Final Report
Leibniz Competition

Predicting the future from signatures of the past:

using living sediment archives and ancient DNA to understand responses of marine primary producers to environmental changes

PHYTOARK

Project number K314/2020

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Leibniz Institute for Baltic Sea Research Warnemünde (IOW)

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Executive Summary

The **PhytoArk** project investigated the long-term dynamics of phytoplankton communities in the Baltic Sea, utilizing the past recovery of these ecosystems to improve future predictions regarding biodiversity loss and climate change. By bridging the fields of paleo-ecology, evolutionary genomics, and ecosystem modeling, the consortium reconstructed community dynamics throughout the Holocene and traced evolutionary adaptations in key species over millennia. The project achieved unprecedented success in "resurrection ecology." We successfully revived **40 strains of the diatom *Skeletonema marinoi* from sediment layers dating back ~6000 years**. This unique biological archive allowed for the direct measurement of phenotypic traits (growth rates, photosynthetic activity) and their linkage to whole-genome sequences across millennia. Additionally, the project reconstructed Holocene community structures using sedimentary ancient DNA (sedaDNA), identifying a **"Holocene baseline"** of stable community composition prior to the Common Era. To overcome limitations in existing tools, PhytoArk developed significant **novel resources** for the scientific community. Custom primers, hybridisation enrichment and ddPCR assays were designed and validated for targeted phytoplankton groups, including the proposition of a new indicator for Good Ecosystem Status using molecular genetic tools. Two open-source pipelines, ObiMAGIC and PR2-wormifier, were developed to solve specific analytical challenges in processing degraded sedaDNA. An advanced ecosystem model was created that integrates three functional groups (including Cyanobacteria) and allows for evolutionary adaptation, a significant advance over static ecosystem models. The project results have direct implications for environmental management. We demonstrated that sedaDNA and biomarkers can extend **HELCOM biomonitoring by centuries**, overcoming the limitations of short-term observational records. PhytoArk has been highly productive, yielding **nine peer-reviewed Open Access publications** to date. The project served as a catalyst for career development: all junior researchers published successfully, with two PhD candidates graduating with distinction (*summa* and *magna cum laude*) to date. The results directly supported PI Laura Epp's successful application for a **Heisenberg Professorship**, ensuring long-term sustainability of this research line. The methodological frameworks established by PhytoArk are now being transferred to new geographic regions and collaborative initiatives.

1. Achievement of objectives and milestones

PhytoArk investigated the millennial-scale dynamics of phytoplankton communities in the Baltic Sea to leverage the past recovery of these communities from biodiversity loss to improve predictions for the future. The project was structured around four core objectives: (1) reconstructing Holocene phytoplankton dynamics to identify responses to climate change and human impact (WP1); (2) tracing intraspecific genomic changes and evolutionary adaptation in key species (WP2); (3) determining the ecosystem consequences of altered traits under past and future climate scenarios (WP3); and (4) formulating strategies for long-term assessment based on pre-Anthropocene (>200 years) baselines (WP4).

WP1: Holocene Phytoplankton Community Dynamics We successfully secured long sediment cores (covering the Holocene) and short cores (last 200 years) during cruise EMB 262 - PHYTOARK in April 2021 (**M1.1**). Age models were developed, biomarker analyses were completed (**M1.2**) and sedimentary ancient DNA (sedaDNA) was extracted from over 300 horizons. While reconstructing community structure (**M1.3**), we addressed gaps in short barcode availability by developing and publishing new primers for two of the three targeted phytoplankton groups (Romahn et al. 2024), a significant methodological output of the project. Furthermore, we developed two novel bioinformatic pipelines to solve phytoplankton-specific analytical challenges (currently under review). These innovations allowed us to establish a robust pipeline for linking eDNA data to functional traits (**M1.4**).

WP2: Evolutionary changes in phytoplankton Resurrection experiments yielded a unique biological archive. We successfully revived 40 strains of the diatom *Skeletonema* *marinoi* from the Gotland Basin, covering 10 time points over the past ~6000 years (Bolius et al., 2025, **M2.1**). This unprecedented success allowed for detailed trait change analysis (**M2.2**), including growth rates under temperature/salinity gradients and high-sequence phenotyping (Bolius et al., 2025b, **M2.3**). The phenotypic data were successfully linked to whole-genome sequences. In parallel, we established *A. malmogiense* strains from the Gulf of Finland, though resurrection success for this species was lower than for *S. marinoi*. We adapted our approach to organismal constraints: as chain-forming *S. marinoi* precluded standard flow cytometry, we focused on interaction analyses using time-matched strains (Bolius et al., in rev., **M2.4**). Millennial-scale population genomics (**M2.5**) were conducted via a hybridisation capture methodology developed specifically for this system (Schmidt et al. 2025). This required droplet digital PCR screening to optimize sample selection (Schmidt et al. 2024). We successfully tracked genomic variation and spatial distribution over time, targeting both organelles and the whole genome of *S. marinoi* (Schmidt et al. 2025, in prep).

WP3: Modeling changes in Baltic phytoplankton We developed an ecosystem model for the Baltic Sea that integrates major functional groups and allows for evolutionary adaptation (**M3.1**). To better represent natural ecosystem complexity, we expanded the original scope to include three functional groups (adding Cyanobacteria) rather than the planned two. Simulations were performed to project community changes (Hochfeld & Hinners, 2024, **M3.2**) and assess the impact of phytoplankton adaptation on ecosystem functioning (Hochfeld & Hinners, 2024b, **M3.3**). A key finding was the comparative role of intraspecific variability versus interspecific competition. To bridge the timeline difference between the availability of WP1/WP2 data and the modeling schedule, we validated the model using literature data and published a collaborative "Ideas and Perspectives" paper outlining the roadmap for integrating sediment archive data into future ecosystem models (Hochfeld et al., 2025).

WP4: Management and Baselines The project successfully demonstrated that sedaDNA and biomarkers can extend the HELCOM biomonitoring scope by centuries (Romahn et al. 2025, **M4.1)**. We applied the HELCOM Dia/Dino index to sedaDNA data (Schmidt et al. 2024), highlighting that long-term biodiversity assessment requires a multi-observational approach. We identified a multi-millennial period of stability in community composition prior to the Common Era (Romahn 2025, **M4.2**) as a robust "Holocene baseline." Intraspecific genetic diversity of key diatoms also remained largely stable (Schmidt et al. 2025). These findings have been communicated to HELCOM to integrate them into monitoring assessment recommendations (**M4.3**). The development of the GES tool (**M4.4**) was delayed due to personnel reasons (detailed in *Activities and obstacles*), but the conceptual framework established by the project remains valid for future implementation.

2. Activities and obstacles

Most planned activities were executed successfully, with strategic adjustments made to navigate external factors. Although the project launch coincided with the onset of the Covid-19 pandemic, sediment coring and laboratory analyses were initiated with minimal latency. We maintained a high level of exchange through hybrid communication formats and regular cross-lab activities, ensuring excellent data flow. This close collaboration resulted in a high volume of joint, interdisciplinary publications.

WP1: Fieldwork required an early strategic adjustment: due to authorization constraints in the Gulf of Finland, the coring site was relocated westward to a position that successfully approximated the originally targeted environmental conditions. Sampling planned at sites in the Bothnian Sea was also not possible. Developing the age models required a rigorous, iterative refinement process to resolve uncertainties in ^{14}C measurements within the oldest horizons. This ensured high robustness for the final geological and biomarker data. A major innovation of WP1 was the development of novel molecular tools, driven by the limitations of existing resources. As literature searches yielded no suitable dinoflagellate-specific primers and standard cyanobacterial primers proved too long for degraded sedaDNA, we designed and validated new primer sets. Furthermore, we developed **ObiMAGIC** (https://github.com/jromahn/ObiMAGIC/), a bioinformatic pipeline for flexible sedaDNA processing, and **PR2-wormifier** (https://github.com/jromahn/PR2-wormifier) for curating marine protist databases. These tools fill a significant gap in the field of phytoplankton sedaDNA analysis. While this necessary methodological development extended the timeline for compositional analyses, these are now complete and central to the associated PhD dissertation (Romahn submitted). Joint species distribution modeling tools were implemented in collaboration with O. Ovaskainen, laying the groundwork for imminent functional analyses.

WP2: The population genomic analyses required adaptive solutions to overcome reference data scarcity. First, the lack of functional annotation for *Apocalathium malmogiense* was addressed by generating a dataset of common SNPs. Second, for *Skeletonema marinoi*, we overcame the initial limitation of a single reference genome by sequencing our own resurrected strains and extracting variable loci from organelle sequences via hybridisation enrichment. While sequencing service delays and a challenging resurrection process for *A. malmogiense* impacted the original schedule, we achieved an unexpected breakthrough: the revival of *S. marinoi* strains from millennial time scales. In response to this unique opportunity we strategically shifted the focus from eco-evo competition experiments to a deep trait analysis of these millennia-old *S. marinoi* cultures (Bolius et al. 2025b), maximizing the scientific novelty of the results.

WP3: Evolutionary processes were successfully integrated into the ecosystem model as planned. To mitigate a timeline offset in data availability from project partners, we utilized high-quality literature data for model setup and validation, ensuring that simulations of phytoplankton groups under climate change scenarios proceeded without interruption. The publication of the first community change manuscript (Hochfeld et al., 2025) was slightly delayed by an extended peer-review process. Personnel changes were managed to ensure continuity: Following Prof. Inga Hense's sabbatical and subsequent career change, Dr. Jana Hinners (Hereon) assumed scientific leadership of the work package, becoming the lead author of its three key publications. Prof. Hense maintained administrative oversight until August 2024, after which Prof. Dr. Elisa Schaum (U. Hamburg) took over administration.

WP4: From 2023 onward, we engaged in intensive data harmonization and dialogue with HELCOM. Key outcomes include a systematic comparison of decadal HELCOM monitoring datasets with WP1 sedaDNA/biomarker data (Romahn et al. 2025) and the development of a DNA-based Dia/Dino index to extend eutrophication monitoring back by thousands of years. Additionally, we defined a baseline time interval of stable community composition using multiple biodiversity metrics. The finalization of the Good Environmental Status (GES) software tool (M4.4) was constrained by the long-term illness of a key team member; however, the conceptual framework for the tool has been established.

3. Results and successes

The project achieved a high level of productivity, with **nine peer-reviewed papers published** by the time of this report submission, and several additional manuscripts currently under review. Notably, all funded junior researchers (three PhD candidates and one Postdoc) successfully published as lead and co-authors, demonstrating the collaborative nature of the consortium. The project served as an excellent platform for early-career researchers. Two PhD candidates have already defended their theses with outstanding success (one ***summa cum laude***, one ***magna cum laude***), while the third defense is scheduled for January 2026. The project integrated research-based teaching, supervising **five Bachelor's and Master's theses**, introducing the next generation of scientists to state-of-the-art paleo-ecological methods. Findings were presented at prestigious international conferences, including the Gordon Research Conference on Marine Microbes, INQUA, and the European Phycological Congress. The team actively engaged in science communication, presenting at events such as the *Warnemünder Abend* (IOW), *Open Ship* events, and the *Match Day of the Initiative NAT*. The expertise and data generated by PhytoArk have catalyzed significant follow-up activities. The project outcomes were instrumental in the **successful Heisenberg Programme application** of PI Epp, securing long-term leadership in the field. The developed methodologies are being transferred to new geographic regions and contexts, including new initiatives for sedaDNA analyses in Africa and the preparation of a collaborative DFG proposal in Southern Germany.

4. Equal opportunities, career development and internationalisation

The project successfully supported the career progression of its junior staff through integration into structured graduate programs and targeted mentoring. **Alexandra Schmidt** (University of Konstanz) benefited from the training environment of the *International Max Planck Research School for Quantitative Behaviour, Ecology and Evolution*. She defended her PhD with distinction (*summa cum laude*) and was immediately retained as a postdoctoral researcher at the University of Konstanz (from June 2025). **Juliane Romahn** (Senckenberg/Frankfurt) took on a leadership role as Speaker of the Young Scientists at the LOEWE Centre for Translational Biodiversity Genomics (TBG). She has submitted her thesis (defense scheduled for January 2026) and successfully secured a postdoctoral position at Senckenberg starting February 2026. This position is part of a specific qualification program designed to support her in developing independent research proposals to establish her own junior research group. **Isabell Hochfeld** was embedded in the *School of Interdisciplinary Climate System Sciences* (SICSS), which provided specific career measures for female scientists. She defended her thesis with high honors (*magna cum laude*) and has successfully transferred to the public sector, now working as a scientist at the Federal Waterways Engineering and Research Institute (BAW) in Hamburg. **Dr. Sarah Bolius** (IOW) utilized the project's success to drive her independence. Currently employed on a follow-up project at IOW, she built on PHYTOARK results to submit a proposal to the German Research Foundation (DFG) for her own position (*Eigene Stelle*).

The project has been instrumental in consolidating the careers of senior female scientists and managing leadership transitions effectively. **Prof. Dr. Laura Epp** successfully leveraged the project's results on sedaDNA-based intraspecific diversity to apply for a **Heisenberg Professorship**. Her appointment procedure for a permanent W3 professorship in "Environmental Genomics" at the University of Konstanz is currently near completion. **Dr. Anke Kremp** used the project to reintegrate into the German research landscape after a long tenure in Finland. The project allowed her to demonstrate her expertise at the IOW in Warnemünde and significantly strengthen her professional network within Germany. **Dr. Jana Hinners** (Hereon) demonstrated strong adaptive leadership. Following Prof. Inga Hense's career move to the non-academic sector, Dr. Hinners successfully assumed the scientific lead of WP3, establishing herself as a principal investigator and lead author of the work package's output. **Dr. Miklós Bálint** further developed his management skills through selected participation in the *Leibniz Leadership Academy* (2024).

5. Structures and collaboration

The project established a deeply integrated research structure across the four partner teams, evidenced by the joint execution of all WPs and a high output of co-authored publications. The collaboration was sustained through a multi-tiered communication strategy: yearly in-person retreats facilitated deep scientific exchange, while bi-monthly online meetings (held separately for PIs and for PhDs/Postdocs) ensured continuous operational alignment. This framework supported seamless cooperation on all operational levels, from joint field campaigns (coring) and lab work (sedaDNA, proxies) to cross-disciplinary synthesis (model parameterization, bio-monitoring harmonization).

The consortium successfully expanded its expertise through targeted external collaborations. We partnered with Prof. Otso Ovaskainen (University of Jyväskylä/Helsinki) to adapt joint species distribution models and the Laboratoire d'Ecologie Alpine (LECA Grenoble) for advanced ancient eDNA processing. Prof. Markus Pfenninger (SGN) provided critical guidance on genomic characterization and bioinformatics for resurrected strains. Access to high-throughput phenotyping infrastructure was secured through a new collaboration with the University of Rostock. Collaboration with Dr. Elinor Andren (Södertörn University) and Dr. Sirpa Lehtinen (Finnish Environment Institute/HELCOM) strengthened our analysis of fossil diatom records and Gulf of Finland interaction dynamics.

The collaborative "Ideas and Perspectives" paper (Hochfeld et al., 2025) exemplifies the project's international visibility. It integrated the PhytoArk consortium with leading global experts in phytoplankton ecology and ecosystem modeling, including Ben Ward

(Southampton), Elena Litchman and Chris Klausmeier (Michigan State), and Lutz Becks (Konstanz), alongside Jerome Kaiser and Helge Arz (IOW).

6. Quality assurance

The project was conducted in strict compliance with the guidelines of the DFG and the Leibniz Association. No animal testing was required or conducted during the course of this research. Field sampling activities were carried out in accordance with international regulations, and all necessary permits for sampling in national waters were secured. In alignment with the Leibniz Association's Open Access Policy, 100% of the peer-reviewed publications resulting from this project have been published under Open Access licenses. To ensure the long-term reusability of the project's extensive datasets, we strictly followed the FAIR principles. Published sequencing data have been deposited in the European Nucleotide Archive (ENA) / NCBI Sequence Read Archive (SRA). The bioinformatic pipelines developed within the project (ObiMAGIC, PR2-wormifier) are open source and hosted on GitHub. Remaining sediment material and DNA extracts are stored at the participating institutes (SGN, IOW) and are available for future research upon request.

7. Additional resources

The participating partners contributed significant core funded personnel resources to complement the grant funding and ensure the project's successful implementation. SGN dedicated 6 person-months of the W3 Principal Investigator and 2 person-months of an E11 Lab Manager to the project. Furthermore, SGN financed 19 person-months of the PhD candidate (E13 65%), ensuring the completion of the dissertation and publication outputs beyond the grant period. University of Konstanz contributed 2 person-months of the W1 Principal Investigator and 1 person-month of technical support (E8). IOW provided essential ship time for the project: the central sampling campaign was conducted on the Research Vessel Elisabeth Mann Borgese in April 2021 (Cruise No. EMB 262). The ship time, including the associated logistical and technical support, was a prerequisite for the successful retrieval of the sediment archives used in WP1 and WP2. To ensure the long-term continuation of this research line and the sustainable management of the generated data and biological resources, SGN has committed to funding a full scientific position (E13, 36 person-months) from its core budget for the period 2026–2029. This position will focus on building upon the PhytoArk results and integrating them into the institute's long-term research strategy.

8. Outlook

The PhytoArk project has opened several critical directions for future research. Immediate follow-up work includes the finalization of a manuscript analyzing phytoplankton community shifts over the last 7,000 years using the generated sedaDNA metabarcoding data, as well as a publication on the full genomic details of the resurrected strains of *S. marinoi* over 7000 years. Building on this foundation, future efforts will move beyond single-group analyses to integrate top-down pressures and broader biotic interactions, providing a more holistic view of climate-driven community dynamics. Furthermore, we aim to systematically evaluate the potential of sedaDNA as a quantitative tool for paleoenvironmental reconstruction, benchmarking it against established methods such as diatom microfossil analysis. A key strategic priority is the operational integration of sediment archive data into ecosystem modeling, as outlined in our collaborative "Perspectives" paper (Hochfeld et al., 2025). This will be essential for validating models against long-term historical variability. Finally, we will continue our dialogue with HELCOM to implement the newly tested DNA-based diversity indices as standard tools for monitoring eutrophication and biodiversity baselines, effectively bridging the gap between paleo-science and modern environmental management.