leading to their underrepresentation in metabarcoding datasets (Martin et al. 2022). These would be of particular interest for comparing and validating various approaches, as some haptophyte biomarkers (alkenones) are used as a sea surface temperature proxy (Brassell 1993; Conte et al. 2006). Coolen et al. (2009) showed with 18S and cytochrome c oxidase subunit I (COI) data that shifts in haptophytes abundance and alkenone concentration are not only temperature-driven but also salinity-driven in the Black Sea, highlighting the importance of further validation. To avoid primer bias, metagenomics or hybridization capture would be an alternative (Nguyen et al. 2023). Nevertheless, the issue with incomplete reference databases and therefore a low assignment success is not only known for metabarcoding but also for other DNA approaches (Lammers et al. 2021; Nguyen et al. 2023; Parducci et al. 2019). A comparable problem exists for interpreting biomarker results. The most considerable challenges are the identification of their origin and linking their varying concentrations to particular taxa. Many biomarker molecules are not sufficiently specific to inform about individual species (Ellegaard et al. 2020). Therefore, more studies investigating the lipid composition of various taxonomic groups under various conditions are necessary to determine their taxonomic specificity and variance in concentration (Galloway and Winder 2015; Peltomaa et al. 2023). At the same time, the comparability of biomonitoring data over different time periods and between sites can be affected by various factors that cause potential biases. These challenges arise from technology improvement, potential loss of expertise, and changing taxonomy (Douda et al. 2023; Straile et al. 2013). Furthermore, observation-based data are often limited to macro- to microorganisms. Smaller organisms are detected, even though picoorganisms can make up a large part of the biomass (Zufia et al. 2022). For sedimentary approaches, it is much discussed how spatially representative sedimentary DNA and other sedimentary biomolecules are preserved in a single core (Wang et al. 2023). Nevertheless, sedimentary approaches are shown to detect general spatial and temporal trends, which we were also able to show here.

Recommendation and conclusions

Based on our results, we recommend careful validation of sedimentary approaches with observation-based approaches like count or biovolume monitoring data and historical records of environmental changes. Nevertheless, more research is necessary to investigate the reliability of these approaches as proxies. Therefore, comparison with microfossil data would help to investigate not only absolute abundance but also preservation issues in time periods prior to monitoring. Additionally, the focus on improving and increasing the reference databases should be prioritized to improve taxonomic resolution of metabarcoding results. Higher taxonomic resolution would allow us to interpret the results more accurately and gain more ecological information. By comparison with observation-based methods, we observed spatial variation due to environmental differences in the applicability of the different approaches. This highlights the need for validation for every location and the need for extensive monitoring data. Despite some differences, our results show that combining sedimentary approaches, sedaDNA metabarcoding, and biomarkers can provide complementary information on past historical trends. Therefore, our results show that observation-based data allow us to validate sedimentary approaches and use them as multiproxies to extend monitoring records.

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Conflicts of Interest

None declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

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