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Getting to the core of sea-ice reconstructions: Tracing Arctic sea ice using sedimentary ancient DNA

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A significant gap exists in our understanding of sea-ice variability on geological timescales. Recent advances using *sed*aDNA captures a larger fraction of the marine biodiversity than classical approaches. Accompanied by developments of new quantifiable *sed*aDNA-based proxies, a new era in paleo reconstructions may be on the horizon.

In a changing world with accelerating temperature rise, Arctic sea ice is declining at an unprecedented pace. Understanding past conditions of the Arctic cryosphere is key to building future climate projections, which are essential for decision-making and resolutions, e.g. towards our common UN Sustainable Development Goals (Fig. 1). For several decades, the Earth-science community has been looking for proxies (indicators) that can improve reconstructions of past sea-ice changes. Most proxies for past sea ice are records from marine sediments, alongside ice cores and other indicators, such as driftwood and whale macrofossils (reviewed in de Vernal et al. 2013). The most widely used proxies are archives of single-celled marine eukaryotes, termed protists. Several protists preserve well in the sediments owing to their silica frustules (e.g. diatoms), calcium carbonate tests (e.g. foraminifera), or refractory organic compounds (e.g. dinoflagellate cysts). Protist-derived biogeochemical tracers, including highly-branched isoprenoid (HBI) biomarkers, such as sea-ice biomarker IP_{25} (Kolling et al. 2020) and alkenones (Wang et al. 2021), are also widely used for paleo sea-ice reconstructions. All established sea-ice proxies have considerable limitations, preservation biases, and low taxonomic resolution or coverage, highlighting the need to identify new proxies to corroborate current paleo reconstructions.

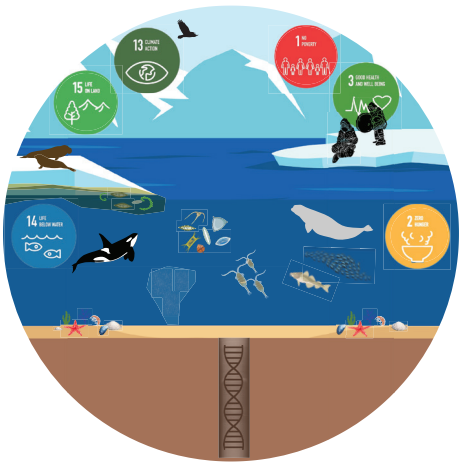

Figure 1: Simplified sketch of Arctic biodiversity, sea ice to the seafloor, illustrating organisms that may leave traces of DNA in the below sediment. As detailed in the United Nations Sustainable Development Goals (SDGs), sea ice can have a large impact on human societies, especially local communities that directly rely on sea ice and the biodiversity related to it. Sea ice is a source of food and income, for example, relevant for SDGs (1) no poverty, (2) zero hunger and, (3) good health and wellbeing. Recognized by the Intergovernmental Panel on Climate Change, the ongoing change in the cryosphere is a global concern; SDG 13 calls for climate actions to sustain the quality of life below water (SDG 14) and life on land (SDG 15). The authors of this article support the SDGs.

In recent decades, sedimentary ancient DNA (*sed*aDNA) has become a promising new tool for paleo reconstructions. The universal presence of DNA in all cellular organisms and some virus genomes makes it an ideal target molecule. In this article, we describe the latest developments in the application of *sed*aDNA in paleo sea-ice research, discuss the major challenges in the field, and suggest avenues for advancements.

Current advances in *sed*aDNA applications for sea-ice reconstructions

The unique diversity of Arctic sea-ice microbiota relative to the water column or seafloor sediments provides distinct targets for *sed*aDNA queries. *sed*aDNA application for sea-ice reconstructions does not demand prior knowledge about the site- or time-relevant paleobiodiversity. Analyses can be "tuned" for selective enrichment of functional groups, such as diatoms (Zimmermann et al. 2021), pan-Arctic microbial eukaryotes (Poulin et al. 2011), foraminifera (Pawłowska et al. 2020), taxa associated with sea-ice melting events (Boetius et al. 2013), sea-ice brine-associated viruses (Zhong et al. 2020), prey organisms and parasites of foraminifera (Greco et al. 2021), protist sources of sea-ice biomarkers (Brown et al. 2020), and sea-ice-dependent mammals (Kovacs et al. 2011) (Fig. 1).

*sed*aDNA measurements uniquely allow us to capture a broad spectrum of organisms in a single sample. Investigations embrace several sequencing technologies that can be used for diverse types of assessments, such as qualitative descriptions of Arctic sea-ice communities (De Schepper et al. 2019; Zimmermann et al. 2021) and quantitative measurements of sea-ice indicator taxa (De Schepper et al. 2019). Many sequencing applications depend on polymerase chain reaction (PCR) technology, which can introduce biases during amplification and significantly impact interpretations. The most advanced approach that both quantifies targeted marine *sed*aDNA sources and overcomes some PCR biases is metagenomics, using direct shotgun sequencing of the DNA extract. Shotgun sequencing combined with hybridization capture baits for research defined taxa have recently been applied to Southern-Hemisphere sediment records, providing new information about the diversity and authenticity of marine protist DNA signatures up to one million years old (Armbrecht et al. 2022). These advances highlight an exciting and important new avenue of *sed*aDNA research that can complement classical multi-proxy Arctic sea-ice reconstructions dating back to the Last Interglacial and beyond.

The molecular technologies used in *sed*aDNA studies are constantly evolving, allowing for developments of quantitative *sed*aDNA proxies. Specifically, a recent development has been the incorporation of Droplet Digital PCR (ddPCR), which was successfully implemented to quantify the low-abundance of highly informative sea-ice taxa (De Schepper et al. 2019). ddPCR is a powerful tool because it allows the absolute quantification of DNA targets from complex environmental samples. In contrast to the traditional method of real-time quantitative PCR, the ddPCR platform uses end-point quantification of target DNA, which makes it less susceptible to poor amplification efficiencies and PCR-inhibiting molecules commonly found in *sed*aDNA samples (Hindson et al. 2011; Kokkoris et al. 2021). The immense number of generated droplets delivers highly reproducible measurements and an excellent range in sensitivity that increases the potential to detect lowly abundant taxa (Hindson et al. 2011).

Challenges

The rapidly evolving field of paleogenomics was initially applied to study human evolution, and there is still much to be learned to accomplish optimal applications for past Arctic sea-ice reconstructions. The mineralogical composition of sediments plays a major role in DNA preservation, and we have a limited understanding of DNA-sediment interactions, leading to significantly variable DNA yields across sediment types (Sand and Jelavić 2018); we suspect that the conditions in the Arctic might be favorable. The differences in the preservation of extracellular

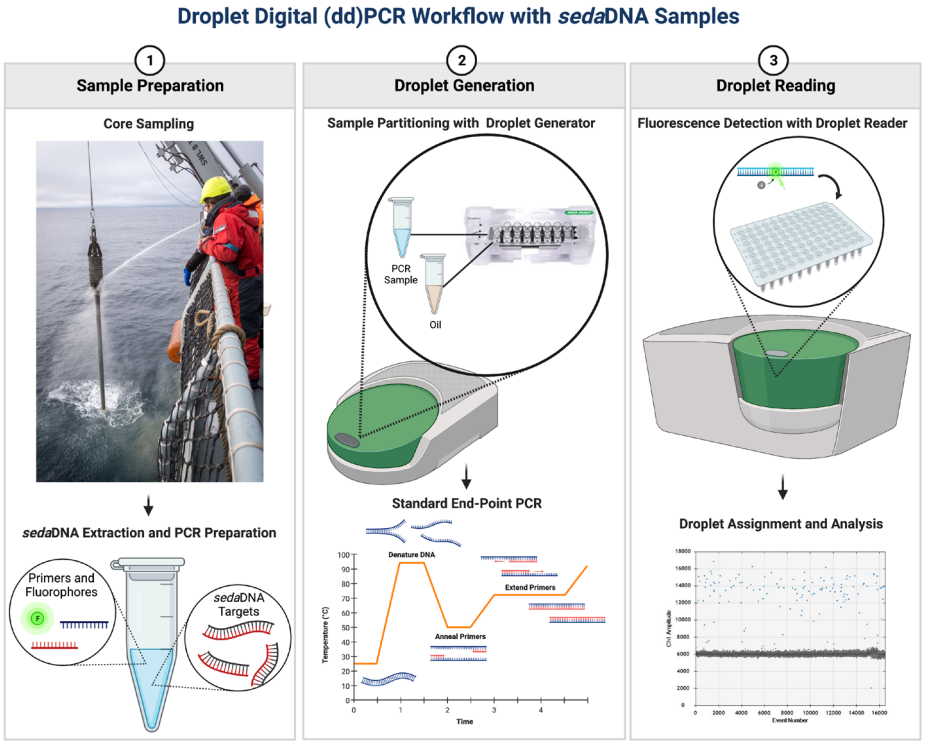

Figure 2: Essentials of the general ddPCR workflow for use with *seda*DNA applications (created with BioRender.com). Following the acquisition of *seda*DNA samples, 20-µL PCR samples (containing a fluorescent DNA reporting dye and *seda*DNA template) are partitioned into 20,000 droplets and then PCR-amplified to the end-point. The PCR-amplified droplets in each sample are then analyzed individually for fluorescence intensity, with each droplet classified as positive or negative. Poisson statistics are then applied to the proportion of positive droplets to calculate the absolute number of target DNA molecules in a sample.

vs. intracellular DNA and DNA degradation rates in sediments are poorly known. The conditions at the water–sediment interface and bioturbation may also affect the long-term preservation of DNA. Ancient DNA sequences are short, averaging around 100 base pairs (Armbrecht et al. 2021), and most often damaged, which hinders amplification if primers cannot bind to the targets. Available *seda*DNA extraction techniques have only moderately been compared. Consequently, the obtained results are difficult to relate and compare, causing a bias as the DNA acquired may not accurately reflect past biodiversity. Temporal constraints have yet to be established, determining how far back in time the method can be applied.

Specifically challenging for *seda*DNA application for sea-ice reconstructions is that data from modern conditions such as changes in community structure, and spatial distribution of environmental proxies are rare (e.g. Limoges et al. 2018). The taphonomy of DNA derived from sea-ice-associated organisms, vertical export from sea ice to the seafloor, and its eventual incorporation into marine sediment records are poorly understood. There is also uncertain ontology, as the surface of the DNA can differ between sea ice, the water column, or sediment. Although progress has been made, protists are still largely unrepresented in DNA databases. Many, if not the majority, of the "true" sea-ice-specific species (sea-ice algae), lack DNA and/or morphological references. This occurs for several reasons: sea-ice algae are difficult to collect, culture, and maintain; scientific investigations are limited and often conducted with a high taxonomic resolution; and there are likely still many species unknown to science.

Call for activities

(1) *Support taxonomists*: The importance of skilled taxonomists in keeping reference databases up to date, and thus making the accurate identification of sea-ice organism genetic signatures in *seda*DNA possible, cannot be overstated. "Blue sky" investments must be prioritized for maintaining and cultivating this invaluable expertise. Curated contributions to reference barcode, e.g. Protist Ribosomal Reference database PR2 (Guillou et al. 2012) and metaPR2 (Vaulot et al. 2022), metagenome, plastid, and mitogenome databases with rich associated metadata are essential for the identification of sympagic and sea-ice-associated genetic signatures in *seda*DNA.

(2) *Build the archive*: Bioinformatic advances using supervised machine learning (SML) can be applied to *seda*DNA records to extract the sea-ice "needle" from the *seda*DNA "haystack". Such data-driven scientific advances are empowered by coordinated research efforts to fill environmental-DNA (eDNA) archives with data from present-day sea ice, the water column, and surface sediments, which reflect different types, thicknesses, and ages of sea-ice cover. eDNA analyses generate community profiles similar to dinoflagellate cyst and diatom assemblages that are used to generate transfer functions. Transfer functions for sea-ice reconstruction based on eDNA community profiling is a tempting possibility. A rich and diverse sea-ice eDNA archive would facilitate rigorous validation to avoid statistical pitfalls. Transfer functions and/or SML algorithms trained on modern eDNA observations, in combination with remote satellite observations and traditional geochemical sea-ice proxies, could then be applied to multi-proxy paleorecords that include *seda*DNA to conduct qualitative and, ideally, quantitative extrapolation of past sea-ice extent in the Arctic. It is uncertain to which extent *seda*DNA analysis can be applied to historical sediment samples, i.e. non-archive samples that have been collected during the last decades and have been stored either freeze-dried or at 4°C, but their inclusion would certainly make a low-cost contribution toward archive development.

(3) *Collaboration and recruitment*: The development of *seda*DNA into a tool that is informative for sea-ice reconstructions in the Arctic will depend upon continued collaboration between geologists, paleoceanographers, paleoclimatologists, paleoecologists, taxonomists, and molecular ecologists. Shared research cruises, dedicated sessions at international conferences, theoretical and practical training courses, hackathons, international sea-ice *seda*DNA collaborative research projects, and cross-disciplinary recruitment programs can help to ensure sample acquisition, strengthen data analysis, encourage competence exchange, and develop training programs for the next generation of Arctic sea-ice researchers.

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