A Phytoplankton Species under Global Warming

Insights from Resurrection Experiments and Ecosystem Modelling

Jana Hinners

A Phytoplankton Species under Global Warming
— *Insights from Resurrection Experiments and Ecosystem Modelling*

DISSERTATION

zur Erlangung des Doktorgrades der Naturwissenschaften
an der Fakultät für Mathematik, Informatik und Naturwissenschaften
im Fachbereich Biologie der Universität Hamburg

Jana Hinners

Hamburg 2018

Gutachter:

Prof. Inga Hense

Dr. Anke Kremp

Tag der mündlichen Prüfung:

12. Oktober 2018

Cover design by Sophia Schönborn

Acknowledgements

I would like to thank everyone who supported me throughout the course of this PhD project.

First, I want to thank Inga Hense for all her time and support, always being there if I needed advice, for thoughtful corrections as well as freedom to find my own way. I am equally grateful to Anke Kremp for her support, for sharing her scientific expertise, for lots of good advice and inspiring scientific discussions.

I am thankful to Gerhard Schmiedl for chairing my PhD panel and being part of my PhD committee. Thanks also to Elisa Schaum and Dieter Hanelt for interesting scientific discussions and for being part of my committee.

I wish to acknowledge the help provided by all technical staff as well as my student assistants at the IMF, who helped me with the conduction of my experiments. Thanks to Bettina Walter and Justus van Beusekom for their help in getting started in the lab. I would like to thank Niko Lahajnar for the conduction of elemental analyses. Thanks moreover to my students, Anselm and Felicitas, for nice discussions and good work. Special thanks also to the SICSS graduate school and my fellow SICSS PhD students for support, as well as informative and enjoyable events. I am also grateful to Karin and Berno S. Mattern for awarding me as early career researcher in marine science and enabling me thereby to explore further opportunities for myself.

I want to express my warm thanks to all people at the IMF, who made this place become a second home, namely Rémy, Camilla, Romain, Xo, Elisa, Marilisa, Eleanor, Heike, Katharina and Dominik. Many thanks to Camilla for becoming such a good companion and flatmate! And special thanks to Rémy for brightening up the final year of my PhD! I would like to thank all my friends, for helping to remind me that there is a life outside science. Warm thanks to Sophia, for designing the cover of this thesis. And finally, I would like to thank my family for always being there for me.

Thank you.
Jana

ABSTRACT

Phytoplankton represent a crucial component of the marine ecosystem – not only as a major food source for higher trophic levels and as oxygen producers, but also due to their important role for global biogeochemical cycles and climate in general. Owing to their large population sizes and short generation times, phytoplankton can adapt rapidly to changing environmental conditions; this has been previously demonstrated in laboratory studies. Whether recent climatic changes have already caused adaptive responses of phytoplankton in nature, remains hitherto unknown.

Resurrection experiments with phytoplankton resting stages from the sediment allow to travel back to the past by digging deeper into the sediment. Analyses of resurrected phytoplankton from different sediment layers can indicate whether and how changing environmental conditions lead to adaptive responses in phytoplankton. Since many environmental factors usually change simultaneously in nature, it remains however a challenge to fully understand the causes of phytoplankton adaptation. Ecosystem modelling can be of help to interpret such changes in phytoplankton. Here, I combined resurrection experiments and ecosystem modelling for the first time to understand whether a phytoplankton species has already adapted to the past century of global warming.

In preparation for resurrection experiments, cysts of three spring-blooming dinoflagellate species from a dated sediment core from the central Gulf of Finland were analysed. This region experienced a substantial temperature increase over the past century. Increasing cyst abundances over the past decades were detected for two species, correlating well with monitoring data. Additionally, one species turned out to be particularly suitable for resurrection experiments: *Apocalathium malmogiense* expressed a cyst longevity of more than hundred years.

Resurrected strains of *A. malmogiense* from two different sediment layers were used to perform experiments comparing temperature-dependent traits of recent, ca. 2 years old, and historic, ca. 100 years old, strains. No significant change in the growth reaction norm or cell size was observed, leading to the conclusion that both traits did not alter in response to increasing temperatures over the past century. However, a significant reduction in the cyst formation (encystment) rate in recent compared to historic strains was detected. The formation of resting cysts is an annually occurring process which ensures survival under unfavourable environmental conditions and leads to the termination of the spring bloom. The observed decrease in the encystment rate may thus represent an adaptive response to global warming, since it could allow for a prolonged spring bloom, even if the temperature threshold for encystment is already exceeded.

To test this hypothesis, an advanced ecosystem model which allows for adaptation was used. Based on this model, it could be shown that global warming can indeed have caused an adaptive response in the encystment rate. The magnitude of change observed in resurrection experiments was however only reproduced if additional factors, such as

eutrophication of the Baltic Sea or a temperature-dependent cyst mortality, were considered. Furthermore, since extreme weather events are expected to increase in intensity and frequency in the future, the influence of short-term temperature fluctuations on the adaptive response in the encystment rate was investigated. These analyses revealed that temperature fluctuations, by occasionally causing extreme conditions, represent a stronger selection pressure and can severely accelerate the adaptive response.

This thesis represents a comprehensive analysis of how a phytoplankton species responded to the past century of global warming. Combining resurrection experiments and ecosystem modelling, I could demonstrate that there is a strong indication that phytoplankton adaptation has already been taking place in nature in response to the past century of global warming. Moreover, adaptive responses to warming may not necessarily manifest itself in changes in the reaction norm or cell size, but can also occur in temperature-dependent life cycle traits. These findings illustrate the huge potential of both resurrection experiments as well as advanced ecosystem modelling to understand how phytoplankton respond to environmental changes.

ZUSAMMENFASSUNG

Phytoplankton stellt eine wesentliche Komponente des marinen Ökosystems dar – nicht nur als Nahrungsquelle für höhere trophische Ebenen und als Sauerstoffproduzent, sondern auch durch seine wichtige Rolle für globale biogeochemische Zyklen und für das Klima im Allgemeinen. Aufgrund großer Populationsgrößen und kurzer Generationszeiten kann sich Phytoplankton schnell an sich ändernde Umweltbedingungen anpassen; dies wurde zuvor in Laborstudien gezeigt. Ob die jüngsten klimatischen Veränderungen bereits eine Anpassung von Phytoplankton in der Natur ausgelöst haben, ist bisher nicht bekannt.

"Wiederbelebungsexperimente" mit Phytoplankton-Ruhestadien aus dem Sediment erlauben in die Vergangenheit zu reisen, indem man tiefer ins Sediment gräbt. Untersuchungen von wiederbelebtem Phytoplankton aus unterschiedlichen Sedimentschichten können Hinweise geben, ob und wie sich ändernde Umweltbedingungen zu Anpassungen bei Phytoplankton führen können. Da sich in der Natur normalerweise viele Umweltfaktoren gleichzeitig ändern, bleibt es jedoch eine Herausforderung, die Gründe für Anpassungen bei Phytoplankton vollends zu verstehen. Ökosystem-Modellierung kann hilfreich sein um solche Phytoplankton-Veränderungen zu interpretieren. Hier habe ich zum ersten Mal Wiederbelebungsexperimente und Ökosystem-Modellierung kombiniert, um zu verstehen, ob sich eine Phytoplankton-Art bereits an die Klimaerwärmung des letzten Jahrhunderts angepasst hat.

Zur Vorbereitung von Wiederbelebungsexperimenten wurden Zysten von drei frühjahrsblühenden Dinoflagellaten-Arten von einem datierten Sedimentkern aus dem zentralen Finnischen Meerbusen analysiert. Diese Region hat sich innerhalb des letzten Jahrhunderts bereits stark erwärmt. Es wurden zunehmende Zystenzahlen über die letzten Jahrzehnte für zwei Arten festgestellt; diese Ergebnisse korrelieren mit Beobachtungsdaten. Eine Art stellte sich außerdem als äußert gut geeignet für Wiederbelebungsexperimente dar: *Apocalathium malmogiense* zeigte eine Zysten-Langlebigkeit von über 100 Jahren.

Wiederbelebte *A. malmogiense* Stämme aus zwei unterschiedlichen Sedimentschichten wurden genutzt um temperaturabhängige Eigenschaften rezenter, ca. 2 Jahre alter und historischer, ca. 100 Jahre alter Stämme in Experimenten zu vergleichen. Es wurden keine signifikanten Veränderungen in der Reaktionsnorm der Wachstumsrate und der Zellgröße festgestellt, was zu der Schlussfolgerung führt, dass beide Eigenschaften sich nicht im Zuge der Klimaerwärmung verändert haben. Jedoch wurde eine signifikante Verringerung in der Zystenproduktionsrate (Enzystierungsrate) in rezenten, verglichen mit historischen Stämmen entdeckt. Die Produktion von Ruhestadien ist ein jährlich auftretender Prozess, der das Überleben unter ungünstigen Umweltbedingungen ermöglicht und zur Beendigung der Frühjahrsblüte führt. Die beobachtete Verringerung in der Enzystierungsrate könnte demnach eine Anpassung an die Klimaerwärmung darstellen, da dies ermöglichen könnte, dass die Frühjahrsblüte weiter besteht, auch wenn

die Temperaturschwelle zur Enzystierung bereits überschritten wurde.

Diese Hypothese wurde mithilfe eines erweiterten Ökosystem-Modells, das Anpassung zulässt, getestet. Mithilfe dieses Modells konnte gezeigt werden, dass die Klimaerwärmung tatsächlich zu einer adaptiven Reaktion in der Enzystierungsrate geführt haben kann. Die Stärke der Reaktion konnte jedoch nur reproduziert werden, wenn weitere Faktoren, wie die Eutrophierung der Ostsee oder eine temperaturabhängige Zystenmortalität, berücksichtigt wurden. Da angenommen wird, dass extreme Wetterereignisse in der Zukunft an Intensität und Häufigkeit zunehmen werden, wurde desweiteren der Einfluss kurzfristiger Temperaturschwankungen auf die adaptive Reaktion in der Enzystierungsrate untersucht. Diese Analysen zeigten, dass Temperaturschwankungen, durch gelegentlich extreme Temperaturbedingungen, einen stärkeren Selektionsdruck erzeugen und die adaptive Reaktion massiv verstärken können.

Diese Thesis stellt eine umfassende Analyse dar, wie eine Phytoplankton-Art auf die Klimaerwärmung der letzten 100 Jahre reagiert hat. Durch die Verbindung von Wiederbelebungsexperimenten mit Ökosystem-Modellierung konnte ich zeigen, dass es starke Hinweise dafür gibt, dass es bei Phytoplankton in der Natur bereits eine Anpassung an die Klimaerwärmung des letzten Jahrhunderts gegeben hat. Außerdem müssen sich adaptive Reaktionen nicht zwingend in einer Veränderung der Reaktionsnorm oder der Zellgröße offenbaren, sondern können auch bei temperaturabhängigen Lebenszyklus-Eigenschaften auftreten. Diese Ergebnisse veranschaulichen das große Potential von Wiederbelebungsexperimenten und erweiterter Ökosystem-Modellierung, die Reaktionen von Phytoplankton auf sich ändernde Umweltbedingungen zu verstehen.

Contents

Acknowledgements **i**

ABSTRACT **iii**

ZUSAMMENFASSUNG **v**

1 INTRODUCTION **1**

2 STUDIES OF THIS THESIS **9**

2.1 Study I: Cyst profiles and viability across the sediment 13

2.2 Study II: Thermal resurrection experiments 27

2.3 Study III: Ecosystem modelling on adaptation to global warming 41

2.4 Study IV: Modelling the influence of temperature fluctuations on adaptation 55

3 SUMMARY & CONCLUSIONS **63**

4 APPENDICES **67**

4.1 Appendix I 69

4.2 Appendix II 71

4.3 Appendix III 73

4.4 Appendix IV 77

REFERENCES **79**

Eidesstattliche Versicherung **91**

1 | INTRODUCTION

In Frank Schätzing's novel *The Swarm*[1] powerful microorganisms from the ocean manipulate dynamics on Earth to fight against humans and their increasing threats to nature. Even though this biological thriller is fictional, it contains some essential, true elements. There actually exist mighty marine microorganisms, namely phytoplankton, which shape life in water as well as on land. Furthermore, they have the potential to respond to anthropogenic changes to the environment. Due to their large population sizes and short generation times, phytoplankton have the potential to rapidly adapt to changing environmental conditions, such as global warming. Some of these phytoplankton species form resting stages, which can stay alive in the sediment for more than a century, allowing them to survive even long-lasting unfavourable environmental conditions. These dormant resting stages, buried deep in the sediment, represent a unique opportunity to travel back in time. Thereby, they allow to trace how environmental changes may have affected phytoplankton over time. In this thesis, I utilise the long-term survival capacity of a phytoplankton species, to look for signs of adaptation to the past century of global warming. To do so, I follow a comprehensive approach which combines resurrection experiments together with advanced ecosystem modelling.

In this introduction I firstly explain the importance of phytoplankton for the global ecosystem, secondly portray the recent climate change and thirdly describe the potential of phytoplankton to adapt to such an environmental change. I continue by explaining the two state of the art research approaches which I apply in this thesis; resurrection experiments and modelling. Finally, I describe the outline of this thesis and present the study organism, which this dissertation is focused on.

The role of phytoplankton in the ecosystem

Although tiny in size[2], phytoplankton organisms have a huge influence on the ecosystem. They represent the main primary producer in the ocean and are the base of the marine food web. Every year, phytoplankton produce about 50 Pg of C, which is comparable to the amount of primary production on land (Field et al. 1998). They are therefore also responsible for half of the global oxygen production.

In the process of photosynthesis, phytoplankton take up dissolved CO_2 from the

1. Schätzing, F. 2012. *The swarm: a novel of the deep*. Hachette UK.
2. the size of phytoplankton organisms ranges between 0.2 μm – 2 mm, (Johnson and Sieburth 1982; Finkel et al. 2009)

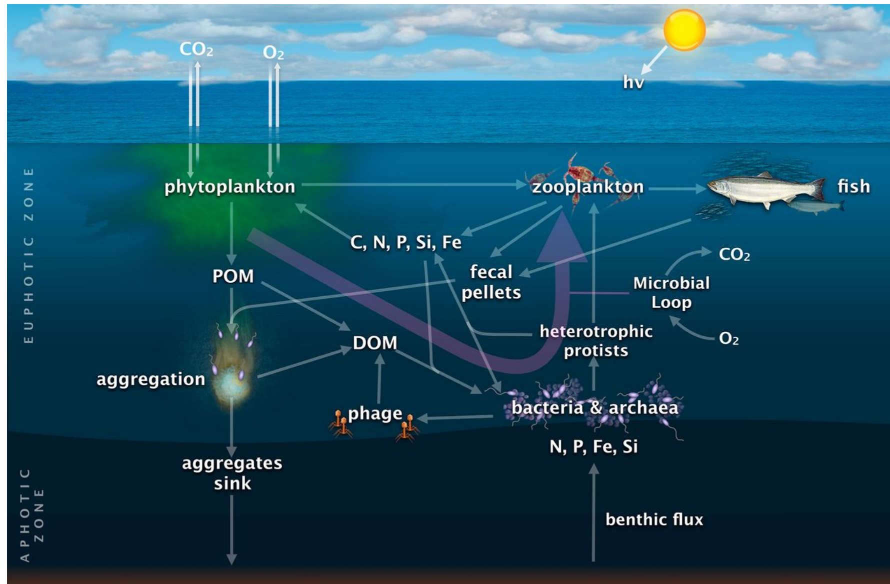


Figure 1.1: The marine food web. Phytoplankton grow close to the water surface, fixing CO_2 and releasing O_2 in the process of photosynthesis. Phytoplankton biomass is transferred to higher trophic levels, such as zooplankton or fish, or released into the water as particulate and dissolved organic matter (POM & DOM). The uptake of POM and DOM by bacteria and archea, which in turn are a food source for zooplankton, comprises the microbial loop (purple arrow). Part of the phytoplankton biomass sinks into deeper water layers where it is stored for thousands of years. Graphic from (Worden et al. 2015).

surrounding sea water and release O_2 to produce organic matter. One part of the phytoplankton biomass is then transferred to higher trophic levels such as zooplankton and fish (Pomeroy 1974), comprising the classical marine food chain, see Fig. 1.1. Another part is transformed into particulate and dissolved organic matter (POM & DOM), for example due to viral attacks and cell death, and is taken up by bacteria and archea, which in turn also represent a food source for zooplankton. This recycling pathway of organic matter within the surface ocean is called microbial loop (Worden et al. 2015).

About one third of the biomass is not recirculated in the upper ocean, but eventually sinks to deeper water layers, where it can remain for thousands of years (Raven and Falkowski 1999). This export production of biomass into deeper water layers is referred to as biological pump (Falkowski et al. 1998), since it allows the ocean to take up CO_2 and transport it into the ocean interior, making the ocean a net carbon sink which partly buffers the increasing concentrations of greenhouse gases in the atmosphere (Khatiwala et al. 2013).

Next to their role in the global carbon cycle, phytoplankton also influence the biogeochemical cycling of nitrogen (Zehr and Kudela 2011), silica (Tréguer and De La Rocha 2013) and phosphorous (Benitez-Nelson 2000), and even feedback on ocean physics. Dense blooms of phytoplankton at the sea surface can increase the light absorption and surface albedo, and decrease the input of wind energy, thereby affecting the stratification of the water column (Sathyendranath et al. 1991; Kahru et al. 1993;

Joehnk et al. 2008; Hense et al. 2017).

All in all, phytoplankton represent a central component of the marine food web, as well as of global biogeochemical cycles and the climate in general, and in this way they influence life in water as well as on land. Changes in phytoplankton productivity, distribution, community structure or seasonality can therefore have far-reaching consequences, making phytoplankton essential study objects under ongoing environmental changes.

A century of global warming

Over the past century humans have severely reshaped the earth's environment. Since the beginning of industrialisation around 1850, more and more greenhouse gases are being released into the atmosphere, causing a global warming. On a worldwide scale, the increase in greenhouse gases has caused a temperature increase by 1°C since the beginning of industrialisation (Pachauri et al. 2015). On a local scale however, the changes may diverge, with regions in the northern high latitudes warming at a faster rate (Cohen et al. 2014).

One region, which experienced a drastic temperature increase over the past century, is the Northern Baltic Sea. New long-term data from a monitoring station in the Gulf of Finland revealed that the sea surface temperature here has increased by 3°C over the past 100 years (Laakso et al. 2018). The warming also manifests itself in the earlier breakup of sea ice in spring. Over a period of 70 years, the start of sea ice melting has advanced by 10-15 days (Jevrejeva 2000).

This change in temperature has already resulted in changes in the biosphere (Reusch et al. 2018). In combination with eutrophication, increasing temperatures have caused a more than 10-fold increase of dead zones on the Baltic Sea floor (Carstensen et al. 2014) and in combination with fishing pressure, they have led to a severe regime shift in the fish community (Möllmann et al. 2009). On the side of the primary producers, the increasing temperatures have been accounted for a shift in the phytoplankton spring bloom composition (Suikkanen et al. 2007; Klais et al. 2011), an earlier onset of spring blooms (Klais et al. 2013) and, together with eutrophication, to increasing cyanobacteria blooms (Finni et al. 2001; Kahru et al. 2007).

Within the next hundred years, a further increase in temperature is expected, along with an increase in intensity and frequency of extreme climatic events (Easterling et al. 2000; Beniston et al. 2007; Field 2012). These environmental changes can represent strong drivers influencing phytoplankton and altering the composition and functioning of marine ecosystems.

Evolutionary potential of phytoplankton

Owing to their large population sizes and short generation times, phytoplankton have the potential to rapidly adapt to environmental changes. In experimental evolution studies, phytoplankton adapted within less than 1000 generations (corresponding to a

time frame between few months and one year under laboratory conditions) to altered environmental conditions. Specifically, adaptation has been described among others in response to elevated CO_2 conditions (Collins and Bell 2004), to high temperatures (Padfield et al. 2016), to the combination of both drivers (Schlüter et al. 2014), as well as to multiple environmental changes simultaneously (Brennan et al. 2017). In the latter case however, few drivers, temperature among them, play the dominant role in shaping the outcome of the adaptive response (Brennan et al. 2017).

As ectotherm organisms, phytoplankton are very sensitive to temperature changes. Important functional traits affecting their life cycle, growth and metabolic rates are temperature-dependent (Kremp and Parrow 2006; Thomas et al. 2012; Padfield et al. 2016). Global warming may therefore act on various temperature-dependent traits, leading to evolutionary changes. In experimental evolution, adaptations to warming have been observed in reaction norms (Schlüter et al. 2014; Listmann et al. 2016; Padfield et al. 2016), which describe the performance or growth rate of organisms for a range of different temperatures. Moreover, the cell size of most phytoplankton organisms decreases in direct response to increasing temperatures (Atkinson et al. 2003), but can increase again after adaptation to high temperatures (Brennan et al. 2017). Thus, the cell size is also a trait with a high evolutionary potential. Temperature is further known to influence life cycle processes of phytoplankton, such as resting stage formation and germination (Bravo and Anderson 1994; Ellegaard et al. 1998; Kremp et al. 2009; Figueroa et al. 2011), and can control the success of bloom formation (Kremp et al. 2008). As a result, temperature-dependent life cycle traits may also respond to global warming.

Hence, global warming may act and may have already acted on phytoplankton due to their high evolutionary potential and can cause changes in their temperature-dependent traits. Evolutionary experiments can, due to their constrained laboratory conditions, however only indicate potential changes in phytoplankton. How phytoplankton evolve in response to increasing temperatures in nature, where many biotic and abiotic factors act together, still remains fairly hypothetical.

Resurrecting phytoplankton

Many phytoplankton species form resting stages to allow for survival under unfavourable environmental conditions. Some of these resting stages can stay alive in the sediment for more than 100 years (Ellegaard and Ribeiro 2018). If the sediment remains undisturbed (e.g. by bioturbation, human activity or strong currents), these living sediment archives provide the possibility to travel back in time by going down into the sediment (Ellegaard et al. 2018). Resurrected phytoplankton from different sediment layers can then give an insight into the impact of environmental changes on genomic and phenotypical characteristics over time.

Previous resurrection experiments focused mainly on genetic patterns of phytoplankton populations over time. Härnström et al. (2011) and Lundholm et al. (2017) documented stable population structures and large genetic diversity in phytoplankton

revived from fjord sediments. They detected local adaptation and a reduced gene flow with adjacent populations (Härnström et al. 2011). Watts et al. (2013) estimated the effective population size from resurrected dinoflagellate cysts and found them comparable to macroscopic species. Ribeiro et al. (2013) investigated phenotypic responses of revived dinoflagellates to salinity and pH changes and detected a high intra-specific variability, but no significant differences between dinoflagellate strains from different sediment ages. How the past century of global warming may have affected temperature-dependent phytoplankton traits has not yet been investigated in resurrection experiments.

Here, I use a resurrection approach to shed light on phytoplankton adaptation to global warming, stored in living sediment archives. For this purpose, my co-authors and I firstly analyse the presence and survival potential of phytoplankton resting stages across a sediment core (Study I) and secondly perform temperature tolerance experiments with phytoplankton strains resurrected from different sediment layers (Study II).

Modelling adaptation

In marine ecosystem models, the potential of phytoplankton organisms to adapt to changing environmental conditions has so far been widely disregarded. There exist numerous ecosystem models with "fixed" functional phytoplankton traits, which may only allow for selection, but not for evolutionary changes (Bruggeman and Kooijman 2007; Follows et al. 2007; Steinacher et al. 2010; Bopp et al. 2013; Dutkiewicz et al. 2015; Laufkötter et al. 2015). In contrast to these ecosystem models, there exist many modelling studies following more conceptual approaches of evolution. Previous studies looked at the adaptative changes in pigment composition in a cyanobacterium (Stomp et al. 2004), or the thermal adaptation of phytoplankton in the global ocean (Grimaud et al. 2015). Collins (2016) explains how cellular damage and repair affect growth rate adaptation under improved environmental conditions, and Denman (2017) reconstructed the adaptation in a coccolithophore to higher temperatures based on data from a laboratory evolution experiment. These studies miss a concrete environmental context, though. In a recent study, Beckmann et al. (in rev.) developed a modelling concept which allows to include adaptation into ecosystem models, but this concept has not been applied to real data, yet.

In two subsequent studies, my co-authors and I investigate phytoplankton adaptation to global warming within an ecosystem model framework. We use the data obtained from our resurrection experiments and include them into an advanced ecosystem model, which allows for adaptation. Based on this model approach, we investigate how a functional, temperature-dependent phytoplankton trait respond to global warming (Study III). We additionally investigate the role of temperature fluctuations. It is under discussion whether environmental fluctuations slow down (Hao et al. 2015) or accelerate evolution (Schaum et al. 2015; Schaum et al. 2018). We therefore use our advanced ecosystem model to analyse which influence short-term temperature fluctuations have on the adaptive response to global warming (Study IV).

Aims and outline of the thesis

In this thesis, I aim for a comprehensive analysis of phytoplankton from a living sediment archive to explore whether and how adaptation to the past century of global warming has already occurred in nature. I base this work on a case study organism, the dinoflagellate *Apocalathium malmogiense*, which forms long-living resting stages and which occurs in a region which experienced a strong warming over the past century, the Gulf of Finland, N Baltic Sea. Together with my co-authors, I perform resurrection experiments with a focus on temperature-dependent phytoplankton traits and combine this approach with advanced ecosystem modelling.

The study organism

Over the past decades, there has been a shift in the spring bloom community in the Northern Baltic Sea from diatom- to dinoflagellate-dominance (Klais et al. 2013). This shift has been related to global warming; dinoflagellates benefit more from higher temperatures in comparison to diatoms (Klais et al. 2013; Lee et al. 2018), indicating that they may be better adapted to higher temperature conditions.

One of the species that is part of the dominating dinoflagellate complex is *Apocalathium malmogiense* (formerly *Scrippsiella hangoei*). Apart from its growth and metabolic traits, an important life cycle trait of *A. malmogiense*, the formation of resting cysts, is also temperature-dependent (Kremp and Parrow 2006; Kremp et al. 2009). As a cold-water, stenotherm species with a temperature window spanning about 0-10 °C (Kremp et al. 2005; Sundström et al. 2009), *A. malmogiense* may show a stronger adaptation to global warming compared to eurytherm species. Its narrower temperature window implicates a stronger response to temperature changes, and thus a higher plasticity, which, in turn, favours adaptation (Schaum et al. 2013). This species moreover forms thick-walled resting cysts which allow for long-term survival (Kremp and Parrow 2006).

Thus, due to its successful bloom formation even under increasing temperatures over the past decades, due to its stenotherm temperature window, and especially due to the long-term survival potential of its resting cysts, the dinoflagellate *A. malmogiense* represents an ideal model organism to examine potential adaptive responses to the past century of global warming.

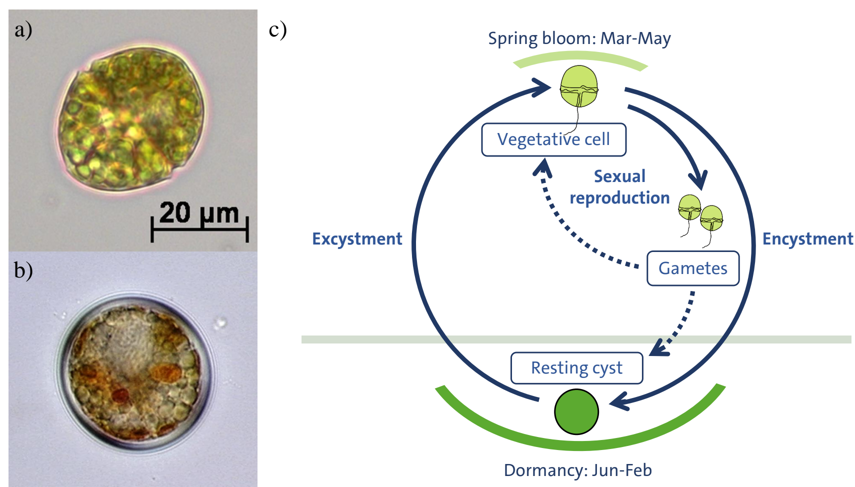


Figure 1.2: The study organism *A. malmogiense*. a) Vegetative cell, and b) resting cyst (picture by Phillip C. Watts), c) schematic life cycle: In early spring, cysts of the dominating dinoflagellate complex, which includes *A. malmogiense*, are resuspended into the water column and germinate (excyst) to become vegetative cells (Kremp and Anderson 2000; Kremp 2001; Kremp et al. 2005). Vegetative cells of these dominating dinoflagellates form a spring bloom, which lasts from March to May (Kremp and Heiskanen 1999; Kremp et al. 2005; Fleming and Kaitala 2006). During this time frame also gametes are formed (Kremp and Heiskanen 1999), but in the case of *A. malmogiense* it is unclear whether the fusion of gametes leads to the formation of resting stages or to new vegetative cells; most *A. malmogiense* resting cysts are however produced asexually, when water temperatures rise above a certain threshold (Kremp and Parrow 2006; Kremp et al. 2009). This onset of encystment causes the termination of the growth period for *A. malmogiense*. Its resting cysts sink to the bottom of the water column, where they remain for a dormancy period of several months (Kremp and Parrow 2006).

Objectives

In this work, I analyse if and how the dinoflagellate *A. malmogiense*, revived from the Gulf of Finland, has adapted to the past century of global warming. This comprehensive analysis is structured into 4 studies:

In **Study I**, we analyse the presence and viability of spring-blooming dinoflagellate resting cysts from a sediment core retrieved from the central Gulf of Finland. For this purpose, we date the sediment layers, investigate which dinoflagellate resting stages can be found in different sediment layers, and study the viability of resting cysts from different sediment layers to identify potential species for resurrection experiments. Our main research question here is:

Do we see changes in dinoflagellate cyst abundance and composition across the sediment and are cysts suitable to be resurrected?

In **Study II** we move on and perform resurrection experiments with strains of the

dinoflagellate *A. malmogiense*, which we successfully resurrected from historic (100 years old) and recent sediment layers. We compare three temperature-dependent traits, growth reaction norm, cell size and life cycle processes of historic and recent strains to answer the question:
Have temperature-dependent traits of a phytoplankton species changed in response to global warming over the past 100 years?

In **Study III**, we use the insights obtained from our resurrection experiments to integrate them into an advanced ecosystem model which allows for adaptation. Here, we investigate:
May global warming be the cause of the observed change in a phytoplankton functional trait?

In **Study IV**, we use our advanced ecosystem model to further understand:
How do short-term temperature fluctuations influence the adaptive response of phytoplankton to global warming?

2 | STUDIES OF THIS THESIS

Pre-published work included in this dissertation

Study I

Kremp, A., **Hinners, J.,** Klais, R., Leppänen, A. P., & Kallio, A. (2018). Patterns of vertical cyst distribution and survival in 100-year-old sediment archives of three spring dinoflagellate species from the Northern Baltic Sea. Eur. J Phycol., 53(2), 135-145.

Study II

Hinners, J., Kremp, A., & Hense, I. (2017). Evolution in temperature-dependent phytoplankton traits revealed from a sediment archive: do reaction norms tell the whole story?. Proc. R. Soc. B, 284(1864), 20171888.

2.1 Study I: Cyst profiles and viability across the sediment

Patterns of vertical cyst distribution and survival in 100-year-old sediment archives of three spring dinoflagellate species from the Northern Baltic Sea

Anke Kremp, **Jana Hinners**, Riina Klais,
Ari-Pekka Leppänen & Antti Kallio

The history of expansion of bloom-forming cold water dinoflagellates in the Northern Baltic Sea was studied using 100-year-old sediment archives of their resting cysts. Vertical cyst distributions of *Biecheleria baltica* and *Apocalathium malmogiense*, two dinoflagellates indistinguishable by light microscopy and not recognised as distinct species in monitoring, and chain-forming *Peridiniella catenata* were analysed in Pb^{210} and Cs^{137} dated layers of a sediment core from deep, hypoxic accumulation bottoms of the Gulf of Finland. Cyst profiles showed that *B. baltica* and *A. malmogiense* were already present in the Baltic spring phytoplankton community at the beginning of the 20th century. This confirms that *B. baltica*, which was only recognised in the late 1980s, is a native species in the area. A drastic increase in *B. baltica* cyst concentrations in the 1930s to 1960s coincided with the acceleration of anthropogenic eutrophication. Large cyst deposits accumulated over several decades in the sediment which, by the 1980s, amounted to the seed stock necessary to inoculate dominant blooms. In the cyst records *A. malmogiense* always contributed a minor fraction of the two species. *P. catenata* had a relatively short cyst record in Gulf of Finland sediments despite demonstrated long-term presence in the plankton, which emphasises that cyst-based historic surveys are not suitable for all cyst-forming dinoflagellates. This was corroborated by correspondence analyses of long-term plankton and cyst records which validated the trends from the sediment archive for *B. baltica* and *A. malmogiense*, but failed to do so for *P. catenata*. Germination experiments with 100-year-old cysts revealed a remarkable long-term survival capacity of *A. malmogiense*, making this species a suitable model for resurrection studies testing adaptation in heavily impacted systems such as the Baltic Sea.

Keywords: Baltic Sea, bloom expansion, dinoflagellate cysts, eutrophication, sediment archives

Published in European Journal of Phycology

Introduction

Phototrophic dinoflagellates are important constituents of phytoplankton in temperate marine and freshwater systems. Under favourable conditions they often form dense blooms which can have toxic or otherwise harmful effects on humans and co-occurring biota. When dinoflagellates dominate primary production, they can play an important role as drivers of carbon fixation and biogeochemical cycles. Many dinoflagellate species form resistant resting cysts as a part of their life cycle (Dale 1983). Dormant cysts are primarily thought to ensure survival through periods of unfavourable conditions. However, various other biological and ecological functions of these life cycle stages have been recognised: in the majority of dinoflagellate species with known life cycles, cyst formation is part of the sexual reproduction process (Stosch 1973; Figueroa and Bravo 2005) and supports genetic diversity (Garcés et al. 2002). The important role of cysts in dinoflagellate bloom dynamics and seasonal succession has been extensively documented (Anderson 1998; Rengefors 1998; Anglès et al. 2012). Pools of resting cysts in lake and marine sediments form 'seed banks' that anchor dinoflagellate populations in an area (Tahvanainen et al. 2012) and, being diverse genetic reservoirs (Lundholm et al. 2011), support their persistence through changing environmental conditions.

The walls of dinoflagellate resting cysts typically consist of resistant materials that effectively protect the cells from degradation (Zonneveld et al. 1997). When buried in sediment deposits, cysts can remain intact and alive for a long time (Lewis et al. 1999; McQuoid et al. 2002; Lundholm et al. 2011). The revival potential of some species is remarkable even after decades of burial, suggesting that these propagules are effective means to ensure persistence of populations through prolonged periods of unfavourable conditions (Ribeiro et al. 2011). Resurrected individuals or populations of planktonic organisms can provide valuable information on past environmental conditions and species responses to long-term changes in a water body. The fossilizable cyst walls of some dinoflagellates remain intact over geological time spans, making cysts useful proxies for environmental change. Cyst records have been extensively used in geology to reconstruct past marine and freshwater environments (Vernal et al. 2013). Cyst profiles from more recent sediments were found to reflect effects of industrialization and eutrophication (Harland et al. 2006; McCarthy et al. 2011). Moreover, cyst distributions in dated sediment cores have helped to explain recent expansions of harmful dinoflagellate blooms by relating vertical cyst dynamics to decadal scale environmental fluctuations (Feifel et al. 2012) or reconstructing invasion histories (Ribeiro et al. 2012). It has been suggested that dinoflagellate sediment records could replace or extend plankton monitoring time-series (Klouch et al. 2016).

In the Baltic Sea, cold-water adapted dinoflagellates regularly dominate the spring phytoplankton community and may in some years contribute up to 80% of the annual new production (Lignell et al. 1993). The species involved – *Peridiniella catenata* (Levander) Balech, *Biecheleria baltica* Moestrup, Lindberg & Daugbjerg, *Apocalathium malmogiense* (G.Sjöstedt) Craveiro, Daugbjerg, Moestrup & Calado comb. nov. and *Gymnodinium corollarium* A.M.Sundström, Kremp & Daugbjerg – are phylogenetically diverse.

While the chain-forming *P. catenata* is morphologically distinct, the other three species cannot be distinguished from one another by means of light microscopy. They were only recently recognised as individual species (Kremp et al. 2005; Sundström et al. 2009). In Baltic phytoplankton monitoring programmes the three species have not been differentiated but pooled in the '*Scrippsiella* complex', referring to the long-used genus name of the only species of the three that had been properly identified from the spring bloom, *Scrippsiella hangoei* (Larsen et al. 1995), now named *A. malmogiense* (Craveiro et al. 2016). All four spring bloom dinoflagellates produce distinct dormant resting cysts (Kremp 2000; Kremp et al. 2005; Sundström et al. 2009). Except for *G. corollarium*, which has an exceptionally small cyst stage that cannot be captured by standard sediment processing methods, these have been identified from Northern Baltic surface sediment samples.

The dominance of dinoflagellates during spring in the northern Baltic Sea is a recent phenomenon which has developed over the past four decades (Klais et al. 2011). It has been proposed that climate-mediated effects on life cycle transformations of *B. baltica* are a major driver of the bloom expansion (Klais et al. 2013; Warns et al. 2012). *B. baltica* has caused massive cyst sedimentation events since the late 1980s (Heiskanen 1993; Kremp et al. 2005) suggesting that this species plays an important role in this development. However, it remains unclear whether the new *B. baltica* blooms are caused by an expanding native population or whether the rapid expansion of the species might be a result of a recent invasion. It is also unclear to what extent the other 'look-alike' species, *A. malmogiense* and *G. corollarium*, are involved.

The present study investigates the distribution of distinct resting cysts of spring bloom dinoflagellates in a hundred-year-old sediment archive from the Gulf of Finland to unravel the history of their recent expansion and resolve the role of different species therein. We also examine to what extent the patterns found in the sediment reflect trends inferred from time-series of phytoplankton monitoring data and test the viability of cysts retrieved from old sediment layers to assess their potential for future resurrection studies.

Material and Methods

Sample collection

Sediment cores were collected in February 2015 onboard RV Aranda at HELCOM monitoring station LL7 (latitude 59°50.79', longitude 24°50.27', water depth 100 m) located in the Gulf of Finland, Baltic Sea (Fig. 2.1.1). Due to severe eutrophication and the inflow of anoxic deep water from the Baltic Proper, this area is characterised by hypoxic bottom water that prevents bioturbation (Conley et al. 2002; Vallius 2006) resulting in a vertically undisturbed sediment. For this study, two replicate core samples of 32–33 cm length were taken simultaneously using a GEMAX gravity corer. Samples were stored in their tubes under cold and dark conditions until further processing. In the laboratory cores were extruded from sampling tubes using a piston and sliced into 1 cm layers.

To avoid contamination by smear from other sediment layers, the outer 5 mm of each slice were removed using a cutting device designed for this purpose. Individual slices were transferred immediately to plastic bags submersed in water to remove air. Tightly closed bags were stored at 4°C in the dark.

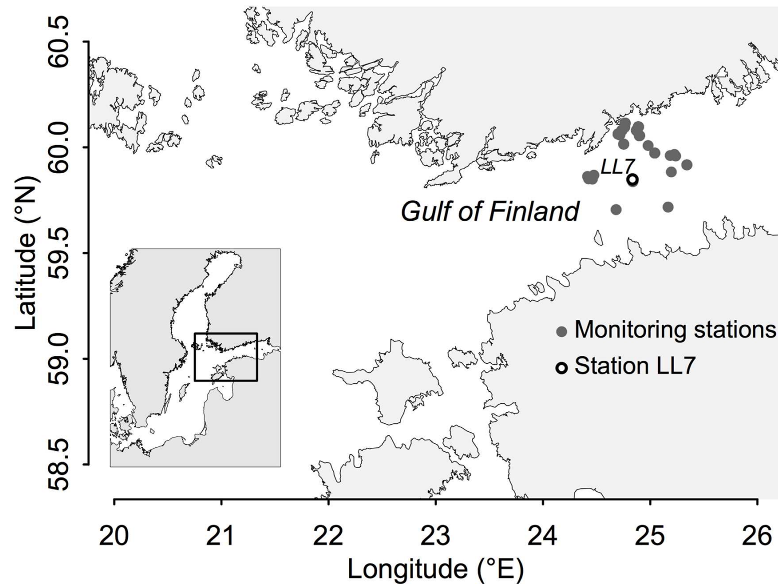


Figure 2.1.1: Map of the study area showing the location of core sampling station LL7 (black dot) in the Gulf of Finland and phytoplankton monitoring stations considered in the sediment archive – plankton correspondence analysis.

Sediment dating

Slices of one core were used for gamma-spectrometric dating of the sediment layers. Wet sediment slices were first weighed and then freeze-dried for 3 days in a Christ Beta 2-8 LD plus freeze dryer at 1.0 mbar and −20°C. Dry material was weighed again. Aliquots of dry sediment (3 g) were measured using gamma-spectrometry at the STUK Regional Laboratory of Northern Finland, using electrically cooled high purity germanium detectors (HPGE) with thin carbon fibre windows (Canberra and Ortec). Detector efficiencies were calibrated with certified National Physical Laboratory reference samples containing multiple nuclides with gamma energies ranging from 46.5 keV to 1836.1 keV, including Pb-210 and Cs-137. Activity concentrations were calculated using Gamma-99 software of STUK. The 46.5 and 661.7 keV gamma peaks were used for determination of Pb-210 and Cs-137, and for Ra-226 the 609 keV peak of Bi-214 and/or the 352 keV peak of Pb-214 were used. The water content and porosity of each sediment slice were calculated from dry and wet weights. Dry bulk density and mass depth of sediment slices were calculated using a constant sediment dry density of 2.3 g cm^{-3}.

The measured Pb-210 concentration in the sediment consists of supported and unsupported (or excess) Pb-210 components. Supported Pb-210 is in radioactive equilib-

rium with the decay chain of Ra-226 in the sediment matter, and therefore represented directly by the measured Ra-226 concentration. Unsupported Pb-210 consists of atmospheric deposition and is calculated by subtracting the supported Pb- 210 (Ra-226) from measured total Pb-210 for each sediment slice. The measurement uncertainties of total Pb-210 and Ra-226 are propagated into the uncertainty of unsupported Pb-210. The Pb-210 dating of sediments is based on tracking the record of unsupported Pb-210 in the sediment as a function of depth. After burial the unsupported Pb-210 will decay with a half-life of 22.23 years. However, assumptions have to be made about the supply rate of unsupported Pb-210 and the sedimentation rate, which may change over time or experience significant post-depositional changes through sediment mixing or diffusion. Pb-210 dating was validated by comparison with Cs-137. Cs-137 has been released into the atmosphere by nuclear weapons testing and nuclear accidents and can be used as a marker. Atmospheric nuclear weapons testing occurred mainly in the 1950s and 1960s, with a peak concentration of deposited Cs-137 around 1963. The Chernobyl accident caused a sudden release of Cs-137 into the environment, which produced a second marker in the sediment record during 1986. The Pb-210 chronology of the LL7 sediment core was calculated using the well-known models of constant initial concentration (CIC) and constant rate of supply (CRS), using the methodology described in Appleby (2001) and Lima et al. (2005) and comparing it with Cs-137 data.

Determination of vertical cyst concentrations

To quantify concentrations of dinoflagellate resting cysts in vertical sediment layers, sediment slurries containing the cyst fraction were prepared from stored wet sediment samples of the second core in October 2016, ca. 20 months after initial slicing. Three 2 ml subsamples were processed from each sediment layer down to 16 cm, the deepest layer where dinoflagellate cysts were found during initial screening. Replicate subsamples taken from well-mixed sediment samples were suspended in 20 ml of 0.2 μm-filtered local seawater (FSW, salinity 6) and sonicated for 30 s using a Bandelin Sonoplus Ultrasonication probe to disaggregate particles and remove fine organic material from the surface of resting cysts. Sonicated samples were rinsed through a 70 μm onto a 20 μm sieve using FSW to clean the slurries and concentrate the 20–70 μm size fraction that contained the dinoflagellate cysts of interest. The concentrated samples containing cysts of 2 ml wet sediment were resuspended in 15 ml FSW and transferred to 15 ml centrifuge tubes. These were closed tightly and wrapped immediately in aluminium foil to minimise exposure to light. Cyst slurries were stored in the cold and dark until microscopic examination and setting up of germination experiments. Cysts of *B. baltica*, *P. catenata* and *A. malmogiense* were counted microscopically using an inverted Leica DMI 3000B microscope (Leica Microsystems, Wetzlar, Germany), in Utermöhl chambers containing 1 ml of the processed sample. In this study, only intact cysts with visible cell contents were enumerated, assuming that permanently dark and hypoxic conditions at the sampling site consistently prevented germination of deposited cysts. The record of content-bearing cysts is thus considered to represent deposition from the plankton.

Cyst counts were made in triplicate for each slice and wet sample concentrations were converted to cysts g^{-1} dry weight based on initial wet and dry weight measurements for respective layers.

Correspondence analysis of sediment and plankton records

To estimate how historic cyst records in the sediment correspond to long-term bloom data in the water, quantitative phytoplankton monitoring data available from sampling locations within 30 km radius of LL7 (most of the samples located north of the sediment station) since 1966 were selected from the database established by Olli et al. (2013). Annual means of logtransformed biovolume ($\mu g\ l^{-1}$) were calculated from surface layer samples (0 to 4–10 m depth) for the '*Scrippsiella* complex' and *P. catenata*. The '*Scrippsiella* complex' represents both *B. baltica* and *A. malmogiense*. The correlation between cysts accumulated in sediment layers and respective annual biovolume means from plankton samples was estimated with Kendall's rank correlation and Pearson's linear correlation; in the latter case cyst abundances were log-transformed. Statistical analyses were performed in R.

Assessment of cyst viability

Viability of dinoflagellate cysts through time was assessed in germination experiments. For this purpose, cyst slurries (1 ml) were prepared from sediment slices obtained in February 2015. Subsamples of 1 ml containing intact cysts were incubated in triplicate wells of a 24 well tissue culture plate for each tested layer and species. Based on cyst distributions through the core, different layers were selected to represent cysts of different ages: 1, 6, 12 and 16 cm (= 3, 19, 50 and 106 years at the time of experiment) for *B. baltica* and *A. malmogiense*, and 1, 4, 5 and 6 cm (= 3, 11, 14 and 19 years, respectively) for *P. catenata*. Tissue culture plates were kept in an incubator for 4 weeks at 4°C, 50 μmol photons $m^{-2}\ s^{-1}$ under a 14:10 h light/dark cycle. After 2 and 4 weeks, samples were screened microscopically for the appearance of empty cysts and swimming vegetative cells.

Results

Sediment chronology

Calculated sediment properties, results of gamma spectrometry and results of CRS model calculations are listed in Appendix I, section 4.1. Unsupported Pb-210 concentration ranged from $\sim$ 500 Bq kg^{-1} at the top of the core to practically zero at 18 cm depth (Fig. 2.1.2(A)). The variability of unsupported Pb-210 was too complex to fit the assumption of exponential decrease of the basic CIC dating model (Fig. 2.1.2(A)) and the fit became only marginally better when using the CIC model with compaction correction (not shown). For that reason the CRS model, which assumes a constant flux of unsupported Pb-210 but allows for a variable sedimentation rate, was applied for age

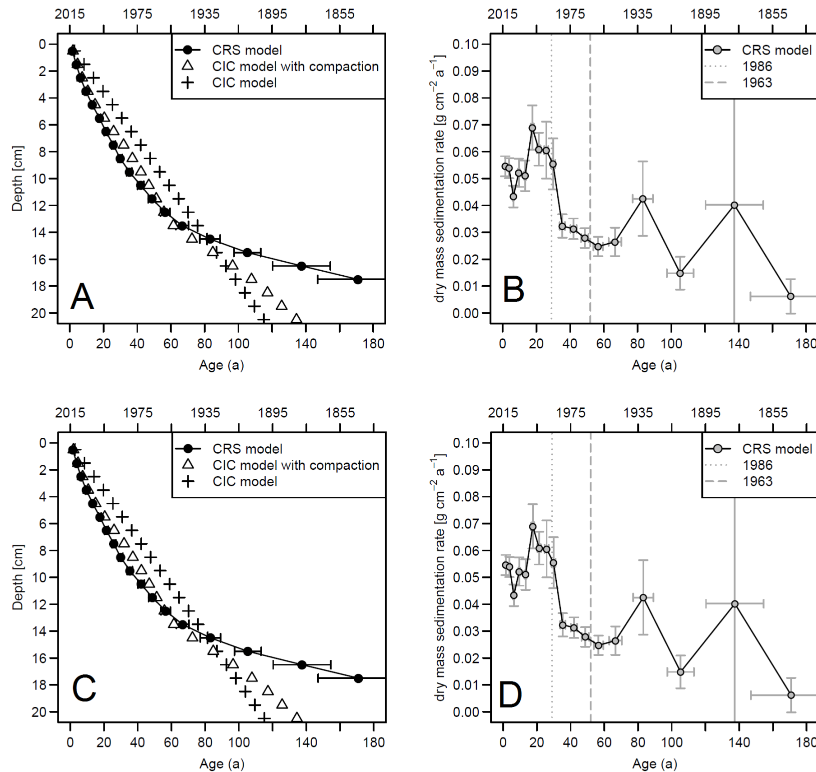


Figure 2.1.2: Gamma spectrometric results. (A) Pb-210 and Ra-226 in the LL7 sediment core: supported Pb-210 is represented by Ra-226, Pb-210 from atmospheric deposition (unsupported) is the difference between measured total Pb-210 and supported Pb-210. (B) Gamma spectrometric results of Cs-137: horizontal lines represent depths where peaks of Cs-137 are expected based on Pb-210 calculations. (C) Age-depth relationships of different Pb-210 dating models, and (D) dry mass sedimentation rate as a function of age, as calculated from the Pb-210 CRS model.

calculation. Because of the very small amount of unsupported Pb-210 in the 16–18 cm region (Fig. 2.1.2(A)) the uncertainties of CRS ages and sedimentation rates were too high below 16 cm depth preventing reliable age estimation. The CRS age for 16 cm depth was 105±8 years, which corresponds to a date of 1910±8 (Fig. 2.1.2(C)).

The Cs-137 data did not exhibit clear sharp peaks that could be definitively identified with Chernobyl or nuclear weapons testing depositional maxima (Fig. 2.1.2(B)). However, the comparison of CRS model Pb-210 ages with Cs-137 shows good agreement with the key dates of 1963 and 1986 and the largest steps in the Cs-137 concentration profile (Fig. 2.1.2(B)), giving confidence in the CRS model ages. The somewhat atypical Cs-137 profiles could result from the thickness of the slices (1 cm) that may have prevented resolution of the Cs-137 record into distinct peaks. Moreover, the concentration of Cs-137 remained high for several sediment slices above the 1986 level in the 6–9 cm depth which is in accordance with the high dry mass sedimentation rates calculated using the CRS model (Fig. 2.1.2(D)) for the period between 1985±2 and 1998±2 compared with other times. This corresponds to the first 15 years after the Chernobyl accident, a period of increased deposition of Cs-137-containing source materials which were trans-

ported to and deposited in the area. Similar patterns were found in other studies from Finnish coastal waters (Katajisto 1996).

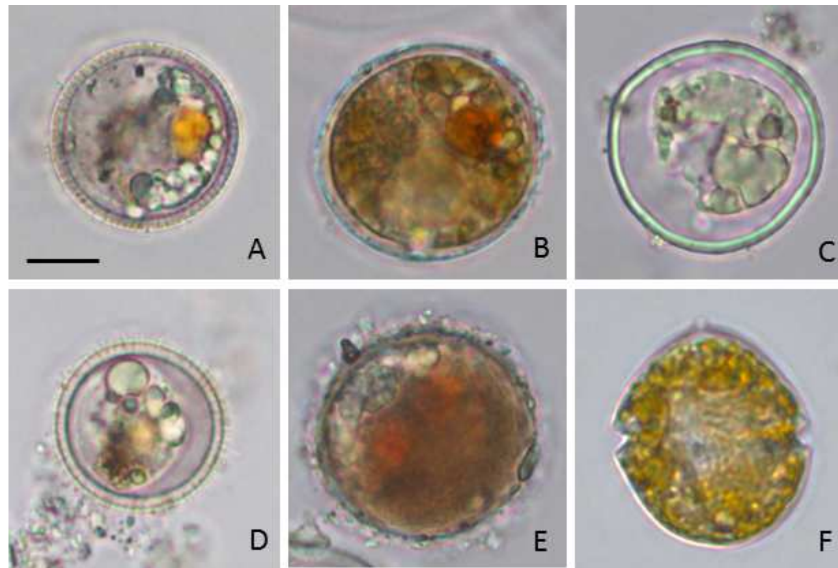


Figure 2.1.3: Light micrographs of *B. baltica* (A, D), *A. malmogiense* (B, E) and *P. catenata* (C) resting cysts. Cysts from surface sediment representing 2-4 years old deposits (A-C) and from the 15-16 cm sediment layer estimated to be ca. 100 years old (D-E). (F) Vegetative cell of *A. malmogiense* germinated from the 15-16 cm layer.

Vertical cyst distributions and long-term trends

The distinct resting cysts of *B. baltica* (Fig. 2.1.3(A)), *A. malmogiense* (Fig. 2.1.3(B)) and *P. catenata* (Fig. 2.1.3(C)) were the most prominent cyst types of phototrophic dinoflagellates found in the surface layer and throughout the LL7 sediment core. Intact *B. baltica* and *A. malmogiense* cysts were still detected at 15–16 cm depth in sediment more than 100 years old (Figs 2.1.3(D), 2.1.3(E)). Here, as in younger sediment layers, newly germinated *A. malmogiense* cells often appeared in the samples while these were being examined for cyst abundances (Fig. 2.1.3(F)).

Patterns of vertical distribution differed among the three species (Fig. 2.1.4). *B. baltica* was the most abundant species in all examined layers of the sediment core. Highest cyst concentrations of this species (740 000 cysts g^{-1} dry weight) were measured at 5 cm depth, corresponding approximately to the year 2002. Though lower before and after this peak, cyst concentrations remained at the same order of magnitude (105 g^{-1} dry weight) between 1958 and 2013. At the beginning of the last century, *B. baltica* cyst concentrations (800 cysts g^{-1} dry weight) were only 0.1–0.2% of concentrations measured a century later. A substantial increase occurred between 1932 and 1958. The distinct cysts of *A. malmogiense* were much less abundant than cysts of the two other species, but occurred consistently in all 16 investigated sediment layers. Their vertical distribution followed a similar pattern to *B. baltica*, with highest cyst concentrations (1700–3800 cysts g^{-1} dry weight) in the most recent sediment layers (1–5 cm) and low

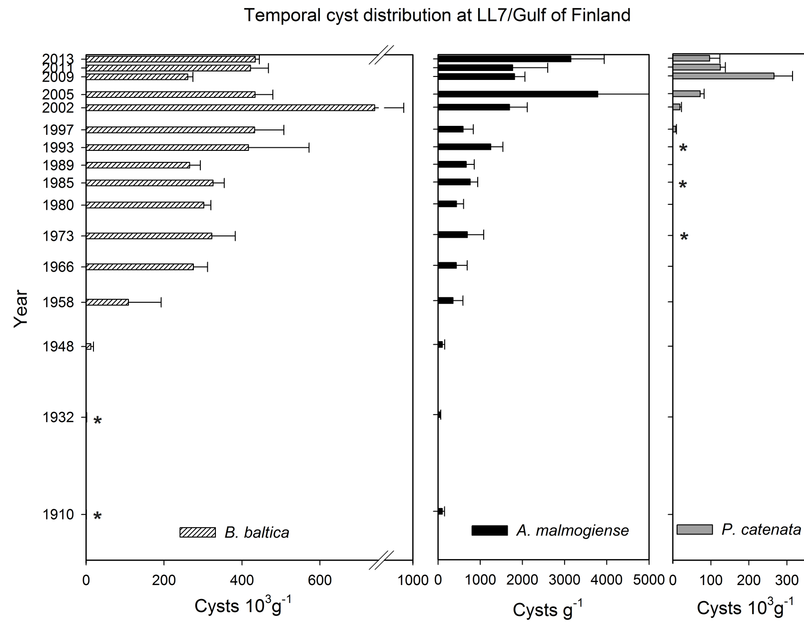


Figure 2.1.4: Concentrations of intact *B. baltica*, *P. catenata* and *A. malmogiense* resting cyst in dated layers of a sediment core collected at station LL7 in the Gulf of Finland, Baltic Sea.

numbers of cysts in the layers representing the first half of the past century. Nevertheless, cyst abundances of *A. malmogiense* did not change as dramatically as those of the other species through the century. Minimum abundances of this species, measured in the 1932 layer, were more than 1% of the maximum. *P. catenata* cysts were only detected down to 10 cm core depth, which corresponds approximately to the year 1980. Continuous cyst records of this species were only found in the uppermost 8 cm, i.e. since ca. 1989. In the late 1990s and early 2000s cyst abundances increased exponentially, reaching peak concentrations of >250 000 cysts g^{-1} dry weight in the year 2009 (3 cm downcore). Since then *P. catenata* cyst concentrations have decreased; approximately 100 000 cysts g^{-1} dry weight were found in the uppermost layer of the core.

When comparing bloom intensities of the investigated spring dinoflagellates to the cyst accumulations shown in Fig. 2.1.4 through the study period, the respective trends were closely correlated for *B. baltica* and *A. malmogiense* constituting the '*Scrippsiella* complex´ (Fig. 2.1.5), both when using Kendall's rank correlation (τ = 0.68) as well as Pearson's linear correlation coefficient (r = 0.75). Cyst densities of *P. catenata*, in contrast, were not correlated with the abundance of this species in the plankton (τ = 0.06, r = 0.17).

Viability of cysts

When resting cysts of the three species from different sediment layers were incubated under suitable temperature, oxygen and light conditions ~20 months after initial core processing, only *A. malmogiense* could be revived (Table 2.1). *B. baltica* cysts did not germinate in any well within 4 weeks. For *P. catenata* one new empty cyst was found

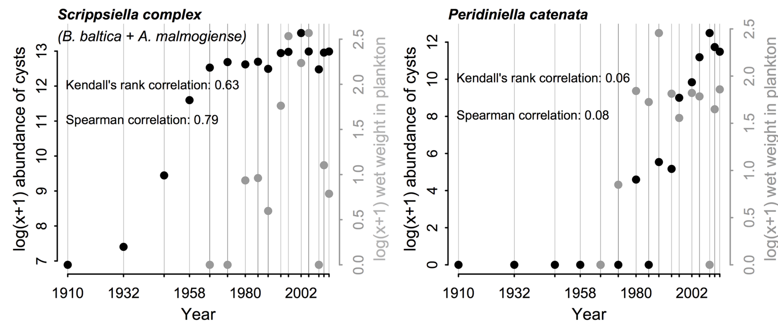


Figure 2.1.5: Correspondence between long-term sediment (resting cyst abundances, black circles) and plankton (grey circles) records of *B. baltica* and *A. malmogiense* (= '*Scrippsiella* complex' in phytoplankton monitoring programs), and *P. catenata*. Quantitative plankton data has been available since 1966 and originates from surface water samples collected by various monitoring programs. Data points for plankton represent annual means of log-transformed biovolumes (μg L-1) from the samples collected within 30 km radius of LL7. Correlation was estimated with Kendall's rank correlation, and Pearson's linear correlation.

after 4 weeks in one of three replicate wells containing material from 5 cm depth (14 year old sediment), but no swimming cells were observed. Germination of *A. malmogiense* occurred in material from all tested sediment layers, including sediment >100 years old. After 2 weeks, new empty cysts had appeared in at least 2 of the 3 replicate wells representing each tested sediment layer. In both, germination had occurred in all replicates of layers aged 19 and 106 years. Swimming cells were found in all wells that contained germinated cysts. The result was unchanged after 4 weeks.

Table 2.1: Germination and viability of germinated cells inferred from appearance of empty cysts and swimming cells in sediment slurries (three replicates) representing different sediment layers and age. Germination was scored positive when empty cysts were found after 2 or 4 weeks of incubation, and cysts considered viable when swimming cells were observed in at least one replicate well. Plus or minus are given for new empty cysts/swimming cells. Scores for all 3 replicates are only given when differences among the individual wells were observed. n.a. = not available.

Sediment depth	Cyst age in years	*B. baltica*	*P. catenata*	*A. malmogiense*
0–1 cm	3	–/–	–/–	− + +/− ++
3–4 cm	11	n.a.	–/–	n.a.
4–5 cm	14	n.a.	–/–	n.a.
5–6 cm	19	–/–	–/–	+/+
11–12 cm	50	–/–	n.a.	− + +/− ++
15–16 cm	106	–/–	n.a.	+/+

Discussion

Gamma-spectrometric radionuclide analyses of sediment layers showed that the sediment cores collected from the 100 m deep central Gulf of Finland consisted of chrono-

logically arranged vertical layers that allowed us to estimate the age of resting cysts retrieved from different depths of the core. Pb-210 and Cs-137 values established for the LL7 core were in agreement with the model assumptions (Appleby 2001) and confirmed that the investigated depth interval corresponded to ~100 years and thus represents nearly the entire 20th century.

The observation of *B. baltica* and *A. malmogiense* cysts in the oldest investigated sediment layers confirms that both species were already present in the Northern Baltic phytoplankton community a century ago, long before the expansion of cold water dinoflagellates began. For *A. malmogiense* the presence of cysts in sediment layers >100 years old is consistent with observations in the plankton. The species, then named *Peridinium gracile*, was identified by Lindemann (1924) from a sample collected in 1905 on the SW coast of Finland. The *B. baltica* cysts from the 1910 sediment layer found in this study represent the oldest record of the species, which was only recognised and described a decade ago. Cyst abundances determined from the 1910 layer suggest that 100 years ago *B. baltica* was much more abundant than *A. malmogiense*, which only amounted to ca. 10% of the total number. It can be assumed that this reflects the actual ratio of the two species at the time, since both have comparable cyst formation strategies with similar triggers and cyst yields (Kremp et al. 2009) allowing direct comparison of cyst abundances.

Cyst concentrations of *B. baltica* and *A. malmogiense* were several orders of magnitude lower in the deep layers compared with the sediment surface, indicating that at the beginning of the 20th century the two species were much less abundant in the water than today. Analyses of historic data (Hällfors et al. 2013) as well as qualitative historic surveys (Levander 1901) indicate that dinoflagellates were a minor component of the diatom–dominated spring phytoplankton community before anthropogenic impact began to affect phytoplankton productivity and composition in the Northern Baltic Sea. The dramatic accumulation of *B. baltica* cyst deposits in the sediment layers representing the 1930s to 1960s coincided with significant anthropogenic nutrient loading to the Baltic Sea (Struck et al. 2000). Within 35 years, *B. baltica* cyst abundances had increased nearly 200-fold. Similar developments have been documented for a number of dinoflagellate species from coastal and lake sediments worldwide using palynological cyst walls as well as whole cyst extraction methods (McCarthy et al. 2011; Miyazono et al. 2012; Ribeiro et al. 2012) and linked to the acceleration of eutrophication in the 1920s to 1960s. The increase of *B. baltica* probably reflects the general increase of phytoplankton biomass, fuelled by the ever-increasing availability of nutrients in the Gulf of Finland during that period. The eutrophication signal was also apparent in the cyst dynamics of *A. malmogiense*, although cyst abundances of this species only tripled through the respective time interval. Interestingly, the expansion of *B. baltica* in the sediment preceded the community shift from diatom to dinoflagellate dominance in the plankton by several decades (Klais et al. 2011). This lag might be related to the low survival rate of *B. baltica* cysts, particularly in anoxic sediments (Kremp and Anderson 2000). A favourable effect of the species' large 'seed beds' as suggested by Klais et al. (2011)

would only be provided at very high cyst concentrations, which had apparently accumulated by the 1980s.

The sediment archive allowed us to trace the history of two species that cannot be distinguished from one another in the plankton monitoring data of the past century, using the patterns of their distinct cysts. As indicated by the cyst record, *A. malmogiense* has remained a minor component of the '*Scrippsiella* complex' throughout eutrophication-induced phytoplankton productivity changes and the expansion of spring dinoflagellates in the Gulf of Finland. The cyst records show that *A. malmogiense* has increased proportionally since the late 1990s compared with *B. baltica*, now making up nearly 1% of the total again after having lost proportionally in the 1960 to 1990s.

To what extent the actual ratio of the two species in the plankton at a given time point is biased by differences in their preservation capacities remains unclear. Compared with the most recent sediment layers, *B. baltica* cyst concentrations decreased by a factor of 500 downcore, compared with *A. malmogiense* which was only 30 times less abundant in the 100-year-old sediment layer than at the sediment surface. Lundholm et al. (2011) detected large differences in the depth distribution of cysts with cell content among different dinoflagellate species, suggesting that resistance to degradation can vary considerably. In fact, a relatively low preservation potential has been indicated for members of the Suessiales, *Biecheleria* sp. and *Polarella glaciales* (Heikkilä et al. 2016), suggesting that mineralization of cysts happens rapidly, leading to substantial loss of cysts within a year after sedimentation. The data presented here show that this is certainly not the case for *B. baltica* from the Gulf of Finland. So far, palynological techniques have not been applied to *B. baltica* or *A. malmogiense* and it remains unclear whether their cyst walls are preserved in the fossil and sub-fossil records.

Despite these uncertainties, the established cyst record of *B. baltica* and *A. malmogiense* correlates well with the plankton record of the '*Scrippsiella* complex' for the investigated period between 1966 and 2013 when plankton monitoring data were available. Though there must have been some bias due to the fact that the cyst concentrations represent a cumulative value whereas cell concentrations in the plankton are a current value, we are confident that the sediment archive of the '*Scrippsiella* complex' represents a realistic trend through the past century and thus extends the plankton time-series data by 50 years to the beginning of the past century. The availability of a plankton monitoring time-series spanning nearly 50 years for the study area allowed us to validate the 'time series' represented by the cyst record. Typically, sediment archives are studied where plankton records do not exist, making it difficult to assess how realistically the cyst dynamics in the sediment core reflect trends in the plankton. So far, only quantitative DNA measurements from sediment archives have been systematically validated by plankton data (Klouch et al. 2016), though for a much shorter time span than the present study. Sometimes, indirect evidence, such as toxin monitoring data for a toxic dinoflagellate species, might provide orientation (Cox et al. 2008).

However, as the case of *P. catenata* presented here demonstrates, the cyst archive approach is not universally applicable for the reconstruction of dinoflagellate species his-

tories in an area of interest. For this species trend correlation of plankton and cyst data was not successful. Cyst records of *P. catenata* could only be traced back 30–40 years, although historic reports refer to the presence of the species in the Gulf of Finland as early as 1894 (Levander 1894). *Peridiniella catenata* (then *Peridinium catenatum*) was already referred to then as a prominent species of the spring phytoplankton community (Levander 1901). In the Gulf of Finland it has been highly abundant and sometimes even dominant during spring (Niemi 1975), but in the past 40 years the proportion of the species in the phytoplankton community has decreased steadily (Klais et al. 2013). The cyst profile of the LL7 core suggests a reverse pattern. The rapid decline of cyst abundances downcore is almost certainly related to the delicate nature of the cysts and the resulting low preservation capacity. As described in Kremp and Anderson (2000), the cyst wall of *P. catenata* is thin, resembling a temporary cyst rather than a resistant resting cyst. Nevertheless, these cysts have the demonstrated function of a dormant resting stage – preserving the cell from degradation at least through a mandatory dormancy period of 6 months. Low preservation potential of cysts of a number of dinoflagellate species was indicated by Lundholm et al. (2011), who reported relatively short-term records of intact cysts (less than 30 years) in a sediment core from the Swedish West Coast e.g. for *Alexandrium margalefii*, *Cochlodinium polykrikoides* and several *Diplopelta* and *Protoperidinium* cyst types.

Of the three investigated species only *A. malmogiense* cysts germinated readily from old sediment layers. When examined under the light microscope, even the 100-year-old cysts of this species quickly showed signs of impending germination such as Brownian motion or the greenish colour of developing chloroplasts. Often, germinated cells would even appear in the slide within the 30–60 minutes of observation. Such high revival potential has also been shown for the peridinioid dinoflagellate *Pentapharsodinium dalei*, which has been extensively used in resurrection studies (Ribeiro et al. 2011; Ribeiro et al. 2013; Lundholm et al. 2011). We established several clonal cultures from 100-year-old cysts and compared temperature-related traits to isolates from recent sediment layers (Hinners et al. 2017). This shows that *A. malmogiense* can be used as a model organism for evolutionary adaptation studies, particularly since it has experienced significant environmental changes during the past century in the heavily impacted Baltic Sea.

In contrast, *B. baltica* and *P. catenata* could not be revived from any sediment layer, not even from the surface which, at the time of the study, was 1.5–3 years old. It is likely that the long storage time after initial slicing of the sediment core (18 months), before germination experiments were performed, affected the results of the germination experiments as described by Lundholm et al. (2011) who found that storage after core processing reduces cyst viability of some dinoflagellate groups significantly. Interestingly, in that study the tested peridinioid taxa were not affected, which is consistent with our results. However, for *B. baltica* lack of excystment in any sediment layer is consistent with a steep decrease in germination potential within the first year of burial, and a negative effect of anoxic conditions on cyst survival (Kremp and Anderson 2000). Although healthy-looking *B. baltica* resting cysts were observed in deeper layers of the

sediment cores, the amount of cysts lacking granular storage products – an indicator of cyst viability (Feifel et al. 2015), (Fig. 2.1.3(D)) – increased significantly downcore. Published information on cyst survival is not available for *P. catenata*. Here, excystment, but no germling survival, was observed on one occasion in a slurry of 14-year-old cysts. This indicates that *P. catenata* might have shown a different germination behaviour, implying longer survival times, had experiments been set up immediately after sediment processing.

2.2 Study II: Thermal resurrection experiments

Evolution in temperature-dependent phytoplankton traits revealed from a sediment archive: do reaction norms tell the whole story?

Jana Hinners, Anke Kremp & Inga Hense

The high evolutionary potential of phytoplankton species allows them to rapidly adapt to global warming. Adaptations may occur in temperature-dependent traits, such as growth rate, cell size and life cycle processes. Using resurrection experiments with resting stages from living sediment archives, it is possible to investigate whether adaptation occurred. For this study, we revived resting cysts of the spring bloom dinoflagellate *Apocalathium malmogiense* from recent and 100-year-old sediment layers from the Gulf of Finland, N Baltic Sea, and compared temperature-dependent traits of recent and historic strains along a temperature gradient. We detected no changes in growth rates and cell sizes but a significant difference between recent and historic strains regarding resting cyst formation. The encystment rate of recent strains was significantly lower compared to historic strains which we interpret as an indication of adaptation to higher and more rapidly increasing spring temperatures. Low encystment rates may allow for bloom formation even if the threshold temperature inducing a loss of actively growing cells through resting cyst formation is exceeded. Our findings reveal that phenotypic responses of phytoplankton to changing temperature conditions may include hidden traits such as life cycle processes and their regulation mechanisms. This study emphasises the potential of living sediment archives to investigate plankton responses and adaptation to global warming.

Keywords: adaptation, global warming, phytoplankton, sediment archives, temperature-dependent traits

Published in Proceedings of the Royal Society B

Introduction

Due to their large census population sizes and short generation times, phytoplankton are expected to rapidly adapt to changing environmental conditions (Collins et al. 2014). In the light of global warming, we investigate whether and how phytoplankton, here represented by the cyst-forming cold-water dinoflagellate *Apocalathium malmogiense*, made use of this potential within the past hundred years and responded to increasing sea surface temperatures (SST) in the Baltic Sea.

Temperature represents one of the important environmental variables essentially influencing phytoplankton productivity, phenology, range expansion and community composition (Edwards and Richardson 2004; Hallegraeff 2010; Hinder et al. 2012; Thomas et al. 2012). Global warming may cause drastic changes in temperature- dependent phytoplankton traits including thermal reaction norms, cell size and life cycle transitions. Thermal reaction norms describe the performance or growth rate of organisms for a range of different temperatures; and due to global warming their shape may vary by shifting vertically, horizontally, or by changing in width (Kingsolver 2009). Laboratory studies using experimental evolution approaches for phytoplankton organisms confirmed that such adaptive changes in reaction norms can take place after 100-500 generations (Schlüter et al. 2014; Listmann et al. 2016; Padfield et al. 2016). Elevated temperatures also cause a linear decrease in cell size in most phytoplankton species for the range of preferred growth conditions, and diverging responses in cell size for high sublethal temperature conditions (Atkinson et al. 2003). Cell size is generally regarded as a trait with high adaptation potential; for example in response to ocean acidification and nutrient limitation (Litchman et al. 2009; Collins et al. 2014; Nakov et al. 2014). Thus, natural selection via increasing temperatures may lead to smaller cell sizes in general and changes in the cell size response at high sublethal temperatures. Temperature is moreover known to affect phytoplankton life cycle processes such as resting stage formation and germination (Bravo and Anderson 1994; Ellegaard et al. 1998; Kremp et al. 2009; Figueroa et al. 2011) and may thereby control the success of bloom formation (Kremp et al. 2008). It can be expected that such temperature-regulated life cycle processes are particularly sensitive to long-term temperature changes over times scales of global climate change.

Previous research on thermal adaptation of phytoplankton has concentrated on experimental evolution in laboratory and mesocosm studies and on field studies comparing organisms from different temperature environments (Schlüter et al. 2014; Zhang et al. 2014; Irwin et al. 2015; Listmann et al. 2016; Padfield et al. 2016). However, sediment archives of living resting stages provide a so far unused opportunity to examine long-term responses of phytoplankton to temperature changes. Living sediment archives are formed by resting stages conserved in undisturbed, stratified sediments. Many phytoplankton species form such resting stages to outlast short-term or seasonally unfavorable environmental conditions, and in some species resting stages are capable to stay alive for as long as hundred years (Härnström et al. 2011; Lundholm et al. 2011; Ribeiro et al. 2013). Previous resurrection studies using sediment archives have focused

on genetic patterns and phenotypic responses to salinity and pH changes of phytoplankton from different sediment layers (Härnström et al. 2011; Ribeiro et al. 2013; Watts et al. 2013; Klouch et al. 2016). Temperature-dependent traits of phytoplankton trapped in sediment archives have so far remained unexplored though.

For this study we chose a site with a significant temperature trend over the past hundred years. In the cold-temperate Gulf of Finland, N Baltic Sea, global warming is particularly noticeable by an earlier breakup of sea ice. The onset of melting is now 10-15 days earlier compared to 1923 (Jevrejeva 2000); and consistent with global trends (Pachauri et al. 2015), SST re-analyses for the central Gulf of Finland from the BALTIC dataset (Feistel et al. 2008) reveal an increase in spring SST by 0.8°C over the past hundred years. One of the most noticeable consequences of this development is an earlier onset of Baltic spring blooms (Klais et al. 2013).

The warming trend should particularly affect cold-adapted spring bloom phytoplankton species with a stenotherm temperature tolerance spanning ca. 10°C (Kremp et al. 2005; Sundström et al. 2009). Stenotherm organisms may adapt faster than eurytherm ones, as their narrow temperature window usually implicates a steeper temperature tolerance curve and thus a higher phenotypic plasticity, which in turn favors evolutionary adaptation (Schaum et al. 2013). One of these stenotherm species is the is the dinoflagellate *Apocalathium malmogiense*, formerly known as *Scrippsiella hangoei*. This species belongs to a group of dinoflagellates that dominates the spring bloom in the Baltic Sea and whose seasonal dynamics is regulated by temperature-dependent life cycle events (Kremp et al. 2008). *A. malmogiense* produces resistant asexual resting cysts when water temperatures increase above a threshold temperature (Kremp and Parrow 2006). The transition of a large fraction of the population to a benthic resting phase secures the survival of the species through periods of adverse warm conditions when growth cannot be sustained and the species disappears from the water column (Kremp et al. 2009). After a mandatory dormancy period of several months the cysts germinate at low temperatures (Kremp and Parrow 2006) leading to the return of a large part of the encysted population to the water column in late winter/early spring. *A. malmogiense* cysts have a high long-term survival capacity and remain viable in century old layers of Gulf of Finland bottom sediments (Kremp et al. 2018). These features make the species an ideal model organism to examine potential long-term responses of phenotypic traits to increasing temperatures in a living sediment archive.

Here, we use revived individuals of the spring-blooming dinoflagellate *A. malmogiense* from recent and 100-year-old sediment layers and compare the temperature-dependent traits growth rate, cell size and cyst formation. This approach will allow us to detect trait changes that indicate adaptation of the organism to increasing temperatures. In addition, these data are useful for ecosystem modeling, specifically for long-term simulations that need to take into account changes in the functional relationship between temperature and key traits.

Material and Methods

Sediment processing

Sediment cores were collected onboard RV Aranda at HELCOM monitoring station LL7 in the Gulf of Finland (lat 59°50.79', lon 24°50.27', water depth 100 m) during a HELCOM monitoring cruise in February 2015. A gravity corer (Gemax) was used to retrieve 3 replicate cores from anoxic sediments. The cores were sliced into 1 cm slices and individual slices were stored cool and dark in plastic bags under oxygen-free conditions until further processing. The material of one of the 3 replicate cores was freeze-dried and used for gamma-spectrometric dating. Radioactive Pb- and Cs-isotope analyses were performed and the age of the sediment slices was estimated using the CSR model. The procedures are described in detail in Kremp et al. (2018). Model estimates showed that the surface layer of the sediment corresponds to an age of approximately 2 years; while 16 cm core depth was estimated to be ca. 100-110 years old. For the isolation of *A. malmogiense* strains, subsamples from both, the surface sediment layer and the 16 cm deep layer were processed as described by Kremp et al. (2018) to extract the dinoflagellate cyst fraction. In short, 2.5 mL of well mixed sample material were suspended in 20 mL filtered sea water (FSW, 0.2 μm filter, 6 psu), sonicated for 30 sec. at 30 percent intensity using a Bandelin Sonoplus Ultrasonicator and sieved through a 70 μm sieve onto a 20 μm sieve using FSW. The resulting 20-70 μm fraction, potentially containing dinoflagellate cysts, was collected into a 15 mL centrifuge tube and stored dark and cool until germination experiments were set up.

Culture establishment

The processed samples were microscopically examined for the presence of intact *A. malmogiense* resting cysts before sediment slurries were distributed into wells of 24-well tissue culture plates. Each well was filled with 500 μL of the sediment slurry and 1000 μL f/8-Si medium. The cyst slurries were placed in a temperature and light controlled culturing cabinet and incubated at 4°C, 50-100 μmol photons m^{-1} s^{-1} for 16:8 h light/dark cycle (L:D) to allow for germination. After six weeks of incubation, well plates were checked for emergence of motile *A. malmogiense* cells and several cells were isolated from each well. To grow clonal cultures single cells were transferred through several washes into separate new wells containing f/8-Si culture medium using a micropipette. The isolated cells were grown for 2 months at 4 °C, 50-100 μmol photons m^{-1} s^{-1} and L:D 14:10 h. 4 well-growing strains were established from the recent sediment surface layer and 5 strains from the 16 cm deep, 100-year-old sediment layer.

Determination of temperature tolerance ranges

For the temperature tolerance experiment 3 recent and 3 historic strains were randomly chosen. Stock cultures of the strains were maintained in 6 psu f/2- Si medium (made from North Sea water that was diluted using MilliQ water) at 3.5°C, 50-100 μmol pho-

tons m^{-1} s^{-1}, and L:D 16:8 h. To examine temperature tolerances of recent and historic *A. malmogiense* individuals temperature reaction norms were generated using a temperature gradient table (Thomas et al. 1963; Walter et al. 2015). The table contained 10 horizontal rows à 6 vertical slots for culturing containers. Along the horizontal axis a temperature gradient from 0-10°C was built up in steps of 0.9-1.4°C, along the vertical axis the temperatures differs slightly by ca. 0.4°C. In each of the 10 horizontal rows the samples of 3 recent and 3 historic strains were randomly distributed among the 6 vertically oriented slots. In this way, each of the 3 recent and 3 historic strains was investigated at 10 temperature steps between 0 and 10°C, at 50 μmol photons m^{-1} s^{-1} and L:D 16:8 h. The experiment was repeated for the overlapping temperature range from 6.5 to 16.5°C.

Inoculum cultures for the temperature gradient experiment were set up from exponentially growing stock cultures by transferring 5 mL of stock culture into experimental vessels filled with 200 mL medium. Vessels were distributed across the table as described above to allow inoculum cultures to acclimate to the respective experimental temperatures. After 2 weeks, the acclimated subsamples were inoculated into new culture vessels containing fresh medium and placed in the same temperature slots. Starting cell concentrations were set to 700 cells/mL. The 0-10°C-experiment was run for 18 days, the 6.5-16.5°C-experiment was run for 14 days to capture the exponential growth phase. Cell concentrations were monitored every 2-3 days from 1 mL subsamples fixed with a drop of Lugol's solution. Cells were counted microscopically in a gridded Sedgewick Rafter chamber. At least 400 cells were counted per sample, except when cell concentrations were very low. Here, at least 200 squares of the chamber were examined. The cell diameter was determined several times during the experiment to examine the effect of temperature on cell size. For this purpose 7 mL sample material were fixed with Lugol's solution and analysed with a FlowCam VS-IV. The temperature and pH of all samples were measured twice a week using a WTW 340i pH meter. Nitrate and phosphate concentrations were measured at the start, in the middle and at the end of the respective experimental run. Therefor each 7 mL sample volume were filtered through a 0.2 μmol filter and frozen until later analysis with a Seal-Analytik AA3 Autoanalyzer. Particulate organic carbon and nitrogen (POC/PON) analyses were performed at the end of the acclimation period, in the middle and at the end of both experimental runs. For the first measurement each 50 mL subsamples were extracted from the acclimation culture vessels, for the latter two measurements during the experiment 14 mL were extracted from each experimental subsample. The subsamples were filtered onto precombusted, acid-washed GF/C filters. The filters were dried in a compartment drier and analysed with an Eurovector EA-3000 elemental analyser.

Estimation of reaction norms

The growth rate established based on POC concentrations was calculated as follows: The POC data were interpolated to obtain POC values for all time points for which the cell concentration *(c)* was monitored. For each time point, the cell concentration was

multiplied with the POC concentration and divided by the molecular mass of carbon:

$$POC\left[\frac{mol}{L}\right] = POC\left[\frac{g}{cell}\right] \times c\left[\frac{cell}{L}\right] \times \frac{1}{12}\left[\frac{mol}{g}\right] \tag{2.2.1}$$

The exponential growth rate based on POC was then calculated for all time steps using

$$\mu = \frac{ln(x_2) - ln(x_1)}{t_2 - t_1} \tag{2.2.2}$$

The time interval of the maximum growth rate was identified graphically using Matlab. The mean growth rate for this time interval was calculated including at least 3 data points in the analysis, corresponding to at least 5-7 days in the experiment. A modified gauss function was fitted to the obtained maximum growth rates of the 3 recent and 3 historic strains in the temperature range 0-16.5°C:

$$\mu = \mu_{max} \times exp\left(-\frac{(T - T_{opt})^2}{\left(T_{l1} + T_{l2} \times sgn(T - T_{opt})\right)^2}\right) \tag{2.2.3}$$

This modified gauss function has an advantage compared to other functions used for fitting temperature reaction norms: the parameters have a biological meaning. μ_{max} represents the maximum growth rate at the optimum temperature T_{opt}, $(T_{l1} - T_{l2})$ determines the slope on the left side of the curve, and $(T_{l1} + T_{l2})$ determines the slope on the right side of the curve. We fitted function 2.2.3 to the growth rate data of both recent and historic strains using maximum likelihood to obtain one reaction norm for the recent strains and one for the historic strains.

Examination of life cycle responses to temperature

To study the sensitivity of life cycle processes to temperature changes in recent and historic *A. malmogiense* strains, a separate experiment was performed where the temperature signal which triggers life cycle transitions in this species was modified in a temperature gradient. Based on previous observations, we assumed that cyst formation is triggered by a temperature shift from preferred growth conditions at low temperatures to the higher end of the temperature window allowing growth (Kremp and Parrow 2006; Kremp et al. 2009). To analyse the flexibility of this temperature trigger and to test whether strains isolated from different temperature regimes distinguish, 2 recent and 2 historic strains were acclimated to 3°C and 6°C. After six weeks of acclimation, subsamples were inoculated in experimental containers filled with fresh f/2-Si medium which were then spread in the temperature table set for a temperature range of 0 to 10°C. All experimental cultures were kept in the table at their respective incubation temperatures for 53 days. Numbers of motile cells, distinct small cells (10-16 μm) and resting cysts were determined every 2-3 days in a Sedgewick Rafter chamber. Samples for cell diameter, POC/PON, and nutrient analysis were taken on day 0, 11, 25 and 53 of the experiment and analysed as described for the temperature tolerance experiment.

The POC content of distinct small cells was extrapolated from their diameter using a linear correlation of cell size and POC from experimental data. Diameter estimates were generated from FlowCam data compiled from thousands of small cells measured from samples containing significant amounts of them. Based on the mean abundance of small cells and their POC content, a POC budget was calculated for this cell fraction. The cyst POC budget was calculated using maximum cyst concentrations and the previously measured mean POC content of 2321 pg C/cyst for *A. malmogiense* (Kremp et al. 2009). The encystment rate was calculated for the time interval from the first detection of cysts in a sample until the end of the experiment as the average percentage of the POC concentration of vegetative cells which transforms into cyst POC per day.

Statistical analysis

To investigate the influence of temperature on the growth rate and to validate if there are differences in the growth rate between ages (recent/historic) and between single strains (strain no.), we performed a statistical analysis in R. As the POC based growth rate data were not normally distributed (analysed using Shapiro-Test and visual diagnostic methods), we used a generalised additive model (GAM) for significance tests. We tested for significant influence of temperature, age, and strain no. on the growth rate and identified the model which explains the data best using the Akaike information criterion (AIC). A similar analysis was performed for the cell diameter. The relative plasticity was calculated as the mean slope of the reaction norm between the coldest tested temperature and the optimum temperature, and a Wilcox test was performed to compare the plasticity of recent and historic strains. The differences in cyst and gamete concentrations between recent and historic strains were investigated in R using a linear model; a similar analysis was performed for the encystment rate. The effect of prior acclimation temperatures (3 and 6°C) on the growth rate was analysed using a GAM, and the effect of acclimation temperatures on gamete and cyst production was tested with a linear model.

Results

Reaction norm

Growth experiments in an experimental temperature gradient from 0 to 16.5 °C revealed typical, left-skewed reaction norms for recent and historic strains (Fig. 2.2.1). Generally, the growth rates increased from 0 to 7°C, reached a plateau between 7 and 11°C and decreased drastically thereafter; no growth was observed above 14°C. The variance explained by the fitted gauss functions (R^2) was 0.844 for historic and 0.836 for recent strains. Growth rates of both recent and historic strains were highly variable within each layer; the historic strain hist-3 had noticeably lower growth rates compared to all other strains at low temperatures and contained a high concentration of small, lightly pigmented cells, presumably sexual life cycle stages (gametes). The

performed generalised additive model (GAM) did not reveal significant differences in growth rates between single strains though (Table S1, Appendix II, section 4.2).

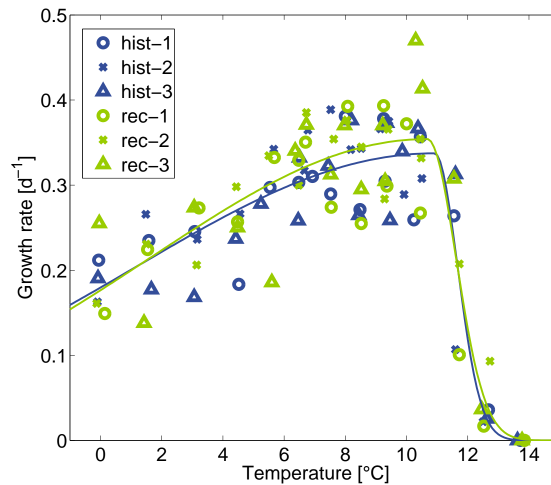


Figure 2.2.1: Reaction norms of recent and historic strains of *A. malmogiense* based on the increase in POC concentrations. Parameter estimates for both fits are summarised in Table S2 (Appendix II, section 4.2).

Recent strains had a slightly higher growth rate compared to historic strains at higher temperatures. But overall, the reaction norms of recent and historic strains were surprisingly similar; the applied GAM did not indicate a statistically significant difference in growth rates between recent and historic strains (Table S1, Appendix II, section 4.2).

Similar to the maximum growth rate, the relative plasticity, expressed by the slope of the reaction norms, was slightly, but not significantly higher in recent compared to historic strains (Table S1, Appendix II, section 4.2).

Cell size

As expected, we observed a linear decrease in cell size with higher temperatures for all strains (Fig. 2.2.2). However, in contrast to what may be assumed under global warming, the linear trend was similar for both recent and historic strains, except for strain hist-3. Excluding this strain, again there was no significant difference in the cell diameter between recent and historic strains (adjusted R^2 = 0.549, Table S1, Appendix II, section 4.2).

Life cycle transitions

In a second experiment, we compared the life cycle transitions of recent and historic strains in a temperature range from 0-10°C, (Fig. 2.2.3, 2.2.4). Prior acclimation temperatures (3 and 6°C) before the start of the experiment did not influence life cycle transitions or growth rates siginificantly (Table S1, Appendix II, section 4.2). Noticeable amounts of distinct small cells, possibly gametes, occured in experimental cultures

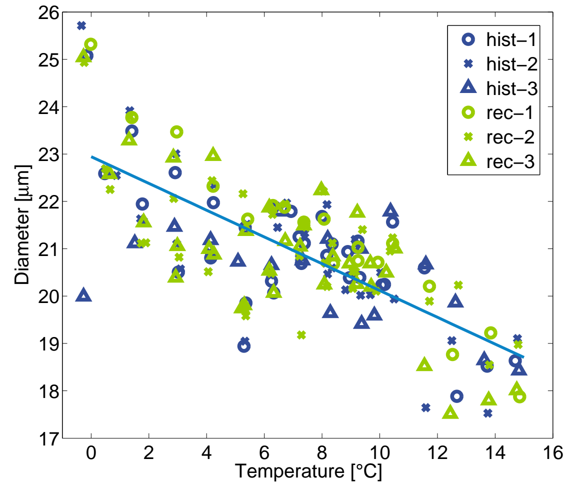


Figure 2.2.2: Cell size of recent and historic strains of *A. malmogiense* in relation to temperature including a linear regression. Visualised are the data from all experimental runs combined. Parameter estimates for the regression are summarised in Table S3 (Appendix II, section 4.2).

incubated at low temperatures; their POC concentrations were elevated at ~0.5-5.5°C. POC concentrations of resting cysts increased at the higher end of the temperature gradient (~8-10°C). Distinct small cells appeared on day 23 and their concentration remained more or less constant from day 28 onwards until the end of the experiment on day 53, whereas the cysts developed later, from day 30 onwards and increased in concentration until the end of the experiment, or until the remaining motile cells died.

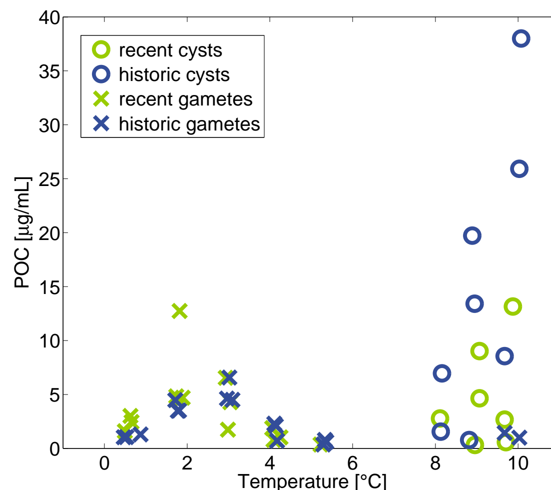


Figure 2.2.3: Life cycle processes in relation to temperature: mean small cell, and maximum cyst POC concentration in the stationary phase for recent (green) and historic (blue) strains.

In contrast to reaction norm and cell size, the differences in life cycle responses between historic and recent strains were pronounced: Significantly less cysts were produced by recent strains compared to historic strains (LM, $F = 7.03$, $p = 0.020$, adjusted

R^2 = 0.499). Likewise, the encystment rate (the POC of vegetative cells which is transformed into POC of cysts per day) was significantly lower in recent compared to historic strains (LM, F = 6.16, p = 0.025, adjusted R^2 = 0.466).

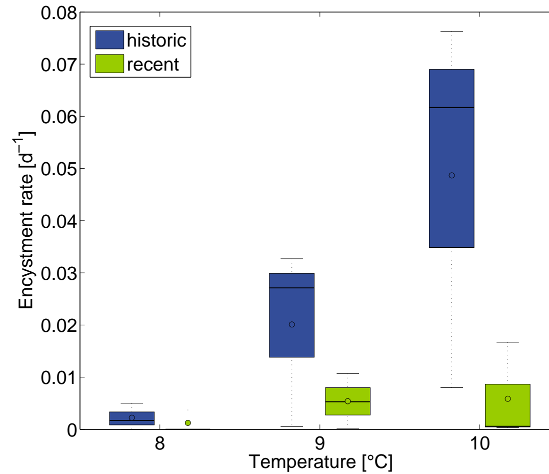


Figure 2.2.4: Encystment rate per day at the end of the stationary phase for two historic and two recent strains; for each temperature step 3 historic and 3 recent samples are averaged. The boxes indicate the interquartile range; the inner line of each box represents the median and the whiskers represent the total range of data.

Discussion and Conclusions

Here, we revived dinoflagellate cysts from recent and 100-year-old sediment layers in the Baltic Sea and compared their temperature-dependent traits to assess potential long-term responses to global warming.

Germination of cysts from different sediment layers has been successfully induced previously (Härnström et al. 2011; Lundholm et al. 2011; Ribeiro et al. 2011; Ribeiro et al. 2013). However, our study is the first looking at potential effects of global warming in revived phytoplankton. The comparison of cultures established from living sediment archives covering periods of significant environmental changes represents an attractive possibility to detect signs of adaptation to such changes. Here, we focus on traits known to be sensitive towards temperature: reaction norm, cell size and life cycle transitions.

We detected a slightly higher growth rate at high temperatures in recent compared to historic strains, but against our expectations the differences were not significant. Apparently, our study organism, *A. malmogiense* has not altered its reaction norm over the past hundred years despite the significant SST increase that has happened in the Baltic since industrialization. Instead, we noticed a high variability in the growth rate among the examined strains from each sediment layer. Such high phenotypic variation likely reflects the reservoir function of the dormant seed pool of cyst-forming phytoplankton species, which is thought to integrate different cohorts of a population and thereby preserve diversity (Lundholm et al. 2011). The lack of a clear change in the thermal reaction norm might be furthermore explained by the presence of simultaneous environmental

changes. It has been suggested that when selection via multiple environmental changes acts simultaneously on phytoplankton traits, genetic correlations among those traits may limit or constrain adaptation (Collins et al. 2014). Apart from temperature, other environmental parameters such as pH, light intensity and nutrient supply have changed due to climate change and likely influence phytoplankton traits (Boyd et al. 2008). In case of the Baltic Sea the nutrient supply has increased particularly due to a severe eutrophication since the 1950s (Fonselius and Valderrama 2003; Gustafsson et al. 2012).

Our results showed a linear decrease in cell with increasing temperature, which is typical for protists (Atkinson et al. 2003). Over a century of global warming the decrease in cell size could become stronger due to selection for earlier cell division; offspring formed earlier can establish a larger fraction of the population than those formed later (Atkinson et al. 2003) and so in the long run, mean cell sizes could decrease. Additionally, temperature-size correlations diverge at high sub-lethal temperatures (Atkinson et al. 2003), and may therefore be more adaptive than the temperature-size correlation at preferred growth conditions. Nevertheless, in this study recent and historic strains did not differ in their cell size - temperature relationship over the whole investigated thermal reaction norm.

Apart from altering their reaction norm and cell size phytoplankton can respond to higher temperatures in other ways, for example by altering life cycle processes. Typically, resting stages are formed to endure unfavorable environmental conditions. In contrast to the majority of dinoflagellate species which form cysts when nutrients are exhausted, Baltic spring bloom dinoflagellates and specifically *A. malmogiense* transform into resting cysts when temperatures shift from preferred cold growth conditions to higher temperatures (Kremp and Parrow 2006; Kremp et al. 2009). In a cold water system such as the Baltic Sea the spring transition from stable cold water conditions to higher surface temperatures is rapidly progressing. Induction of encystment at favorable growth conditions is necessary to ensure successful production of enough resting stages before growth ceases. The amount of produced resting stages then determines the extent of the next year's bloom (Kremp et al. 2008). Our data show that the temperature threshold for cyst formation remained constant in both historic and recent strains. Instead we detect an intrinsic change in the amount of cysts produced: cyst concentrations and encystment rates in recent strains were on average 70% lower compared to historic strains. But what could be the benefit of reduced cyst production in recent *A. malmogiense* populations?

Cyst formation reduces the pool of actively growing cells and can lead to the termination of the spring bloom (Kremp and Heiskanen 1999). Under present climatic conditions with an early and rapidly increasing SST in spring, a high encystment rate would be unfavorable, as it would cause high losses to the vegetative population at an early stage of growth, which would hinder the development of a bloom (Warns et al. 2012). A low intrinsic encystment rate on the contrary, would have a less drastic effect on the growing cell population. Even if the threshold temperature for encystment is exceeded, there remain still enough cells in the water column which can form a bloom. By al-

lowing for bloom formation at rapidly increasing spring temperatures, the strategy of a reduced encystment rate would support population survival and long-term persistence more effectively than a high encystment rate.

As the temperature window of *A. malmogiense* has remained unchanged over the past hundred years, global warming including earlier ice-melting and earlier increase in spring SST can be expected to cause a shift in bloom timing. Indeed, the 10-15 day earlier onset of melting over the past hundred years (Jevrejeva 2000) matches the 10 day phenological shift of phytoplankton spring bloom timing observed over the past 3 decades in the study area (Klais et al. 2013). In this context it is plausible to consider the changed cyst formation behaviour of *A. malmogiense* an adaptation to the changed temperature regime.

The life cycle experiment conducted here revealed a notable increase in small and lightly pigmented cells at the lower end of the temperature gradient. We consider them as sexual life cycle stages as they have the typical appearance of dinoflagellate gametes. We did not find significant differences in small cell POC budgets of historic and recent strains and conclude that sexual reproduction has remained unaffected by changes in the temperature regime. Unlike in most other dinoglagellates, the sexual reproduction of *A. malmogiense* is largely decoupled from resting cyst formation (Kremp and Parrow 2006). Low temperature preference for sexual reproduction implies that this process happens at optimal growth conditions which should increase the chance for the success of immediate recombination and support preservation of genetic diversity (Bengtsson 2003). Being uncoupled from temperature-sensitive encystment, sexual reproduction may not be equally subjected to global warming.

Here, resurrection experiments are used for the first time to investigate responses of phytoplankton temperature traits to global warming. Our results show that trait changes, indicative of temperature adaptation have occured over the past century in the investigated *A. malmogiense* population and that these trait changes do not manifest themselves in reaction norms but involve complex life cycle processes. These findings emphasise that the resurrection approach used here represents a promising tool for studies on phytoplankton adaptation to global warming.

This first attempt to utilise a phytoplankton sediment archive in the context of temperature adaptation however also revealed some challenges that need to be addressed by future studies. First, it is difficult to establish comparable samples with a representative number of revived strains from different sediment layers with traditional experimental approaches. Variation among strains is very common in phytoplankton populations (Alpermann et al. 2010; Reusch and Boyd 2013) and ideally samples should consist of a high number of strains to get representative responses for the respective temporal sub-populations. The present study comprises a fairly small amount of strains that were tested for each layer. This small sample size expresses itsself in low R^2 values for the analyses of cell sizes and encystment rates and implies an uncertainty regarding the interpretation of our results for the whole *A. malmogiense* population. New high throughput phenomic approaches that begin to emerge in phytoplankton experimenta-

tion (Houle et al. 2010; Xu et al. 2015; Cruz et al. 2016; Sackett et al. 2016) will help to overcome these limitations and make phenotypic trait comparisons more powerful. Second, it is challenging to assess the randomity in sample representation when working with living sediment archives: We cannot exclude the possibility of selective cyst germination even though according to (Kremp et al. 2018) the recent and the deep sediment layer had equally good germination success under the same conditions. Similarly, it is impossible to assess potential selection in the regulation of encystment a hundred years ago and selective processes affecting cyst survival. The rapidly advancing field of single cell genomics may provide solutions to these limitations of living sediment archives. When genomic DNA is quantitatively analysed including estimates of effective population sizes and potential storage effects, conclusions on evolutionary adaptation will be possible. Still, genetic analyses cannot replace the more traditional experimental setup applied here. Only if the functional relationships between traits and environmental factors are quantitatively described and mathematically formulated, they are of use for ecosystem modeling.

2.3 Study III: Ecosystem modelling on adaptation to global warming

Modelling phytoplankton adaptation to global warming based on resurrection experiments

Jana Hinners, Inga Hense & Anke Kremp

Due to its crucial role in the ecosystem, phytoplankton is incorporated in marine ecosystem models. Most models however neglect the evolutionary potential of phytoplankton. Previous resurrection experiments with a spring bloom dinoflagellate suggest that the past century of global warming has caused an adaptive response in an important life cycle trait, the encystment rate. Here, we apply an advanced ecosystem model including selection and mutation, to test whether a temperature increase could induce a change in encystment. In line with observations, our results show that in warmer waters strains with a lower encystment rate benefit over those with a higher encystment rate. The magnitude of change in encystment rate is however only reproduced, if additional factors, like eutrophication and a cyst mortality that increases with temperature, are considered. By using this ecosystem model including adaptation, we demonstrate that ecosystem modelling represents a powerful approach to investigate the adaptive potential of phytoplankton.

Keywords: adaptation, ecosystem model, evolution, global warming, life cycle, living sediment archives, phytoplankton, resurrection experiment

Submitted to Evolutionary Applications

Introduction

Phytoplankton organisms represent the base of the marine food web, they are an important component of biogeochemical cycles (Hutchins and Fu 2017) and they even feed back on ocean physics (Sathyendranath et al. 1991; Kahru et al. 1993; Joehnk et al. 2008; Hense et al. 2017). Due to the importance of phytoplankton for the ecosystem, they are explicitly considered in marine ecosystem models (Bruggeman and Kooijman 2007; Follows et al. 2007; Steinacher et al. 2010; Bopp et al. 2013; Dutkiewicz et al. 2015; Laufkötter et al. 2015). Owing to their large population sizes and short generation times, phytoplankton have a high potential to rapidly adapt to environmental changes. Despite the growing body of research demonstrating this high evolutionary potential of phytoplankton, it is so far disregarded in the vast majority of ecosystem models. Here, we apply an advanced ecosystem model which allows for adaptation to investigate, how a phytoplankton life cycle trait may change in response to global warming.

The high evolutionary potential of phytoplankton organisms has been described in many experimental evolution studies (Collins and Bell 2004; Lohbeck et al. 2012; Schaum et al. 2017). There is also a number of conceptual and modelling studies that include evolutionary aspects. Among those, however, only few regard concrete cases of trait changes in planktonic organisms. One example focuses on the adaptative change of pigment composition in a marine cyanobacterium using a simple population model (Stomp et al. 2004). A similar approach is used by Grimaud et al. (2015) to study thermal adaptation of phytoplankton in the global ocean. Denman (2017) simulates observed changes in the growth rate of the phytoplankton *Emiliania huxleyi*, assuming random mutations as well as plasticity. Collins (2016) explains with an individual based model how cellular damage and repair influence the change in cell division rates in phytoplankton under improved environmental conditions, considering an explicit trade-off of resource allocation between growth and repair. The first study, (Stomp et al. 2004), is very specific and uses data from observations, but the model does not allow for evolutionary responses and misses an ecosystem framework. The latter three, (Grimaud et al. 2015; Denman 2017; Collins 2016), apply the theoretical knowledge to the real world, but do not account for ecosystem dynamics. In contrast to these modelling studies including evolution, there are numerous ecosystem models which prescribe functional groups and/or strains with "fixed" traits and only allow for selection (Follows et al. 2007; Steinacher et al. 2010; Bopp et al. 2013; Dutkiewicz et al. 2015; Laufkötter et al. 2015). Bruggeman and Kooijman (2007) regard trait distribution and its changes by immigration but also ignore evolutionary changes. Thus, none of the above mentioned studies exemplify consequences of observed evolutionary changes in a phytoplankton trait in response to environmental changes using an ecosystem model framework. Only recently, a model concept has been developed which allows to include the potential for adaptation into ecosystem models (Beckmann et al., in rev.), but this model concept has not been applied to real data yet.

Lately, life cycle trait changes were detected in resurrected phytoplankton resting

stages from the sediment in the Gulf of Finland, N Baltic Sea (Hinners et al. 2017). Over the past hundred years, the temperature in the Gulf of Finland has increased significantly by 0.3°C per decade (Laakso et al. 2018). Temperature tolerance experiments with revived strains of the dinoflagellate *Apocalathium malmogiense* suggest that in consequence of increasing temperatures changes in resting stage formation (encystment) have already occurred. The encystment rate of recent strains was almost 5 times lower compared to the encystment rate of historic, 100 years old strains (Hinners et al. 2017). Encystment is an annually occurring life cycle process, which terminates the spring bloom of *A. malmogiense* and thus has an important effect on its phenology. The authors hypothesised that the lowered encystment rate represents an adaptation to global warming, allowing for spring bloom formation even if the temperature threshold for encystment is already exceeded. They assumed that under warming conditions natural selection favours strains with a lower encystment rate.

Here, we perform ecosystem modelling for the first time including adaptation, based on experimental data from resurrected *A. malmogiense*. In a life-cycle-ecosystem model we investigate bloom formation and encystment of a model dinoflagellate population over a period of 200 years of global warming (1900-2100). The population comprises a number of strains competing with each other for nutrients and light, and differing only in their encystment rate. Following the assumption that most mutations have a small effect on the fitness of organisms (Fisher 1930; Orr 2005; Matuszewski et al. 2014), we include a mutation rate, which allows for phenotypic evolution by small steps (see Beckmann et al. (in rev.) for details). Using this advanced model we are able to trace concretely if and how a phytoplankton trait responds to global warming.

Moreover, we explore additional factors that could have influenced the adaptive response. The first factor we consider for this purpose is cyst mortality. Cysts are known to play an important role in bloom formation (Klais et al. 2013; Lee et al. 2018) and the survival potential of resting stages can be affected under elevated temperatures (Ellegaard and Ribeiro 2018). We therefore study the sensitivity of the adaptive response under global warming and a concurrent increase in cyst mortality. The second factor we investigate is eutrophication. The Baltic Sea has experienced a significant eutrophication over the past century, which has led to a higher primary production (Rönnberg and Bonsdorff 2004; Gustafsson et al. 2012). The higher nutrient levels may have affected spring bloom dynamics and in turn also evolutionary dynamics.

All in all, we investigate (1) whether the hypothesis that global warming causes an adaptive response in the encystment rate is confirmed by using an advanced marine ecosystem model and (2) whether our model reflects the data from resurrection experiments, as well as how additional factors influence the adaptive response.

Model description

Life-cycle-ecosystem model

Our life-cycle-ecosystem model is similar to the dinoflagellate life cycle model in Warns et al. (2012) and includes 3 life cycle stages of *A. malmogiense*, vegetative cells (V), gametes (G), and resting cysts (C), as well as nutrients (N) and detritus (D), see Fig. 2.3.1. All components are in nitrogen units (in $mmol\ \ N\ m^{-3}$) and mass conservation is ensured. *A. malmogiense* is part of the dinoflagellate complex which dominates spring blooms in the Northern Baltic Sea and shows a similar life cycle: Cysts germinate in early spring after a dormancy period of several months when they are resuspended into the water column (Kremp 2001; Kremp and Parrow 2006). In the 0-dimensional model used here, we simplified this process by prescribing germination to a certain time window in early spring. As long as the water temperature is below freezing point, growth of vegetative cells is inhibited; when the freezing temperature is exceeded, vegetative cells take up nutrients and form a spring bloom. Gamete production takes place under nutrient-replete, low temperature conditions (Hinners et al. 2017), but it is not known what triggers the fusion of gametes to a planozygote and whether planozygotes form new vegetative cells or cysts. Since most cysts are produced asexually (Kremp and Parrow 2006), we assume that sexual reproduction plays a minor role in the life cycle of *A. malmogiense* and do not consider this process in detail. The formation of resting cysts takes place when spring temperatures rise above a certain threshold (Kremp and Parrow 2006; Hinners et al. 2017). A mortality rate for all life cycle stages is assumed, filling the detritus pool. Detritus is subsequently remineralised into nutrients.

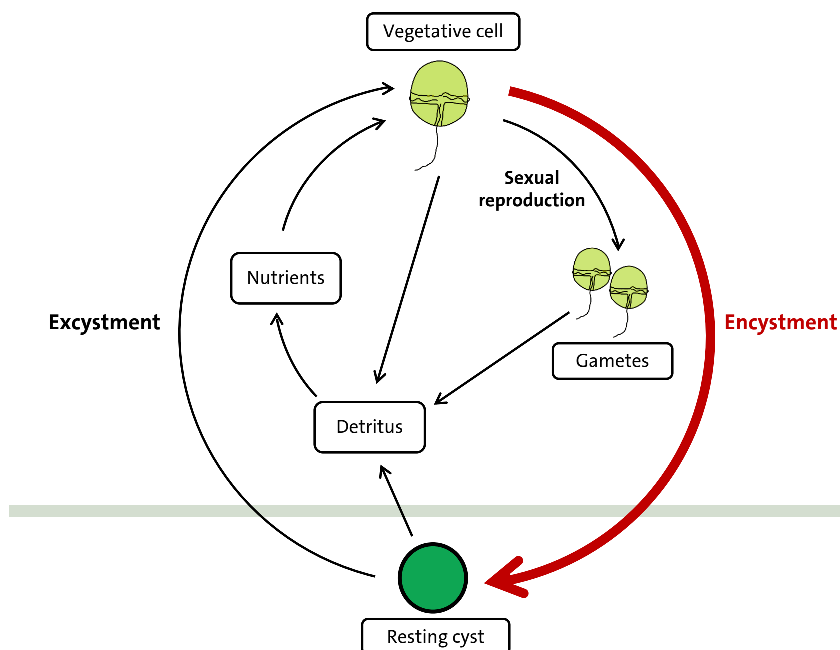


Figure 2.3.1: Schematic life-cycle-ecosystem model of the dinoflagellate *A. malmogiense*.

Table 2.2: Parameters for the life-cycle-ecosystem model

Symbol	Parameter	Value	Unit
α_{VC}	Basic encystment rate from V to C	0.02	d^{-1}
α_{VG}	Max. transition factor from V to G	0.05	-
g_{CV}	Germination rate from C to V	0.1	d^{-1}
i_1	Mean light intensity	142	$W\ m^{-2}$
i_2	Range of light intensity	130	$W\ m^{-2}$
i_3	Initial slope of lim_L	0.0085	$m^2\ W^{-1}\ d^{-1}$
k_N	Half saturation constant for N	0.3	mmol N m^{-3}
m_C	Mortality rate of C	0.0097	d^{-1}
m_G	Min. mortality rate of G	0.005	d^{-1}
m_V	Min. mortality rate of V	0.005	d^{-1}
μ_{max}	Max. growth rate of V	0.35	d^{-1}
r_D	Remineralisation rate of D	0.1	d^{-1}
T_f	Freezing point Gulf of Finland sea water	-0.33	°C
T_{GD1}	Temp. threshold for mortality of G	13.0	°C
T_{GD2}	Temp. slope for mortality of G	2.0	°C
T_{l1}	Constant for slope of temp. reaction norm	7.22	°C
T_{l2}	Constant for slope of temp. reaction norm	6.03	°C
T_{opt}	Temp. at optimum growth rate	10.8	°C
T_{VC1}	Temp. threshold for transition from V to C	6.0	°C
T_{VC2}	Temp. slope for transition from V to C	1.6	°C
T_{VD1}	Temp. threshold for mortality of V	13.0	°C
T_{VD2}	Temp. slope for mortality of V	2.0	°C
T_{VG1}	Temp. threshold for transition from V to G	2.3	°C
T_{VG2}	Temp. slope for transition from V to G	1.5	°C
X_C	Encystment factor	$1-5$	-

The processes describing the life-cycle-ecosystem model can be grouped into growth (eq. 2.3.1), life cycle transition (eqs. 2.3.2- 2.3.4), mortality (eqs. 2.3.5), and remineralisation (eq. 2.3.6). A list of all model parameters is given in Table 2.2.

The growth of vegetative cells, gr_V, is dependent on light, nutrients, and temperature.

$$\mathrm{gr}_V = \mu_{max} \times lim_L \times lim_N \times lim_T \times V \tag{2.3.1}$$

The production of gametes, τ_{VG}, is temperature-dependent and proportional to the growth of vegetative cells.

$$\tau_{VG} = \alpha_{VG} \times exp\left[-\left(\frac{T - T_{VG1}}{T_{VG2}}\right)^2\right] \times \mathrm{gr}_V \tag{2.3.2}$$

The formation of cysts, τ_{VC}, is also temperature-dependent with a strong gradient, centred around 6°C . Previous experiments revealed that *A. malmogiense* strains revived from a sediment layer from 1910 ± 8 yrs exhibit an 4.6 times higher encystment rate in comparison to strains revived from a recent sediment layer from 2013 ± 0.2 yrs (Hinners et al. 2017). Therefore, we include an encystment factor, X_C, into cyst formation,

which varies between 0.1 and 9.8, whereby encystment factor $X_C = 1.0$ represents the factor which was measured for recent, and $X_C = 4.6$ the encystment factor which was measured for historic strains.

$$\tau_{VC} = \alpha_{VC} \times X_C \times 0.5 \times \left(1 + \tanh\left[\frac{T - T_{VC1}}{T_{VC2}}\right]\right) \times V \tag{2.3.3}$$

The germination of vegetative cells from cysts, τ_{CV}, is fixed to the period from day 44 until day 60 of a year.

$$\tau_{CV} = \begin{cases} g_{CV} \times C, & \text{if 44 days} < \text{t [days]} > \text{60 days} \\ 0, & \text{otherwise.} \end{cases} \tag{2.3.4}$$

The mortality of vegetative cells and gametes, mort_V and mort_G, is temperature-dependent, whereas the loss of cysts through mortality and burial, mort_C, is temperature-independent.

$$\begin{aligned} \text{mort}_V &= (1 - m_V) \times 0.5 \times \left(1 + \tanh\left[\frac{T - T_{VD1}}{T_{VD2}}\right] + m_V\right) \times V \\ \text{mort}_G &= (1 - m_G) \times 0.5 \times \left(1 + \tanh\left[\frac{T - T_{GD1}}{T_{GD2}}\right] + m_G\right) \times G \\ \text{mort}_C &= m_C \times C \end{aligned} \tag{2.3.5}$$

The remineralisation, remin_D, is adopted from Enríquez et al. (1993).

$$\text{remin}_D = r_D \times D \tag{2.3.6}$$

The terms describing growth limitation are described in eqs. 2.3.7-2.3.9. The term lim_L represents the light limitation following (Webb et al. 1974), with lower, limiting light conditions in winter and light saturation during the rest of the year. For temperatures below freezing point in the Gulf of Finland, $T_f = -0.33$ °C , light limitation is set to zero due to light absorption and reflection by sea ice.

$$lim_L = \begin{cases} 0, & \text{if } T < T_f \\ 1 - exp\left[\frac{-i_3 \times I_L}{\mu_{max}}\right], & \text{otherwise.} \end{cases} \tag{2.3.7}$$

Nutrient limitation lim_N is defined following Monod (1949).

$$lim_N = \frac{N}{N + k_N} \tag{2.3.8}$$

Temperature limitation of *A. malmogiense*, lim_T, is applied from temperature-dependence experiments (Hinners et al. 2017).

$$lim_T = exp\left[-\frac{\left(T - T_{opt}\right)^2}{\left(T_{l1} - T_{l2} \times sgn\left(T - T_{opt}\right)\right)^2}\right] \tag{2.3.9}$$

The changes in life cycle stages, nutrients and detritus in time are given by:

$$\begin{aligned}
\frac{\delta V}{\delta t} &= \mathrm{gr}_V + \tau_{CV} - \tau_{VC} - \tau_{VG} - \mathrm{mort}_V \\
\frac{\delta C}{\delta t} &= \tau_{VC} - \tau_{CV} - \mathrm{mort}_C \\
\frac{\delta G}{\delta t} &= \tau_{VG} - \mathrm{mort}_G \\
\frac{\delta D}{\delta t} &= \mathrm{mort}_V + \mathrm{mort}_C + \mathrm{mort}_G - \mathrm{remin}_D \\
\frac{\delta N}{\delta t} &= -\mathrm{gr}_V + \mathrm{remin}_D
\end{aligned} \tag{2.3.10}$$

Model setup and environmental forcing

We model the life cycle of the dinoflagellate *A. malmogiense* in Matlab with a time step of 1 h using a 0-dimensional model; the length of each simulated year is fixed at 360 days. To solve our differential equations we use a predictor-corrector method.

The irradiance I_L during the seasonal cycle is adapted from Stramska and Zuzewicz (2013).

$$I_L = i_1 + i_2 \times cos\left[\frac{2 \times \pi \times t - 180 \times 24}{360 \times 24}\right] \tag{2.3.11}$$

The pre-industrial annual temperature curve is obtained from a high resolution reconstruction of atmospheric forcing (HiResAFF) for Northern Europe (Schenk and Zorita 2012). The mean climatic temperature curve for 1900 is calculated based on the air temperature data from 1885-1915 from station LL7 in the Central Gulf of Finland (lat 59°51, lon 24°50), and a *cos*-function (eq. 2.3.7) is fitted to this climatic temperature curve.

$$T_{1900} = 7.5 - 10.5 \times cos\left[\frac{2 \times \pi \times (day - 35)}{360}\right] \tag{2.3.12}$$

To analyse the effect of global warming, a continuous increase of 0.3°C/decade is applied following Laakso et al. (2018), which is similar to the IPCC scenario RCP8.5 (Pachauri et al. 2015).

Additionally, we include short-term temperature fluctuations in form of daily oscillations and weekly random fluctuations into the temperature forcing. The extent of these daily oscillations and weekly fluctuations were obtained as well from the HiResAFF data set (Schenk and Zorita 2012). Daily oscillations comprise ±0.75 °C, weekly fluctuations are set to random values in the range of ±6 °C once per week and interpolated linearly in-between. To account for the varying effect of short-term temperature fluctuations on the model results in single years, we performed an ensemble of 30 runs and averaged the results.

Basic evolution model experiment

In this evolution experiment, we use an advanced model approach (see Beckmann et al. (in rev.) for details) that considers evolution to investigate how an *A. malmogiense* population may adapt to increasing temperatures. We consider a number of strains with fixed characteristics that differ only in one life cycle trait, the encystment rate. In order to represent the effects of mutation, part of the newly grown biomass in each strain is transferred to the neighbouring strains. This transfer is symmetric for all strains (except for the first and last) and penalty-free (no trade-off), since it is unclear whether the cost for producing a cyst is significantly different from that for a vegetative cell. All strains are in competition for nutrients and light, leading to selection of the strain with the most favourable encystment rate for the prevailing temperature regime. In a warming environment, the population will continuously adjust to the temperature change.

$$
\begin{aligned}
\frac{dV_1}{dt} &= (1-\delta)\times \mathrm{gr}_{\mathrm{V}_1} + \tau_{CV_1} - \tau_{VC_1} - \tau_{VG_1} - mo_{V_1} + \delta \times \mathrm{gr}_{\mathrm{V}_2} \\
\frac{dV_i}{dt} &= (1-2\,\delta)\times \mathrm{gr}_{\mathrm{V}_i} + \tau_{CV_i} - \tau_{VC_i} - \tau_{VG_i} - mo_{V_i} \\
&+ \delta \times \mathrm{gr}_{\mathrm{V}_{i+1}} + \delta \times \mathrm{gr}_{\mathrm{V}_{i-1}} \qquad (i = 2, N-1) \\
\frac{dV_N}{dt} &= (1-\delta)\times \mathrm{gr}_{\mathrm{V}_N} + \tau_{CV_N} - \tau_{VC_N} - \tau_{VG_N} - mo_{V_N} + \delta \times \mathrm{gr}_{\mathrm{V}_{N-1}}
\end{aligned}
$$

In our experiment we have chosen N = 98 strains with encystment rates expressed by the encystment factor X_C times the basic encystment rate (α_{VC}) and times a temperature-dependent term, see eq. 2.3.3. The encystment factor ranges from X_{C1} = 0.1 to X_{CN} = 9.8 with an interval of 0.1. Three percent ($\delta = 0.03$) of the biomass from a strain is transferred after mutation to each of the neighbouring strains. For the first and last strain this transfer is one-sided only. Since each strain also receives biomass from its neighbouring strains, the change in net biomass resulting from mutation ranges between 0 and 6% of the growth term. Initial concentrations were set to N = 5.9 mmol N m^{-3}, and V_{46} = 1.1 mmol N m^{-3} (V_{46} corresponds to the strain with an encystment factor X_C = 4.6, measured in revived historic cultures); all other strain concentrations were set to zero. The model is forced for 20 years with the historic temperature curve for 1900 to allow for model spin-up and subsequently for 200 years with increasing temperatures of 0.3 °C/decade. The first 20 years of simulations are excluded from analysis.

Modified evolution model experiments

To explore possible additional reasons for the decrease in encystment rate observed in revived *A. malmogiense* strains, we investigate the influence of two factors, a temperature-dependent cyst mortality and eutrophication, on the adaptive response in encystment.

To explore the influence of a temperature-dependent cyst mortality, we replace the term describing cyst mortality and burial, mort_C, described in eq. 2.3.5 by eq.

2.3.13, where the term $T_{inc}(t)$ represents the continuous increase in temperature by 0.3°C/decade (Laakso et al. 2018).

$$\text{mort}_C = m_C \times (1 + 0.5 \times T_{inc}(t)) \times C \tag{2.3.13}$$

To investigate the development of encystment under eutrophied nutrient conditions, the model was run for 200 years of global warming (plus 20 years for model spin-up), with high initial nutrient concentrations. For this purpose, the model was initialised with a doubled nutrient concentration, $N = 12.0$ mmol N m^{-3}. This nutrient concentration lies in the range of recently measured dissolved inorganic nitrogen concentrations in the Gulf of Finland (Suikkanen et al. 2013).

Results

Here, we use an advanced ecosystem model approach to investigate adaptation to global warming. We confirmed that our model is able to represent the natural spring bloom phenology in the Gulf of Finland, similar to previous studies (Warns et al. 2012; Lee et al. 2018), see S1 in Appendix III, section 4.3. We illustrate the evolutionary changes of the encystment rate, expressed by the encystment factor, which - according to observations - should range from 4.6 in 100 years-old to 1.0 of recent strains (Hinners et al. 2017).

Basic evolution experiment

In an evolution model experiment, we analyse how the mean encystment factor of a population responds to increasing temperatures. This experiment shows a decrease in the encystment factor over 200 years of global warming, see Fig. 2.3.2. Following the increase in temperature, the mean encystment factor decreases from $X_C = 4.60$ at the start of the model experiment to $X_C = 3.74$ at the end of the experiment.

Modified evolution experiments

In modified evolution experiments we investigate how two other factors, namely a temperature-dependent cyst mortality and high nutrient conditions, alter the adaptive response in encystment.

A temperature-dependent cyst mortality causes a stronger decrease in the mean encystment factor in response to increasing temperatures (down to $X_C = 1.75$), see Fig. 2.3.3(a). Eutrophied nutrient concentrations in comparison lead only to a slightly stronger decrease in the encystment factor over 200 years of global warming to $X_C = 3.71$, Fig. 2.3.3(b), when compared to the basic evolution experiment.

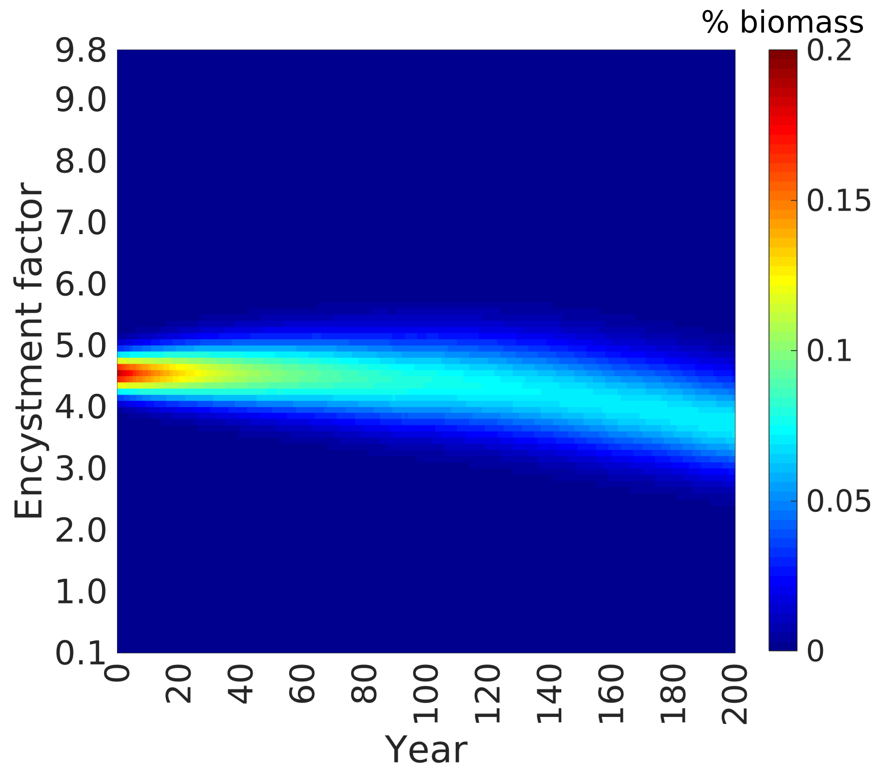


Figure 2.3.2: Relative biomass of all strains over 200 years of global warming. The relative biomass is indicated by color. Strains differ only in their encystment factor, ranging from 0.1-9.8. The model was initialised with the strain with encystment factor 4.6, representing the encystment rate measured in historic, 100 years old cultures.

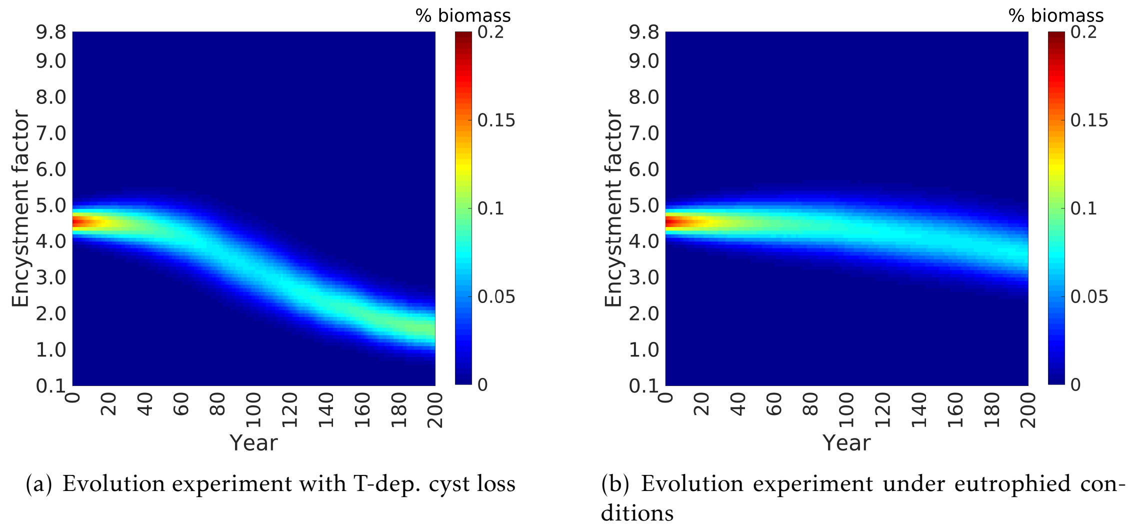
Discussion

To the best of our knowledge, this ecosystem model approach is the first one analysing potential adaptation in phytoplankton to increasing temperatures, based on experimental data from a case study on living sediment archives (Hinners et al. 2017).

Basic evolution model experiment

Our basic evolution model experiment shows a decrease in the encystment factor over 200 years of global warming. Thus, our modelling results qualitatively agree with results from resurrection experiments: an increase in temperature causes a decrease in encystment.

But how can this decrease in the encystment rate evolve? A large inoculum formed by extensive cyst beds is argued to be one major reason why dinoflagellates can compete with the faster growing diatoms (Klais et al. 2013; Lee et al. 2018). The large inoculum of cysts, ready to germinate as soon as conditions become favourable, lead to a rapid input of vegetative cells which can outcompete less abundant vegetative cells of other strains or species in the water column. In a previous resurrection study, Hinners et al. (2017) hypothesised that under cold conditions, strains with a higher encystment



(a) Evolution experiment with T-dep. cyst loss

(b) Evolution experiment under eutrophied conditions

Figure 2.3.3: (a) Relative biomass of all strains over 200 years including global warming, and including a temperature-dependent cyst mortality. The relative biomass is indicated by color. Strains differ only in their encystment factor, ranging from 0.1-9.8. The model was initialised with the strain with encystment factor 4.6, representing the encystment rate measured in historic, 100 years old cultures. (b) Relative biomass of all strains over 200 years of global warming under eutrophied nutrient concentrations.

rate have a competitive advantage over those which form less cysts. Vice versa, they hypothesise, that under warm conditions strains with a lower encystment rate benefit, since their bloom continues, even if the threshold temperature for encystment is exceeded, leading to larger blooms of strains with a low encystment rate. Our model results confirm this hypothesis. Without prescribing the encystment rate, our simulations show that strains with a high encystment rate are dominant under cold conditions (see also S2 in Appendix III, section 4.3), while strains with a lower encystment rate are more abundant under warm conditions. Under cold conditions, strains with a high encystment rate have enough time over the entire spring period to grow and form a bloom, such that after the onset of encystment a major part of the biomass of vegetative cells is transferred to resting stages forming a large cyst pool. In the following year, the high cyst pool leads to a high inoculum after germination, resulting in a larger bloom. Consequently, strains with a high encystment rate become dominant under cold conditions. In contrast, under warm conditions, the temperature threshold for encystment is exceeded early in time leading to high biomass transfer from vegetative cells to cysts early in spring due to the high encystment rate, thereby severely shortening the bloom time span, see left side of schematic Fig. 2.3.4. A lower encystment rate, however, will cause a prolonged spring bloom, since the onset of encystment does not directly terminate the spring bloom (see right side of Fig. 2.3.4). Since more vegetative cells are produced, also the inoculum of newly formed cysts becomes larger, even though the encystment rate is lower. This higher inoculum of cysts formed by strains with a low encystment rate represents an advantage in the following year, because it allows for a

higher amount of germinating cells. Therefore, in response to global warming, strains with a low encystment rate will gradually make up a larger fraction of the population and the mean encystment rate of the population will decrease. By showing a gradual decrease in the mean encystment factor for our model population in response to global warming, our model results support this theory. Thus, regarding our research hypothesis (1), our model confirms that global warming may cause an adaptive response in the encystment rate.

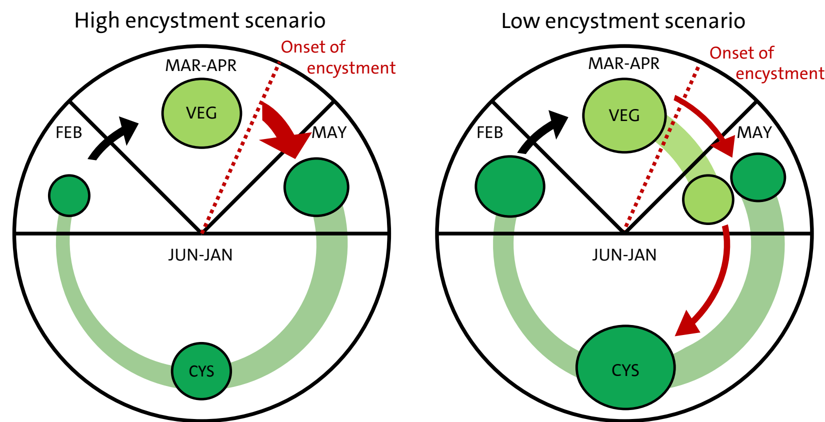


Figure 2.3.4: Schematic sizes of vegetative cell and cyst pool throughout the year under high temperature conditions, for a high (left) and for a low (right) encystment rate. Black arrows indicate germination, red arrows indicate encystment. The left part of the schematic shows the annual development of the vegetative cell and cyst pool for a higher encystment rate: due to the high spring temperature, the onset of encystment takes place already in the middle of April. The high encystment rate then causes a rapidly progressing encystment which terminates the spring bloom. During the following dormancy period (Jun-Jan) the cyst pool decreases due to cyst mortality and burial, until in the next spring (Feb) cysts germinate, initiating a new spring bloom. The right part of the schematic shows the annual development for a lower encystment rate under warm spring conditions. Since the encystment rate is lower, the onset of encystment in April does not lead to an immediate termination of the spring bloom, but to a more gentle decrease of the vegetative cell pool; the remaining vegetative cells are still able to form a prolonged spring bloom. The longer duration of the spring bloom eventually leads to a higher production of cysts, and this higher cyst inoculum will favour a larger vegetative cell pool in the preceding year.

Modified evolution experiments

Even though our results qualitatively agree with the results from resurrection experiments, they show some quantitative difference. The magnitude of change in encyst-

ment in our evolution model experiment is smaller than the change in encystment observed in resurrection experiments, which showed a change in the encystment factor from $X_C = 4.6$ to $X_C = 1.0$ within the past 100 years. Hence, we test whether further factors not yet captured in this model could have enhanced the adaptive response in nature.

For this purpose, we first look at cyst mortality. Cysts are known to have a large influence on the spring bloom formation (Klais et al. 2013; Lee et al. 2018), but it is unclear how cyst mortality might be influenced by global warming. A decrease in the survival potential of resting stages due to elevated temperatures has been previously observed in different phytoplankton species (Ellegaard and Ribeiro 2018). Since not only the sea surface, but also deeper water layers warmed over the past century (Laakso et al. 2018), this temperature increase may have caused an increase in cyst mortality over the past 100 years. We therefore study the sensitivity of the adaptive response under increasing temperature conditions and a simultaneously increasing cyst mortality. Indeed, this experiment shows a stronger decrease in the mean encystment factor in response to increasing temperatures. A temperature-dependent cyst mortality can thus amplify the adaptive response to global warming by representing an additional selection pressure towards a smaller encystment rate. For further exemplification (see also S3 in Appendix III, section 4.3).

Another possible explanation for a stronger change in encystment in nature may lie in the eutrophication, which the Baltic Sea experienced over the past century (Gustafsson et al. 2012), and which could have influenced spring bloom dynamics and thereby also evolutionary dynamics. Our model experiment with eutrophied conditions shows a slightly stronger decrease in the encystment factor compared to the basic evolution experiment. This stronger adaptive response develops, since strains with a lower encystment rate benefit more from eutrophied conditions compared to strains with a high encystment rate. Higher nutrient levels allow for an even longer growth phase and thus a higher production of vegetative cells after the onset of encystment. Furthermore, eutrophication may indirectly enhance cyst mortality by causing a lack of oxygen in bottom waters and sediments. Although cysts are generally considered to withstand anoxia, a general loss of cyst revivability leading to decreased germination at population level, has been observed for a number of species (Kremp and Anderson 2000; Lundholm et al. 2011). Thus, also eutrophication can enhance the adaptive response in encystment.

Summarising, regarding our research question (2), our model results do not exactly mirror the data from resurrection experiments, but show a weaker change in encystment. We therefore conclude that additional factors, such as eutrophication and an increasing cyst mortality due to global warming could have enhanced the change in encystment in nature.

Summary and conclusions

In this study, we use an advanced ecosystem model to investigate for the first time how a life cycle trait may have responded to global warming, based on data from a case study on living sediment archives (Hinners et al. 2017). We find that an increase in temperature can cause a decrease in encystment, because a high encystment rate can prevent the formation of a spring bloom at an early stage, whereas a lower encystment rate allows for the formation of an extensive spring bloom and is thereby more beneficial for the long-term survival of the population. Consequently, our results confirm that global warming can indeed cause adaptive response in a life cycle trait. Since our results show a weaker change in encystment compared to the results from sediment archives, we perform sensitivity experiments to identify potential further factors that could have enhanced the adaptive response in nature. These sensitivity experiments reveal that eutrophication leads to a slightly enhanced change in encystment, whereas an increasing cyst mortality due to global warming strongly enhances the adaptive response in nature.

By using this advanced ecosystem model which includes adaptation, we demonstrate that marine ecosystem modelling represents a powerful, so far unexhausted approach to investigate the evolutionary potential of phytoplankton organisms. Including the evolutionary potential of phytoplankton into ecosystem models will in the short run provide a tool to further exemplify and understand results from resurrection experiments and experimental evolution, and in the long run help improving the predictive force of ecosystem and Earth system models.

2.4 Study IV: Modelling the influence of temperature fluctuations on adaptation

Modelling the influence of short-term temperature fluctuations on the adaptive response to global warming in a phytoplankton trait

Jana Hinners & Inga Hense

Phytoplankton organisms are an important component of the marine ecosystem and they have the potential to rapidly adapt to changing environmental conditions. Due to anthropogenic greenhouse gas emissions, global temperatures are rising and extreme climatic events are expected to become stronger. Here, we investigate which influence short-term temperature fluctuations have on the adaptive response in a phytoplankton life cycle trait using an ecosystem model which allows for adaptation. Our model results show that short-term temperature fluctuations can strongly enhance the adaptive response to global warming by occasionally causing extreme temperature conditions, which lead to a stronger selection pressure. Our model study thereby demonstrates that the influence of variability and extreme events should be considered, when investigating how phytoplankton will develop and evolve in the future.

Keywords: adaptation, ecosystem model, fluctuations, global warming, phytoplankton

Hitherto unpublished

Introduction

Phytoplankton play a central role in the earth's ecosystem, producing almost half of the atmospheric oxygen (Field et al. 1998), representing the base of the marine food web, and being an important component of marine biogeochemical cycles (Falkowski et al. 2008). Since phytoplankton organisms are ectotherm, important traits affecting their life cycle, growth and metabolic rates are temperature-dependent (Kremp and Parrow 2006; Thomas et al. 2012; Padfield et al. 2016). Due to the high evolutionary potential of phytoplankton, their temperature-dependent traits may rapidly respond to environmental changes. In context of climate change, extreme weather events including temperature fluctuations are expected to increase in intensity and frequency in the future (Easterling et al. 2000; Beniston et al. 2007; Field 2012). Here, we investigate the influence of temperature fluctuations on the adaptive response in a phytoplankton life cycle trait to global warming.

As an immediate response to a change in temperature, phytoplankton organisms can acclimate to these changes e.g. by up- or down-regulating growth (Eppley 1972; Geider 1987). Due to large population sizes and short generation times, they moreover have the potential to quickly adapt to changing temperature conditions. Experimental evolution studies suggest that adaptation can occur already after a few hundred generations (Listmann et al. 2016; Padfield et al. 2016; Schaum et al. 2018). The impact of fluctuations on the adaptive response of organisms is however under discussion. Fluctuations in a changing environmental variable may either slow down adaptation by recurrently restoring mild conditions and thus relaxing the selection pressure, or they may accelerate adaptation by allowing for population recovery as a positive demographic effect (Hao et al. 2015). In an experimental evolution study with a bacteriophage, temperature fluctuations reduced the chance for environmental rescue (Hao et al. 2015), whereas in a study on a marine diatom, temperature fluctuations accelerated the adaptive response (Schaum et al. 2018). In another study, fluctuations in CO_2 conditions also caused an accelerated evolutionary response in a green-algae, compared to stable environmental conditions (Schaum et al. 2015).

In nature, the past hundred years of global warming may have already caused an adaptative response in a temperature-dependent life cycle trait of a Baltic Sea dinoflagellate: The encystment rate of the spring blooming dinoflagellate *Apocalathium malmogiense*, which causes the termination of the spring bloom, has decreased significantly (Hinners et al. 2017). This decrease in the encystment rate is interpreted as an adaptation to global warming, since it allows for a prolonged spring bloom, even if the temperature threshold for encystment is already exceeded. An ensuing modelling study using an ecosystem model which allows for adaptation supports this hypothesis (see Study III, section 2.3 of this thesis). Which influence temperature fluctuations have on the adaptive response to global warming, has so far not been addressed in an ecosystem modelling framework though.

So far, there exist only conceptual studies on the genetic principles of evolution of phytoplankton in response to fluctuations (Lande et al. 2009; Chevin et al. 2017),

which lack a realistic environmental framework. (Grimaud et al. 2015) studied how phytoplankton on a global scale become adapted to local thermal conditions; but also in this study an ecosystem framework is missing. Past ecosystem modelling studies focus instead on the role of seasonal temperature changes on the distribution and coexistence of phytoplankton species, (e.g. Thomas et al. 2012; Kremer and Klausmeier 2017), but exclude the potential for adaptation. In a recent study, the adaptive response to global warming in a life cycle trait has been investigated (Study III, section 2.3), but the authors did not address the effect of temperature fluctuations. Here, we apply an ecosystem model which includes adaptation. We compare four scenarios excluding and including long-term global warming, as well as short-term temperature fluctuations to analyse which influence short-term temperature fluctuations have on the adaptive response in a phytoplankton trait.

Model description

Life-cycle-ecosystem model

We use the life-cycle-ecosystem model from Study III (section 2.3), which focuses on the spring-bloom dinoflagellate *A. malmogiense* from the Gulf of Finland, N Baltic Sea. The model includes 3 life cycle stages of *A. malmogiense*, vegetative cells (V), gametes (G), and resting cysts (C), as well as nutrients (N) and detritus (D). The components are in nitrogen units (in $mmol \;\; N \; m^{-3}$) and mass conservation is ensured. In nature, *A. malmogiense* co-occurs with other spring blooming dinoflagellates and shares a similar life cycle. In early spring, cysts are resuspended from the sediment, germinate and develop into vegetative cells (Kremp and Anderson 2000; Kremp 2001; Kremp et al. 2005). This process is simplified in the model by limiting germination to a certain time window in early spring. Vegetative cells subsequently form a spring bloom and take up nutrients. Gamete production takes place under nutrient-replete, low temperature conditions (Hinners et al. 2017), but since we assume that sexual reproduction plays a minor role in the life cycle of *A. malmogiense*, we do not consider this process in detail in the model. When temperatures rise above a certain threshold, vegetative cells transform into resting cysts (Kremp and Parrow 2006; Hinners et al. 2017). For all life cycle stages a mortality rate is assumed, leading to the production of detritus; detritus is remineralised into nutrients.

The dinoflagellate population is modelled with 98 strains, which differ only in their temperature-dependent encystment rate. The encystment rate of each strain is represented by its respective encystment factor, whereby an encystment factor of $X_C = 4.6$ represents the encystment rate measured in revived 100 year old cultures and and encystment factor of $X_C = 1.0$ represents an encystment factor measured in revived recent cultures (Hinners et al. 2017). All strains compete with each other for nutrients and light, leading to natural selection. Moreover, a small mutation rate is included, allowing for evolution by small steps (see Study III, section 2.3 for details).

Model setup and temperature forcing

The life cycle ecosystem model is implemented in Matlab with a time step of 1 h using a 0-dimensional model; the length of one simulated year is fixed at 360 days. The differential equations are solved using a predictor-corrector method.

We apply the same temperature forcing as used in Study III, section 2.3. The temperature forcing stems from a high resolution reconstruction of atmospheric forcing (HiResAFF) for Northern Europe (Schenk and Zorita 2012). The mean climatic temperature curve for 1900 is calculated based on the air temperature data from 1885-1915 from station LL7 in the Central Gulf of Finland (lat 59°51, lon 24°50), to which a *cos*-function is fitted, see eq. 2.4.1. To account for global warming, a continuous increase of 0.3°C/decade is applied following Laakso et al. (2018).

$$T_{1900} = 7.5 - 10.5 \times cos\left(\frac{2 \times \pi \times (day - 35)}{360}\right) \tag{2.4.1}$$

To analyse the effect of short-term temperature fluctuations on the model population, we include daily oscillations and weekly random fluctuations in the temperature forcing. The extent of both daily oscillations and weekly fluctuations were calculated from the HiResAFF data set (Schenk and Zorita 2012). Daily oscillations amount to ±0.75 °C, weekly fluctuations are set to random values in the range of ±6 °Conce per week and interpolated linearly in-between.

Fluctuation-evolution experiment

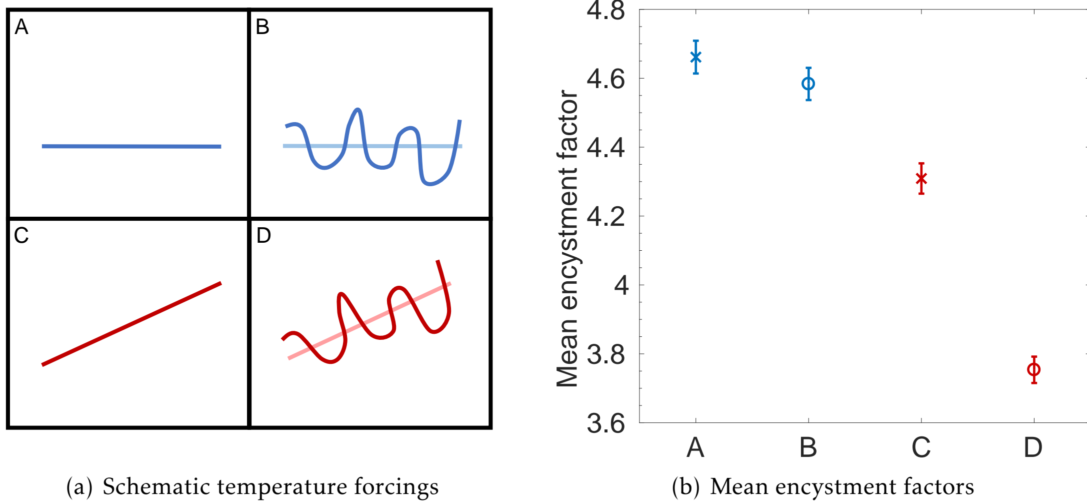
To investigate which effect short-term temperature fluctuations have on the adaptive response to global warming, we performed four model runs (A-D) which differ only in their temperature forcing. Forcing A and B follow the temperature curve from 1900, excluding (A), or including (B) short-term temperature fluctuations. Forcing C and D include an increase in temperature of 0.3 °C/decade, with forcing C excluding short-term temperature fluctuations, and forcing D including them. All experiments are run for 20 years excluding fluctuations and temperature increase to allow for model spin-up. For simulations including temperature fluctuations, an ensemble of 30 model runs is used for analysis. We initially set V = C = G = D = 0.0 mmol N m^{-3}, N = 5.9 mmol N m^{-3}. Only strain N_{46} with encystment factor $X_C = 4.6$, representing the encystment rate measured in revived historic cultures (Hinners et al. 2017), was initialised with $V_{46} = 1.1$ mmol N m^{-3}.

Results & Discussion

Here, we study the impact of short-term temperature fluctuations on the magnitude of an adaptive response to global warming. For this purpose, we use a life-cycle-ecosystem model for the dinoflagellate *A. malmogiense*, which allows for an adaptive response in the encystment rate, a temperature-dependent life cycle trait responsible for the termination of the spring bloom. We perform 4 model experiments with different temper-

ature forcings (A) excluding global warming and fluctuations, (B) excluding warming, but including fluctuations, (C) including warming, but excluding fluctuations, and (D) including warming as well as fluctuations, see also schematic Fig. 2.4.1(a).

For each of the model experiments, we analyse the mean encystment factor after 20 years of model spin-up plus 200 years of the respective temperature forcing. The mean encystment factor of the population is based on the relative biomass of each strain of the population.



(a) Schematic temperature forcings (b) Mean encystment factors

Figure 2.4.1: Model simulations with vs. without short-term temperature fluctuations. (a) shows a schematic of the 4 applied temperature forcings, A and B for historic, cold temperature conditions and C and D for global warming; excluding (A and C) and including (B and D) short-term temperature fluctuations. (b) shows the mean encystment factor in the final year of simulations, based on the relative biomass of all strains, and including a standard deviation.

In the final year of simulations, the population's mean encystment factors for the four different forcings lie at A = 4.66, B = 4.58, C = 4.31, and D = 3.75, see Fig. 2.4.1(b).

As previously shown in Study III (section 2.3), increasing temperatures (in scenarios C & D) cause a decrease in the encystment rate, compared to lower, pre-industrial temperature conditions (in scenarios A & B), see Fig. 2.4.1(b). This decrease has been explained by a prolongation of the spring bloom under warming conditions: Under increasing temperature conditions, a high encystment rate causes an early inhibition of the spring bloom shortly after germination, and consequently a limited formation of new cysts. A lower encystment rate in comparison does not directly terminate the spring bloom as soon as the temperature exceeds the threshold for encystment. Instead, the lower encystment rate leads to a more gradual decrease of the vegetative cell concentration, which allows for a longer and larger spring bloom and thereby also for a larger pool of newly formed cysts. In the following year, strains with a lower encystment rate, germinating from the larger cyst pool, can form a larger fraction in the new spring bloom. Eventually, increasing temperatures will cause a decrease in the population's mean encystment rate.

Our model simulations show a decrease in the mean encystment factor for all scenarios which include global warming and/or short-term-temperature fluctuations (B-D), in comparison to the scenario (A) excluding warming and fluctuations. Thus, not only increasing temperatures but also short-term temperature fluctuations can represent drivers for an adaptive response in a life cycle trait, the encystment rate. Comparing scenarios A & B, which exclude global warming, temperature fluctuations cause a slight decrease in the encystment factor by 0.08. Comparing, scenarios C & D, which include global warming, fluctuations cause an even stronger decrease in the mean encystment factor by 0.56. The combination of increasing temperatures and short-term temperature fluctuations (D) more than double the adaptive response in the mean encystment rate, compared to the adaptive response caused by increasing temperatures alone (C).

These results are in agreement with findings from previous experimental evolution studies on phytoplankton organisms, showing that adaptation is accelerated when the environmental variable under selection is not held steady, but fluctuates (Schaum et al. 2015; Schaum et al. 2018). Schaum et al. (2018) explains the stronger adaptation under fluctuating temperature conditions with the higher population size that can be maintained, favouring an earlier evolutionary rescue. In our model system, due to the ecosystem setting which is based on mass conservation, the biomass of the population does not differ drastically between the different forcings (see S1, Appendix IV, section 4.4). Moreover, neither the standard deviation of the mean encystment factor, which correlates with the effective population size, varies among the four different forcings, see Fig 2.4.1 (b). Thus, both the overall biomass as well as the effective population size are similar in all four forcings and cannot explain the stronger adaptive response in the encystment rate under fluctuating conditions.

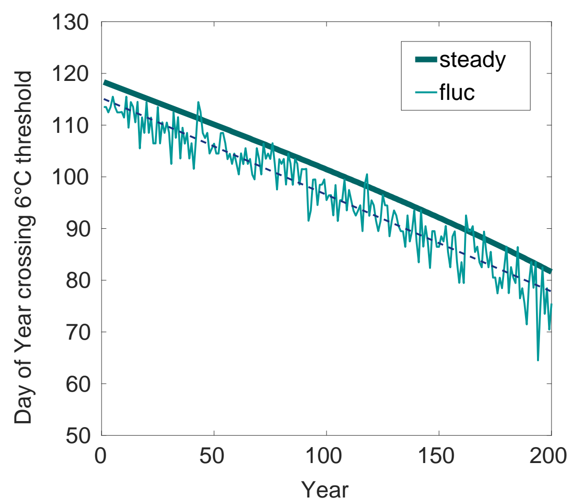


Figure 2.4.2: Day of the year on which the encystment threshold of 6°C is exceeded for the first time after the onset of germination, for a temperature forcing with an increase in temperature by 0.3°C/decade, excluding (steady, forcing C) and including (fluc, forcing D) short-term temperature fluctuations.

In our case, the reason for the stronger adaptive response lies in the earlier onset of encystment under fluctuating temperature conditions. Even though the annual mean temperature including short-term temperature fluctuations remains similar to the temperature under steady conditions, the time point, when the temperature exceeds the encystment threshold for the first time after germination, is earlier under fluctuating conditions, see Fig 2.4.2. This occasional exceeding of the encystment threshold under fluctuating conditions leads to a severe reduction of the vegetative cell concentration for strains with a high encystment factor early on in the growth period. Strains with a lower encystment factor are less affected by the crossing of the encystment threshold, their lower encystment rate allows for the development of a higher vegetative cell concentration, even if the threshold for encystment is occasionally exceeded. Hence, temperature fluctuations, repeatedly causing extreme conditions, lead to an even stronger selection for strains with a lower encystment rate and a stronger adaptive response. Regarding the influence on phenology, in contrast, short-term temperature fluctuations cause a similar, slightly milder shift towards earlier blooming (see S2, Appendix IV, section 4.4).

Conclusions

In this model study we investigate the influence of temperature fluctuations on the adaptive response in a functional phytoplankton trait. Our model results show that fluctuations can strongly enhance the adaptive response to global warming. The reason for the stronger adaptive response lies in the stronger selection pressure caused by the occasional occurrence of extreme temperature conditions. Regarding the expected future increase in extreme climatic events due to climate change, the influence of variability and extreme events should therefore be considered, when investigating how phytoplankton will develop and evolve in the future.

3 | SUMMARY & CONCLUSIONS

In this thesis, I investigated how the past century of global warming may already have affected a phytoplankton species. For this purpose, I combined resurrection experiments on the Baltic Sea spring bloom dinoflagellate *A. malmogiense*, with advanced ecosystem modelling. In the following chapter I will summarise my findings, and answer my initial research questions, grouped into the four studies that comprise this thesis. I will end this thesis with my conclusions and suggestions for future research perspectives.

Study I: Cyst profiles and viability across the sediment

In the first study, my co-authors and I investigated sediment cores from the Gulf of Finland, a region heavily influenced by global warming over the past century, to resolve the distribution and viability of dinoflagellate resting cysts in different sediment layers. For this purpose, we extracted two sediment cores from the central Gulf of Finland. The material from one core was used for a gamma-spectrometric dating; the other core was used for cyst analyses. We analysed the abundance and viability of resting cysts from three dinoflagellate species, which are important for the Northern Baltic Sea spring bloom: *Biecheleria baltica*, *Apocalathium malmogiense* and *Peridiniella catenata*. Moreover, we correlated the results from cyst abundances with monitoring data available for the region. Our main research question for this study was:

Do we see changes in dinoflagellate cyst abundance and composition across the sediment and are cysts suitable to be resurrected?

The cyst profiles of *B. baltica* and *A. malmogiense* revealed that both species have been present in the Baltic Sea since the beginning of the 20th century. The abundance of cysts for both species correlated well with monitoring data of plankton retrieved from the water column. Over the past century, both species increased in abundance, and the increase appears to be correlated to eutrophication and global warming. The short cyst record of *P. catenata* in contrast did not reflect its long-term presence documented in monitoring data, suggesting that cyst profiles are not universally applicable to reconstruct the history of dinoflagellate species.

Regarding the viability of resting cysts, only cysts of *A. malmogiense* successfully germinated from all tested sediment layers. The high survival capacity of more than a

century makes this species an ideal study organism for resurrection experiments.

Study II: Thermal resurrection experiments

In the second study, we performed thermal tolerance experiments with resurrected strains of the dinoflagellate *A. malmogiense*. We compared revived historic, ca. 100-year-old strains, and revived recent, ca. 2-year-old strains, focusing on three temperature-dependent traits, growth reaction norm, cell size and life cycle processes. In doing so, we aimed to answer the following research question:

Have temperature-dependent traits of a phytoplankton species changed in response to global warming over the past 100 years?

We observed no significant differences in the thermal reaction norm and cell size of historic and recent strains. Therefore, we concluded that neither the reaction norm, nor the cell size changed in response to increasing temperatures over the past century. However, we detected a significant change in a life cycle trait. Recent strains showed a 5 times lower encystment rate compared to historic strains. We hypothesised that this lowered encystment rate represents an adaptive response to global warming: usually, the onset of encystment, triggered by the exceeding of a temperature threshold, leads to the termination of the spring bloom. Under high temperature conditions, a high encystment rate will then lead to a shortened spring bloom. A lower encystment rate in comparison will not cause a direct termination of the spring bloom, but will allow for a prolonged growth period, even if the encystment threshold is exceeded. Therefore, under high temperature conditions, a lower encystment rate is beneficial, and, in response to global warming, the encystment rate decreases. Whether this hypothesis actually applies to the changes that occurred in *A. malmogiense*, could not be answered with this experimental setting, though.

Study III: Ecosystem modelling on adaptation to global warming

In the third study, we applied an advanced ecosystem model, which allows for adaptation and comprises the life cycle of *A. malmogiense*. The ecosystem model was set up with a model population comprising a number of strains that only differ in their encystment rate. This model population was analysed for 200 years of global warming to answer the following research question:

May global warming be the cause of the observed change in a phytoplankton functional trait?

Our model simulations revealed that global warming can indeed have led to a decrease

in the encystment rate, which we observed in resurrected dinoflagellate strains (Study II). Concomitantly, we could confirm our initial hypothesis, that a lower encystment rate is beneficial under higher temperature conditions, since it allows for a prolonged spring bloom. The magnitude of change in the encystment rate that we obtained in our simulations was however smaller than the observed change in our previous resurrection experiments. We therefore analysed further plausible factors that could have enhanced the change in encystment rate in nature. We found that eutrophication as well as an increasing cyst mortality in response to global warming represent possible factors, which could have accelerated the adaptive response to global warming.

Study IV: Modelling the influence of temperature fluctuations on adaptation

In the fourth study, we used our advanced ecosystem model, established in Study III, to further explore how the adaptive response in encystment is influenced by short-term (daily and weekly) temperature fluctuations. For this purpose, we compared four scenarios which include or exclude long-term global warming, as well as short-term temperature fluctuations. Our leading research question here was:

How do short-term temperature fluctuations influence the adaptive response of phytoplankton to global warming?

Our model results revealed that short-term temperature fluctuations can substantially accelerate the adaptive response to global warming. By occasionally causing extreme high temperature conditions, fluctuations lead to a stronger selection pressure and thus a stronger decrease of the encystment rate.

Conclusions and future perspectives

This PhD thesis combined two scientific approaches, resurrection experiments and ecosystem modelling, to analyse how a phytoplankton species responded to the past century of global warming. It is very important to understand how phytoplankton, a crucial component of the marine ecosystem, deal with changing environmental conditions; even more so since these changes are expected to increase within and throughout the next century. In my thesis, I demonstrated that phytoplankton adaptation may not only take place under laboratory conditions, but has likely already occurred in nature in response to the past decades of warming. In addition, adaptive responses can reach beyond a change in the reaction norm or cell size (as previously shown), but can stretch into other temperature-dependent traits, such as life cycle processes.

This PhD project has opened up a number of further questions and perspectives.

Regarding my study species *A. malmogiense* in particular, it is not yet resolved under which conditions sexual reproduction occurs and how this process is embedded in

the life cycle. Sexual reproduction, by generating new genomic combinations, may accelerate evolution and thereby affect adaptive responses. Understanding the conditions that trigger sexual reproduction (for example high or low nutrient concentrations) could therefore indicate under which conditions adaptive responses are sped up.

Resurrection experiments, by allowing to sample a population across decades of environmental change, represent a promising approach to understand how phytoplankton adapt to changing conditions in a most realistic setting. Simultaneously, they can give hints on how phytoplankton evolution may proceed in the future. The analysis of living sediment archives is still at its beginning and high-throughput techniques, as well as genomic and transcriptomic analyses will allow for new research questions to be answered with the help of living sediment archives. Since these archives conserve phytoplankton history including multiple functional traits, as well as the response to multiple changing abiotic and biotic factors, there is plenty of room for future analyses. Moreover, hidden adaptive changes in response to global warming, for example in phytoplankton life cycle traits, may be more common and can be further explored using living sediment archives.

Since many environmental factors change simultaneously in nature, it remains a challenge to understand which of the altering environmental drivers may have caused responses in phytoplankton. Here, ecosystem models that include the potential for adaptation can be useful, especially to understand results from resurrection experiments, but also results from evolution experiments (e.g. from mesocosms). In our specific case, an angle for future research is to include a more complex description of the ecosystem model. Both a higher spatial dimensionality (1D to 3D), as well as the consideration of additional factors, such as competition between diatoms and dinoflagellates, may help to understand how adaptive processes may alter phytoplankton productivity, phenology and community structure. However, ecosystem modelling that allows phytoplankton to evolve will not only help to further analyse and understand data from resurrection and evolution experiments. It can also play an important role to assess how phytoplankton adaptation to global change may affect marine ecosystems and biogeochemical cycles and may feedback on climate.

To conclude, living sediment archives provide a unique opportunity to understand how phytoplankton respond to changing environmental conditions in nature and should therefore be further explored. Additionally, ecosystem modelling represents a powerful tool to understand how phytoplankton evolve and what consequences may arise thereof for ecosystems, biogeochemical cycles and climate. Hence, the evolutionary potential of phytoplankton should be more generally considered in ecosystem models. Here, I combined resurrection experiments and ecosystem modelling for the first time and could demonstrate that a tight linkage of these two complementary research approaches can represent a fruitful way to study and understand phytoplankton adaptation.

4 | APPENDICES

4.1 Appendix I

Supplementary Table 1: Calculated sediment properties, gamma-spectrometric results of Pb210, Ra226 and Cs137, and the results of CRS model Pb210 dating .

depth	dry weight	wet weight	water content	porosity	dry bulk density	mass depth	Pb210	Pb210 RSD	Cs137	Cs137 RSD	Ra226	Ra226 RSD	excess Pb210	excess Pb210, 1σ	**CRS Date**	**CRS age**	**CRS age std.error**	CRS dry mass sed.rate	CRS dry mass std.error
[cm]	[g]	[g]	%		[g/cm3]	[g/cm2]	[Bq/kg]	[%]	[Bq/kg]	[%]	[Bq/kg]	[%]	[Bq/kg]	[±Bq/kg]	**[yr]**	**[a]**	**[±a]**	[g/cm2/a]	[±g/cm2/a]
															2015	**0**			
1	6,12	75,82	0,92	0,96	0,08	0,08	539,2	5	190,9	5	66	7	473	27	**2013,5**	**1,5**	**1,5**	0,055	0,0038
2	7,38	64,18	0,88	0,95	0,12	0,21	508,8	5	192,6	5	62	7	447	26	**2011,2**	**3,8**	**1,5**	0,054	0,0038
3	7,20	62,87	0,89	0,95	0,12	0,33	586,8	7	215,9	6	73	7	514	41	**2008,7**	**6,3**	**1,6**	0,043	0,0040
4	8,61	62,33	0,86	0,93	0,15	0,48	472,4	7	217,9	6	86	9	386	34	**2005,5**	**9,5**	**1,7**	0,052	0,0053
5	12,06	68,56	0,82	0,92	0,20	0,68	418,9	8	278	6	68	9	351	34	**2001,7**	**13,3**	**1,7**	0,051	0,0057
6	14,74	68,00	0,78	0,89	0,25	0,92	306,5	7	295,6	5	79	13	228	24	**1997,5**	**17,5**	**1,8**	0,069	0,0083
7	15,20	69,59	0,78	0,89	0,25	1,17	297,6	6	301	5	69	8	229	19	**1993,7**	**21,3**	**1,9**	0,061	0,0061
8	15,73	67,66	0,77	0,88	0,27	1,44	260,4	12	274	6	60	9	200	32	**1989,3**	**25,7**	**2,0**	0,061	0,0107
9	15,24	73,47	0,79	0,90	0,23	1,67	273,1	10	293,2	5	80	12	193	29	**1985,2**	**29,8**	**2,1**	0,056	0,0095
10	13,58	67,08	0,80	0,90	0,23	1,90	339	9	264,6	6	60	8	279	31	**1979,8**	**35,2**	**2,2**	0,032	0,0044
11	12,24	64,97	0,81	0,91	0,21	2,11	295,7	7	175,1	5	61	11	235	22	**1973,2**	**41,8**	**2,5**	0,031	0,0039
12	11,80	66,02	0,82	0,91	0,20	2,31	269,9	7	90,4	6	56	11	214	20	**1966,4**	**48,6**	**2,8**	0,028	0,0037
13	12,78	69,01	0,81	0,91	0,21	2,52	246,5	7	72	6	58	8	189	18	**1958,6**	**56,4**	**3,4**	0,025	0,0036
14	16,31	73,59	0,78	0,89	0,25	2,77	199,1	8	57,5	7	70	7	129	17	**1948,6**	**66,4**	**4,3**	0,027	0,0053
15	30,03	77,35	0,61	0,78	0,50	3,27	104,1	9	17,4	9	56	7	48	10	**1932,1**	**82,9**	**6,5**	0,043	0,0139
16	33,26	78,52	0,58	0,76	0,56	3,83	119,4	14	9,9	22	50	11	69	18	**1910,1**	**104,9**	**8,2**	0,015	0,0061
17	35,61	87,22	0,59	0,77	0,53	4,36	63,4	13	8,3	17	54	10	9	10	**1878,0**	**137,0**	**17,3**	0,040	0,0584
18	32,28	82,18	0,61	0,78	0,50	4,86	71,7	14	2,4	43	50	10	22	11	**1844,7**	**170,3**	**23,9**	0,006	0,0064
19	27,47	79,66	0,66	0,81	0,43	5,29	44,4	14	1,5	44	46	9	-2	7					
20	24,45	78,23	0,69	0,83	0,38	5,67	47,4	18	4,6	22	48	8	-1	9					
21	24,66	76,17	0,68	0,83	0,40	6,07			2,1	43	39	8							
22	27,61	81,81	0,66	0,82	0,42	6,48					37	9							
23	27,47	75,31	0,64	0,80	0,46	6,94					38	9							
24	30,17	75,41	0,60	0,78	0,52	7,46					41	8							
25	30,10	83,87	0,64	0,80	0,45	7,91					49	9							
26	25,18	79,09	0,68	0,83	0,39	8,30					51	10							

4.2 Appendix II

S1: Results of statistical analyses

Table 4.1: Growth rate – GAM

	df	AIC	edf	Ref.df	F	p
Growth rate~Temp	3	−175.473				
Growth rate~s(Temp)	4.977	**-273.498**	2.977	3	58.95	$< 2e-16**$
Growth rate~s(Temp)+Age	5.977	−272.587				
Growth rate~s(Temp)+Strain no.	9.977	−267.229				

Table 4.2: Cell Size – GAM

	df	AIC	edf	Ref.df	F	p
Cell Size~Temp	3	257.918				
Cell Size~s(Temp)	4.903	**248.069**	2.903	2.993	129.2	$< 2e-16**$
Cell Size~s(Temp)+Age	5.901	250.022				
Cell Sizee~s(Temp)+Strain no.	8.581	255.477				

Table 4.3: Plasticity – T test

	t	df	p
Age	−0.957	2	0.440

Table 4.4: Acclimation temperature (3 or 6°C)

	df	Sum Sq.	Mean Sq.	F	p
Cyst – LM					
Acclimation Temp	1	389137	389137	0.0385	0.8451
Residuals	58	585755659	10099235		
Gametes – LM					
Acclimation Temp	1	26154986	26154986	1.6304	0.2121
Residuals	28	449172204	16041864		
Reaction norm – GAM					
Acclimation Temp	1			0.21	0.648

Table 4.5: Cyst POC budget – LM

	df	AIC	Sum Sq.	Mean Sq.	F	p
POC cysts~Temp	3	353.4029				
POC cysts~Temp+Age	4	**348.7149**				
Temp	1		156953200	156953200	13.4423	0.002292**
Age	1		78810891	78810891	6.7498	0.020178*
Residuals	15		175140824	1167605		

Table 4.6: Encystment rate – LM

	df	AIC	Sum Sq.	Mean Sq.	F	p
Encystment rate~Temp	3	−88.18				
Encyst rate~Temp+Age	4	**-92.37**				
Temp	1		0.0028447	0.0028447	10.6911	0.00517**
Age	1		0.0016383	0.0016383	6.1571	0.02542*
Residuals	15		0.0039912	0.0002661		

Table 4.7: Gamete POC budget – LM

	df	AIC	Sum Sq.	Mean Sq.	F	p
POC Gametes~Temp	3	**584.6913**				
POC Gametes~Temp+Age	4	586.3389				
Temp	1		56474904	56474904	3.6835	0.06558
Age	1		4890841	4890841	0.319	0.57687
Residuals	27		413961446	15331905		

S2: Parameter estimates for reaction norm fits

Table 4.8: Reaction norm fit parameter values

Parameter	Historic	Recent
μ_{max}	0.337	0.354
T_{opt}	10.91	10.68
T_{l1}	7.41	7.06
T_{l2}	−6.34	−5.74

S3: Parameter estimates for linear regression of cell size – temperature correlation

$$\text{Cell diameter}[\mu m] = \text{a} \times \text{Temperature}[^\circ\text{C}] + \text{b}$$

Table 4.9: Cell size – temperature correlation

a [μm °C^{-1}]	−0.283
b [μm]	22.945

Supplementary Excel tables S4-S6 with experimental data can be accessed on `http://rspb.royalsocietypublishing.org/content/284/1864/20171888.figures-only`
(Accessed latest: 2018-07-23).

4.3 Appendix III

S1: Model evaluation and seasonal cycle

To analyse the seasonal cycle of our life-cycle-ecosystem model, the model was forced for 10 years with the temperature curve for the year 2000, calculated from the historic temperature curve for 1900 (eq. 7 in main text) including an addition of 3°C. The model was initialised with the strain with an encystment factor $X_C = 4.6$, measured in revived historic strains, excluding the potential for selection or mutation. The last year of simulations is illustrated in Fig. 4.3.1.

Our life-cycle-ecosystem model is able to represent the natural spring bloom phenology for the dominating dinoflagellate complex in the Gulf of Finland, which includes *A. malmogiense*. In agreement with previous studies (Kremp and Heiskanen 1999; Kremp et al. 2005; Fleming and Kaitala 2006; Olli and Trunov 2010), the dinoflagellate spring bloom starts around the middle of March and is terminated by the onset of cyst formation around the middle of May, see Fig. 4.3.1. During the spring bloom, the number of gametes increases similar to observations (Kremp and Heiskanen 1999). The concentration of cysts peaks at the end of May and decreases again over fall and winter due to burial and cyst mortality, as previously described (Kremp and Heiskanen 1999; Kremp et al. 2005).

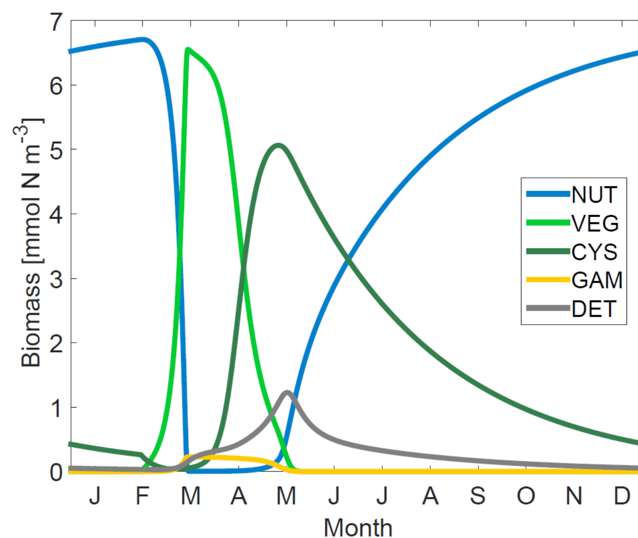


Figure 4.3.1: Modelled annual life cycle of *A. malmogiense*, including vegetative cells (VEG), resting cysts (CYS), gametes (GAM), as well as detritus (DET), and nutrients (NUT). The model is forced with the climatic temperature curve for the central Gulf of Finland for 2000. Ticks on the x-axis mark the middle of the respective month.

S2: Sensitivity experiment excluding temperature increase

To investigate the changes in encystment without global warming, the model was run for 200 years (plus 20 years for model spin-up). The model was initialised with the same

biomass (V = 0.0112 mmol N m^{-3}) for all 98 strains of different encystment factors, ranging from 0.1-9.8.

Without global warming the population evolves to a comparably high encystment rate. The mean encystment factor after 200 years without global warming lies at X_C = 4.56 and thus close to the encystment factor of X_C = 4.6 measured in revived historic strains (Hinners et al. 2017).

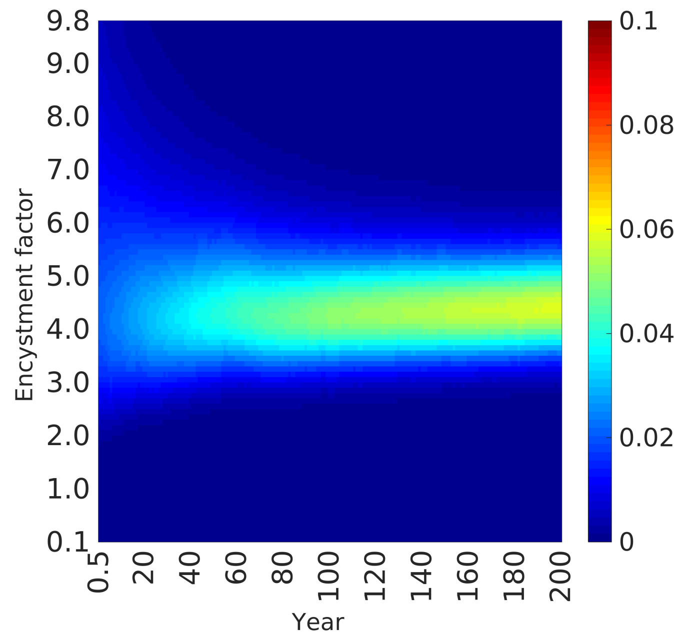
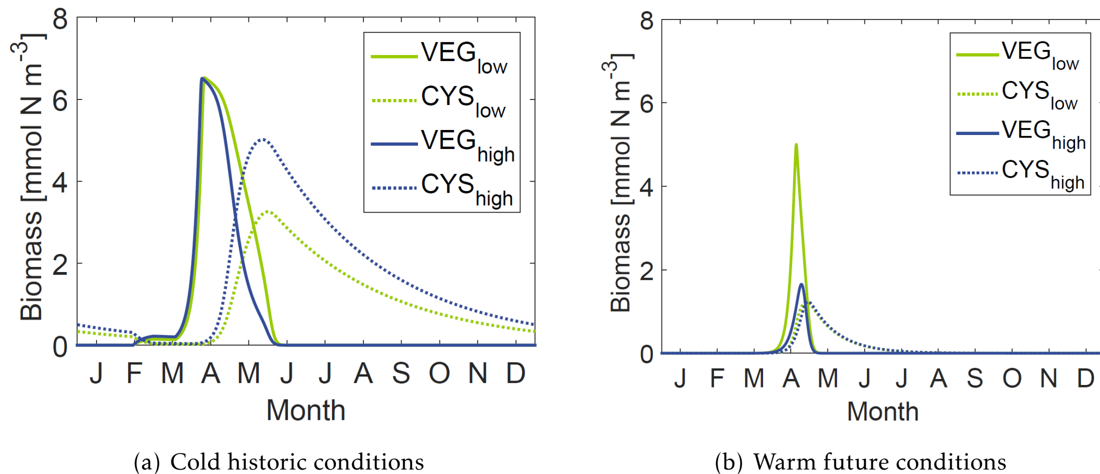


Figure 4.3.2: Relative biomass of all strains over 200 years, excluding an increase in temperature. The model was initialised with the same biomass for all strains. The relative biomass is indicated by color. Strains differ only in their encystment factor, ranging from 0.1-9.8.

S3: Annual life cycle for temperature-dependent cyst mortality

To disentangle how a temperature-dependent cyst mortality affects the vegetative cell and cyst pool for a lower and a higher encystment rate, we compare the seasonal cycle of vegetative cell and cyst concentrations for a low encystment factor (X_C = 1.7) and a high encystment factor (X_C = 4.6) under cold, historic conditions, see eq. 7 in main text, and under warm, future conditions including an addition of 6°C to eq. 7. The model was forced for 10 years with the respective temperature forcing, and each simulation was performed for a homogeneous population, which only expresses one constant encystment factor (low vs. high), thus excluding selection and mutation. The last year of each simulation is illustrated in Figs. 4.3.3.

Since the model was separately run for a population with either a high or a low encystment factor, these simulations exclude selection and competition, as well as mutations. Under cold historic conditions, the spring bloom formed by vegetative cells has a similar shape for both a low and a high encystment factor, while the cyst inoculum is



(a) Cold historic conditions

(b) Warm future conditions

Figure 4.3.3: Seasonal cycle under cold (a) and warm (b) conditions. The vegetative cell concentration (solid line) and cyst concentration (dashed line) of a population made up exclusively by one strain with a low encystment factor is depicted in green; in blue is the vegetative cell and cyst concentration of a population exclusively made up by one strain with a high encystment factor.

much stronger for a high encystment factor. On closer consideration, the spring bloom for a high encystment factor however starts slightly earlier, due to the higher amount of germinating cells from the larger cyst pool. Under selection pressure, this head start of strains with a high encystment factor represents a competitive advantage and will cause a concurrent dominance of strains with a high encystment factor.

Under warm future conditions, the vegetative cell concentration for a low encystment factor is much higher compared to a high encystment rate, since the early onset of encystment shortly after the initiation of the spring bloom hinders the development of an extensive spring bloom for a high encystment factor. Under increasing temperature conditions, a lower encystment factor is therefore beneficial.

4.4 Appendix IV

S1: Integrated annual biomass

To evaluate the population size after 200 years of the respective forcing, we visualised the integrated annual biomass of the population for each forcing in the final year of simulations.

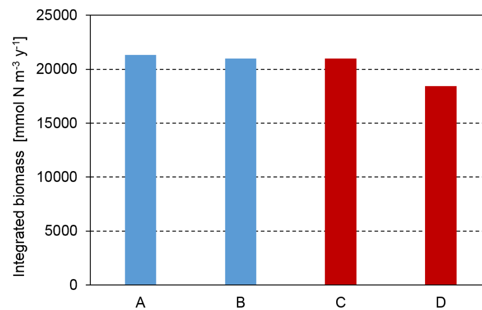


Figure 4.4.1: Integrated biomass in the last year of simulations.

The integrated annual biomass is slightly, but not drastically lower under fluctuating, as well as increasing temperature conditions (forcing B-D), compared to a cold steady temperature forcing (A).

S2: Spring bloom phenology

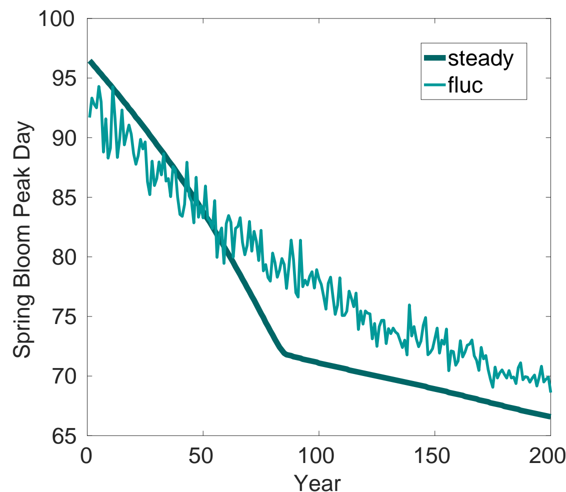


Figure 4.4.2: Day of the year on which the vegetative cell concentration of the whole population reaches its maximum over 200 years of global warming, excluding (steady, forcing C) and including (fluc, forcing D) short-term temperature fluctuations.

In response to the increase in temperature, both simulations excluding and including short-term temperature fluctuations (forcings C and D) show a phenological shift towards an earlier blooming over the time span of 200 years. In accordance to this phe-

nological shift, measurements for the Gulf of Finland show a shift towards a 10 day earlier blooming over the past 3 decades (Klais et al. 2013).

Although the long-term temperature increase shows a linear trend, the phenological shift for a steady increase in temperature (forcing C) does not follow in this linear manner, but shows an abrupt shift in the slope after 85 years of global warming. The reason for this change in slope lies in the time span between germination of resting stages and limitation of growth due to sea-ice presence. During the first 85 years of simulated global warming, the increase in temperature causes an earlier melting of sea-ice, which allows for a strong shift in phenology. After 85 years of global warming however, the melting of sea-ice starts so early in the year that it takes place within the germination time frame. Germination, which is limited in our model to days 44-60 of a year, becomes a factor, which slows down a further acceleration in phenology. Since temperature keeps increasing in the following years, the higher growth rate in response to higher temperatures still causes a slight enhancement of the spring bloom.

Under fluctuating temperature conditions, the acceleration of phenology is initially slightly weaker, and the shift in the rate of change occurs later, ca. after 150 years of global warming. Thus, fluctuating temperature conditions initially buffer against a strong change in phenology, but overall cause a similar acceleration of the spring bloom.

REFERENCES

Alpermann, T. J., U. Tillmann, B. Beszteri, A. D. Cembella, and U. John. 2010. „Phenotypic variation and genotypic diversity in a planktonic population of the toxigenic marine dinoflagellate *Alexandrium tamarense* (Dinophyceae)“. *J Phycol.* 46 (1): 18–32.

Anderson, D. M. 1998. „Physiology and bloom dynamics of toxic *Alexandrium* species, with emphasis on life cycle transitions“. *NATO ASI Series G Ecol. Sci.* 41:29–48.

Anglès, S., E. Garcés, A. Reñé, and N. Sampedro. 2012. „Life-cycle alternations in *Alexandrium minutum* natural populations from the NW Mediterranean Sea“. *Harmful Algae* 16:1–11.

Appleby, P. 2001. „Chronostratigraphic techniques in recent sediments“. *Tracking Environmental Change Using Lake Sediments* 1:171–203.

Atkinson, D., B. J. Ciotti, and D. J. Montagnes. 2003. „Protists decrease in size linearly with temperature: ca. 2.5% $°C^{-1}$“. *Proc. R. Soc. Lond. B Biol. Sci.* 270 (1533): 2605–2611.

Beckmann, A., C.-E. Schaum, and I. Hense. in rev. „Trait adaptation of phytoplankton in ecosystem models“. *Evol. Appl.*

Bengtsson, B. O. 2003. „Genetic variation in organisms with sexual and asexual reproduction“. *J Evol. Biol.* 16 (2): 189–199.

Beniston, M., D. B. Stephenson, O. B. Christensen, C. A. Ferro, C. Frei, S. Goyette, K. Halsnaes, T. Holt, K. Jylhä, B. Koffi, et al. 2007. „Future extreme events in European climate: an exploration of regional climate model projections“. *Clim. Change* 81 (1): 71–95.

Benitez-Nelson, C. R. 2000. „The biogeochemical cycling of phosphorus in marine systems“. *Earth Sci. Rev.* 51 (1-4): 109–135.

Bopp, L., L. Resplandy, J. C. Orr, S. C. Doney, J. P. Dunne, M. Gehlen, P. Halloran, C. Heinze, T. Ilyina, R. Seferian, et al. 2013. „Multiple stressors of ocean ecosystems in the 21st century: projections with CMIP5 models“. *Biogeosciences* 10:6225–6245.

Boyd, P. W., S. C. Doney, R. Strzepek, J. Dusenberry, K. Lindsay, and I. Fung. 2008. „Climate-mediated changes to mixed-layer properties in the Southern Ocean: assessing the phytoplankton response“. *Biogeosciences* 5 (3): 847–864.

Bravo, I., and D. M. Anderson. 1994. „The effects of temperature, growth medium and darkness on excystment and growth of the toxic dinoflagellate *Gymnodinium catenatum* from northwest Spain“. *J Plankton Res.* 16 (5): 513–525.

Brennan, G. L., N. Colegrave, and S. Collins. 2017. „Evolutionary consequences of multidriver environmental change in an aquatic primary producer". *Proc. Natl. Acad. Sci. U.S.A.* 114 (37): 9930–9935.

Bruggeman, J., and S. A. Kooijman. 2007. „A biodiversity-inspired approach to aquatic ecosystem modeling". *Limnol. Oceanogr.* 52 (4): 1533–1544.

Carstensen, J., J. H. Andersen, B. G. Gustafsson, and D. J. Conley. 2014. „Deoxygenation of the Baltic Sea during the last century". *Proc. Natl. Acad. Sci. U.S.A.* 111 (15): 5628–5633.

Chevin, L.-M., O. Cotto, and J. Ashander. 2017. „Stochastic Evolutionary Demography under a Fluctuating Optimum Phenotype". *Am. Nat.* 190 (6): 786–802.

Cohen, J., J. A. Screen, J. C. Furtado, M. Barlow, D. Whittleston, D. Coumou, J. Francis, K. Dethloff, D. Entekhabi, J. Overland, et al. 2014. „Recent Arctic amplification and extreme mid-latitude weather". *Nat. Geosci.* 7 (9): 627–637.

Collins, S. 2016. „Growth rate evolution in improved environments under Prodigal Son dynamics". *Evol. Appl.* 9 (9): 1179–1188.

Collins, S., and G. Bell. 2004. „Phenotypic consequences of 1,000 generations of selection at elevated CO_2 in a green alga". *Nature* 431 (7008): 566–569.

Collins, S., B. Rost, and T. A. Rynearson. 2014. „Evolutionary potential of marine phytoplankton under ocean acidification". *Evol. Appl.* 7 (1): 140–155.

Conley, D. J., C. Humborg, L. Rahm, O. P. Savchuk, and F. Wulff. 2002. „Hypoxia in the Baltic Sea and basin-scale changes in phosphorus biogeochemistry". *Environ. Sci. Technol.* 36 (24): 5315–5320.

Cox, A., D. Shull, and R. Horner. 2008. „Profiles of *Alexandrium catenella* cysts in Puget Sound sediments and the relationship to paralytic shellfish poisoning events". *Harmful Algae* 7 (4): 379–388.

Craveiro, S. C., N. Daugbjerg, Ø. Moestrup, and A. J. Calado. 2016. „Studies on *Peridinium aciculiferum* and *Peridinium malmogiense* (= *Scrippsiella hangoei*): comparison with *Chimonodinium lomnickii* and description of *Apocalathium* gen. nov.(Dinophyceae)". *Phycologia* 56 (1): 21–35.

Cruz, J. A., L. J. Savage, R. Zegarac, C. C. Hall, M. Satoh-Cruz, G. A. Davis, W. K. Kovac, J. Chen, and D. M. Kramer. 2016. „Dynamic environmental photosynthetic imaging reveals emergent phenotypes". *Cell Systems* 2 (6): 365–377.

Dale, B. 1983. „Dinoflagellate resting cysts:"benthic plankton"". *Survival strategies of the Algae:* 69–136.

Denman, K. L. 2017. „A model simulation of the adaptive evolution through mutation of the coccolithophore *Emiliania huxleyi* based on a published laboratory study". *Front. Mar. Sci.* 3:286.

Dutkiewicz, S., J. J. Morris, M. J. Follows, J. Scott, O. Levitan, S. T. Dyhrman, and I. Berman-Frank. 2015. „Impact of ocean acidification on the structure of future phytoplankton communities“. *Nat. Clim. Chang.* 5 (11): 1002–1006.

Easterling, D. R., G. A. Meehl, C. Parmesan, S. A. Changnon, T. R. Karl, and L. O. Mearns. 2000. „Climate extremes: observations, modeling, and impacts“. *Science* 289 (5487): 2068–2074.

Edwards, M., and A. J. Richardson. 2004. „Impact of climate change on marine pelagic phenology and trophic mismatch“. *Nature* 430 (7002): 881–884.

Ellegaard, M., A. Godhe, and S. Ribeiro. 2018. „Time capsules in natural sediment archives — tracking phytoplankton population genetic diversity and adaptation over multi-decadal timescales in the face of environmental change“. *Evol. Appl.* 11 (1): 11–16.

Ellegaard, M., D. M. Kulis, and D. M. Anderson. 1998. „Cysts of Danish *Gymnodinium nolleri* Ellegaard et Moestrup sp. ined.(Dinophyceae): studies on encystment, excystment and toxicity“. *J Plankton Res.* 20 (9): 1743–1755.

Ellegaard, M., and S. Ribeiro. 2018. „The long-term persistence of phytoplankton resting stages in aquatic 'seed banks'“. *Biol. Rev.* 93 (1): 166–183.

Enríquez, S., C. M. Duarte, and K. Sand-Jensen. 1993. „Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C: N: P content“. *Oecologia* 94 (4): 457–471.

Eppley, R. W. 1972. „Temperature and phytoplankton growth in the sea“. *Fish. bull.* 70 (4): 1063–1085.

Falkowski, P. G., R. T. Barber, and V. Smetacek. 1998. „Biogeochemical controls and feedbacks on ocean primary production“. *Science* 281 (5374): 200–206.

Falkowski, P. G., T. Fenchel, and E. F. Delong. 2008. „The microbial engines that drive Earth's biogeochemical cycles“. *Science* 320 (5879): 1034–1039.

Feifel, K. M., S. Fletcher, L. Watson, S. Moore, and E. Lessard. 2015. „*Alexandrium* and *Scrippsiella* cyst viability and cytoplasmic fullness in a 60-cm sediment core from Sequim Bay, WA“. *Harmful Algae* 47:56–65.

Feifel, K. M., S. K. Moore, and R. A. Horner. 2012. „An *Alexandrium* spp. cyst record from Sequim Bay, Washington State, USA, and its relation to past climate variability“. *J Phycol.* 48 (3): 550–558.

Feistel, R., G. Nausch, and N. Wasmund. 2008. *State and evolution of the Baltic Sea, 1952-2005: a detailed 50-year survey of meteorology and climate, physics, chemistry, biology, and marine environment.* John Wiley & Sons.

Field, C. B. 2012. *Managing the risks of extreme events and disasters to advance climate change adaptation: special report of the Intergovernmental Panel on Climate Change.* Cambridge University Press.

Field, C. B., M. J. Behrenfeld, J. T. Randerson, and P. Falkowski. 1998. „Primary production of the biosphere: integrating terrestrial and oceanic components“. *Science* 281 (5374): 237–240.

Figueroa, R. I., and I. Bravo. 2005. „Sexual reproduction and two different encystment strategies of *Lingulodinium polyedrum*(Dinophycae) in culture“. *J Phycol.* 41 (2): 370–379.

Figueroa, R. I., J. A. Vazquez, A. Massanet, M. A. Murado, and I. Bravo. 2011. „Interactive effects of salinity and temperature on planozygote and cyst formation of *Alexandrium minutum* (Dinophyceae) in culture“. *J Phycol.* 47 (1): 13–24.

Finkel, Z. V., J. Beardall, K. J. Flynn, A. Quigg, T. A. V. Rees, and J. A. Raven. 2009. „Phytoplankton in a changing world: cell size and elemental stoichiometry“. *J Plankton Res.* 32 (1): 119–137.

Finni, T., K. Kononen, R. Olsonen, and K. Wallström. 2001. „The history of cyanobacterial blooms in the Baltic Sea“. *Ambio* 30 (4): 172–178.

Fisher, R. A. 1930. *The genetical theory of natural selection.*

Fleming, V., and S. Kaitala. 2006. „Phytoplankton spring bloom intensity index for the Baltic Sea estimated for the years 1992 to 2004“. *Hydrobiologia* 554 (1): 57–65.

Follows, M. J., S. Dutkiewicz, S. Grant, and S. W. Chisholm. 2007. „Emergent biogeography of microbial communities in a model ocean“. *Science* 315 (5820): 1843–1846.

Fonselius, S., and J. Valderrama. 2003. „One hundred years of hydrographic measurements in the Baltic Sea“. *J Sea Res.* 49 (4): 229–241.

Garcés, E., A. Zingone, M. Montresor, B. Reguera, and B. Dale. 2002. „LIFEHAB: Life histories of microalgal species causing harmful blooms“. *Office for the Official Publications of the European Communities, Luxembourg:* 1–189.

Geider, R. J. 1987. „Light and temperature dependence of the carbon to chlorophyll *a* ratio in microalgae and cyanobacteria: implications for physiology and growth of phytoplankton“. *New Phytol.* 106 (1): 1–34.

Grimaud, G. M., V. Le Guennec, S.-D. Ayata, F. Mairet, A. Sciandra, and O. Bernard. 2015. „Modelling the effect of temperature on phytoplankton growth across the global ocean“. *IFAC-PapersOnLine* 48 (1): 228–233.

Gustafsson, B. G., F. Schenk, T. Blenckner, K. Eilola, H. M. Meier, B. Müller-Karulis, T. Neumann, T. Ruoho-Airola, O. P. Savchuk, and E. Zorita. 2012. „Reconstructing the development of Baltic Sea eutrophication 1850–2006“. *Ambio* 41 (6): 534–548.

Hallegraeff, G. M. 2010. „Ocean climate change, phytoplankton community responses, and harmful algal blooms: a formidable predictive challenge". *J Phycol.* 46 (2): 220–235.

Hällfors, H., H. Backer, J.-M. Leppänen, S. Hällfors, G. Hällfors, and H. Kuosa. 2013. „The northern Baltic Sea phytoplankton communities in 1903–1911 and 1993–2005: a comparison of historical and modern species data". *Hydrobiologia* 707 (1): 109–133.

Hao, Y.-Q., M. A. Brockhurst, O. L. Petchey, and Q.-G. Zhang. 2015. „Evolutionary rescue can be impeded by temporary environmental amelioration". *Ecol. Lett.* 18 (9): 892–898.

Harland, R., K. Nordberg, and H. L. Filipsson. 2006. „Dinoflagellate cysts and hydrographical change in Gullmar Fjord, west coast of Sweden". *Sci. Total Environ.* 355 (1-3): 204–231.

Härnström, K., M. Ellegaard, T. J. Andersen, and A. Godhe. 2011. „Hundred years of genetic structure in a sediment revived diatom population". *Proc. Natl. Acad. Sci. U.S.A.* 108 (10): 4252–4257.

Heikkilä, M., V. Pospelova, A. Forest, G. A. Stern, L. Fortier, and R. W. Macdonald. 2016. „Dinoflagellate cyst production over an annual cycle in seasonally ice-covered Hudson Bay". *Mar. Micropaleontol.* 125:1–24.

Heiskanen, A.-S. 1993. „Mass encystment and sinking of dinoflagellates during a spring bloom". *Mar. Biol.* 116 (1): 161–167.

Hense, I., I. Stemmler, and S. Sonntag. 2017. „Ideas and perspectives: climate-relevant marine biologically driven mechanisms in Earth system models". *Biogeosciences* 14 (2): 403–413.

Hinder, S. L., G. C. Hays, M. Edwards, E. C. Roberts, A. W. Walne, and M. B. Gravenor. 2012. „Changes in marine dinoflagellate and diatom abundance under climate change". *Nat. Clim. Chang.* 2 (4): 271–275.

Hinners, J., A. Kremp, and I. Hense. 2017. „Evolution in temperature-dependent phytoplankton traits revealed from a sediment archive: do reaction norms tell the whole story?" *Proc. R. Soc. Lond. B Biol. Sci.* 284 (1864): 20171888.

Houle, D., D. R. Govindaraju, and S. Omholt. 2010. „Phenomics: the next challenge". *Nat. Rev. Genet.* 11 (12): 855–866.

Hutchins, D. A., and F. Fu. 2017. „Microorganisms and ocean global change." *Nat. Microbiol.* 2:17058.

Irwin, A. J., Z. V. Finkel, F. E. Müller-Karger, and L. T. Ghinaglia. 2015. „Phytoplankton adapt to changing ocean environments". *Proc. Natl. Acad. Sci. U.S.A.* 112 (18): 5762–5766.

Jevrejeva, S. 2000. „Long-term variability of sea ice and air temperature conditions along the Estonian coast“. *Geophysica* 36 (1-2): 17–30.

Joehnk, K. D., J. Huisman, J. Sharples, B. Sommeijer, P. M. Visser, and J. M. Stroom. 2008. „Summer heatwaves promote blooms of harmful cyanobacteria“. *Glob. Change Biol.* 14 (3): 495–512.

Johnson, P. W., and J. M. Sieburth. 1982. „In-situ morphology and occurrence of eucaryotic phototrophs of bacterial size in the picoplankton of estuarine and oceanic waters“. *J Phycol.* 18 (3): 318–327.

Kahru, M., J.-M. Leppanen, and O. Rud. 1993. „Cyanobacterial blooms cause heating of the sea surface“. *Mar. Ecol. Prog. Ser.* 101:1–7.

Kahru, M., O. Savchuk, and R. Elmgren. 2007. „Satellite measurements of cyanobacterial bloom frequency in the Baltic Sea: interannual and spatial variability“. *Mar. Ecol. Prog. Ser.* 343:15–23.

Katajisto, T. 1996. „Copepod eggs survive a decade in the sediments of the Baltic Sea“. *Hydrobiologia* 320 (1-3): 153–159.

Khatiwala, S., T. Tanhua, S. Mikaloff Fletcher, M. Gerber, S. Doney, H. Graven, N. Gruber, G. McKinley, A. Murata, A. Rıos, et al. 2013. „Global ocean storage of anthropogenic carbon“. *Biogeosciences* 10 (4): 2169–2191.

Kingsolver, J. G. 2009. „The Well-Temperatured Biologist“. *Am. Nat.* 174 (6): 755–768.

Klais, R., T. Tamminen, A. Kremp, K. Spilling, B. W. An, S. Hajdu, and K. Olli. 2013. „Spring phytoplankton communities shaped by interannual weather variability and dispersal limitation: Mechanisms of climate change effects on key coastal primary producers“. *Limnol. Oceanogr.* 58 (2): 753–762.

Klais, R., T. Tamminen, A. Kremp, K. Spilling, and K. Olli. 2011. „Decadal-scale changes of dinoflagellates and diatoms in the anomalous Baltic Sea spring bloom“. *PloS One* 6 (6): e21567.

Klouch, K. Z., S. Schmidt, F. Andrieux-Loyer, M. Le Gac, D. Hervio-Heath, Z. N. Qui-Minet, J. Quéré, E. Bigeard, L. Guillou, and R. Siano. 2016. „Historical records from dated sediment cores reveal the multidecadal dynamic of the toxic dinoflagellate *Alexandrium minutum* in the Bay of Brest (France)“. *FEMS Microbiol. Ecol.* 92 (7): fiw101.

Kremer, C. T., and C. A. Klausmeier. 2017. „Species packing in eco-evolutionary models of seasonally fluctuating environments“. *Ecol. Lett.* 20 (9): 1158–1168.

Kremp, A. 2000. „Morphology and germination pattern of the resting cyst of *Peridiniella catenata* (Dinophyceae) from the Baltic Sea“. *Phycologia* 39 (3): 183–186.

Kremp, A. 2001. „Effects of cyst resuspension on germination and seeding of two bloom-forming dinoflagellates in the Baltic Sea“. *Mar. Ecol. Prog. Ser.* 216:57–66.

Kremp, A., and D. M. Anderson. 2000. „Factors regulating germination of resting cysts of the spring bloom dinoflagellate *Scrippsiella hangoei* from the northern Baltic Sea". *J Plankton Res.* 22 (7): 1311–1327.

Kremp, A., M. Elbrächter, M. Schweikert, J. L. Wolny, and M. Gottschling. 2005. „*Woloszynskia halophila* (Biecheler) comb. nov.: A bloom-forming cold-water dinoflagellate co-occuring with *Scrippsiella hangoei* (Dinophyceae) in the Baltic Sea". *J Phycol.* 41 (3): 629–642.

Kremp, A., and A.-S. Heiskanen. 1999. „Sexuality and cyst formation of the spring-bloom dinoflagellate *Scrippsiella hangoei* in the coastal northern Baltic Sea". *Mar. Biol.* 134 (4): 771–777.

Kremp, A., J. Hinners, R. Klais, A.-P. Leppänen, and A. Kallio. 2018. „Patterns of vertical cyst distribution and survival in 100-year-old sediment archives of three spring dinoflagellate species from the Northern Baltic Sea". *Eur. J Phycol.* 53 (2): 135–145.

Kremp, A., and M. W. Parrow. 2006. „Evidence for asexual resting cysts in the life cycle of the marine peridinoid dinoflagellate, *Scrippsiella hangoei*". *J Phycol.* 42 (2): 400–409.

Kremp, A., K. Rengefors, and M. Montresor. 2009. „Species-specific encystment patterns in three Baltic cold-water dinoflagellates: The role of multiple cues in resting cyst formation". *Limnol. Oceanogr.* 54 (4): 1125–1138.

Kremp, A., T. Tamminen, and K. Spilling. 2008. „Dinoflagellate bloom formation in natural assemblages with diatoms: nutrient competition and growth strategies in Baltic spring phytoplankton". *Aquat. Microb. Ecol.* 50 (2): 181–196.

Laakso, L., S. Mikkonen, A. Drebs, A. Karjalainen, P. Pirinen, and P. Alenius. 2018. „One hundred years of atmospheric and marine observations at Utö Island in the Baltic Sea". *Ocean Sci.* 14:617–632.

Lande, R., S. Engen, and B.-E. Sæther. 2009. „An evolutionary maximum principle for density-dependent population dynamics in a fluctuating environment". *Philos. Trans. Royal Soc. B* 364 (1523): 1511–1518.

Larsen, J., H. Kuosa, J. Ikävalko, K. Kivi, and S. Hällfors. 1995. „A redescription of *Scrippsiella hangoei* (Schiller) comb. nov. – a 'red tide' dinoflagellate from the northern Baltic". *Phycologia* 34 (2): 135–144.

Laufkötter, C., M. Vogt, N. Gruber, M. Aita-Noguchi, O. Aumont, L. Bopp, E. Buitenhuis, S. Doney, J. Dunne, T. Hashioka, et al. 2015. „Drivers and uncertainties of future global marine primary production in marine ecosystem models". *Biogeosciences* 12:6955–6984.

Lee, S., R. Hofmeister, and I. Hense. 2018. „The role of life cycle processes on phytoplankton spring bloom composition: a modelling study applied to the Gulf of Finland". *J Marine Syst.* 178:75–85.

Levander, K. M. 1894. „*Peridinium catenatum* n. sp. eine kettenbildende Peridinee im finnischen Meerbusen“. *Acta Societatis pro Fauna et Flora Fennica* 9:1–18.

Levander, K. M. 1901. „Zur Kenntnis des Planktons und der Bodenfauna einiger seichten Brackwasserbuchten“. *Acta Societatis pro Fauna et Flora Fennica* 20:1–34.

Lewis, J., A. Harris, K. Jones, and R. Edmonds. 1999. „Long-term survival of marine planktonic diatoms and dinoflagellates in stored sediment samples“. *J Plankton Res.* 21 (2): 343–354.

Lignell, R., A. Heiskanen, H. Kuosa, K. Gundersen, P. Kuuppo-Leinikki, R. Pajuniemi, and A. Uitto. 1993. „Fate of a phytoplankton spring bloom: sedimentation and carbon flow in the planktonic food web in the northern Baltic“. *Mar. Ecol. Prog. Ser.* 94:239–239.

Lima, A. L., J. B. Hubeny, C. M. Reddy, J. W. King, K. A. Hughen, and T. I. Eglinton. 2005. „High-resolution historical records from Pettaquamscutt River basin sediments: 1. 210Pb and varve chronologies validate record of 137Cs released by the Chernobyl accident“. *Geochim. Cosmochim. Acta* 69 (7): 1803–1812.

Lindemann, E. 1924. „Ueber finnische Peridineen“. *Archiv fuer Hydrobiologie* 15:1–4.

Listmann, L., M. LeRoch, L. Schlüter, M. K. Thomas, and T. B. Reusch. 2016. „Swift thermal reaction norm evolution in a key marine phytoplankton species“. *Evol. Appl.* 9 (9): 1156–1164.

Litchman, E., C. Klausmeier, and K. Yoshiyama. 2009. „Contrasting size evolution in marine and freshwater diatoms“. *Proc. Natl. Acad. Sci. U.S.A.* 106 (8): 2665–2670.

Lohbeck, K. T., U. Riebesell, and T. B. H. Reusch. 2012. „Adaptive evolution of a key phytoplankton species to ocean acidification“. *Nat. Geosci.* 5 (5): 346–351.

Lundholm, N., S. Ribeiro, T. J. Andersen, T. Koch, A. Godhe, F. Ekelund, and M. Ellegaard. 2011. „Buried alive-germination of up to a century-old marine protist resting stages“. *Phycologia* 50 (6): 629–640.

Lundholm, N., S. Ribeiro, A. Godhe, L. Rostgaard Nielsen, and M. Ellegaard. 2017. „Exploring the impact of multidecadal environmental changes on the population genetic structure of a marine primary producer“. *Ecol. Evol.* 7 (9): 3132–3142.

Matuszewski, S., J. Hermisson, and M. Kopp. 2014. „Fisher's geometric model with a moving optimum“. *Evolution* 68 (9): 2571–2588.

McCarthy, F. M., K. N. Mertens, M. Ellegaard, K. Sherman, V. Pospelova, S. Ribeiro, S. Blasco, and D. Vercauteren. 2011. „Resting cysts of freshwater dinoflagellates in southeastern Georgian Bay (Lake Huron) as proxies of cultural eutrophication“. *Rev. Palaeobot. Palynol.* 166 (1-2): 46–62.

McQuoid, M. R., A. Godhe, and K. Nordberg. 2002. „Viability of phytoplankton resting stages in the sediments of a coastal Swedish fjord“. *Eur. J Phycol.* 37 (2): 191–201.

Miyazono, A., S. Nagai, I. Kudo, and K. Tanizawa. 2012. „Viability of *Alexandrium tamarense* cysts in the sediment of Funka Bay, Hokkaido, Japan: Over a hundred year survival times for cysts“. *Harmful Algae* 16:81–88.

Möllmann, C., R. Diekmann, B. Müller-Karulis, G. Kornilovs, M. Plikshs, and P. Axe. 2009. „Reorganization of a large marine ecosystem due to atmospheric and anthropogenic pressure: a discontinuous regime shift in the Central Baltic Sea“. *Glob. Change Biol.* 15 (6): 1377–1393.

Monod, J. 1949. „The growth of bacterial cultures“. *Annu. Rev. Microbiol* 3 (1): 371–394.

Nakov, T., E. C. Theriot, and A. J. Alverson. 2014. „Using phylogeny to model cell size evolution in marine and freshwater diatoms“. *Limnol. Oceanogr.* 59 (1): 79–86.

Niemi, Å. 1975. „Ecology of phytoplankton in the Tvärminne area, SW coast of Finland II. Primary production and environmental conditions in the archipelago and the sea zone“. *Acta Botanica Fennica* 105:1–73.

Olli, K., O. Trikk, R. Klais, R. Ptacnik, T. Andersen, S. Lehtinen, and T. Tamminen. 2013. „Harmonizing large data sets reveals novel patterns in the Baltic Sea phytoplankton community structure“. *Mar. Ecol. Prog. Ser.* 53–66.

Olli, K., and K. Trunov. 2010. „Abundance and distribution of vernal bloom dinoflagellate cysts in the Gulf of Finland and Gulf of Riga (the Baltic Sea)“. *Deep-Sea Res. Pt II* 57 (3): 235–242.

Orr, H. A. 2005. „The genetic theory of adaptation: a brief history“. *Nat. Rev. Genet.* 6 (2): 119–127.

Pachauri, R. K., L. Meyer, G.-K. Plattner, T. Stocker, et al. 2015. *IPCC, 2014: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* IPCC.

Padfield, D., G. Yvon-Durocher, A. Buckling, S. Jennings, and G. Yvon-Durocher. 2016. „Rapid evolution of metabolic traits explains thermal adaptation in phytoplankton“. *Ecol. Lett.* 19 (2): 133–142.

Pomeroy, L. R. 1974. „The ocean's food web, a changing paradigm“. *Bioscience* 24 (9): 499–504.

Raven, J., and P. Falkowski. 1999. „Oceanic sinks for atmospheric CO_2“. *Plant Cell Environ.* 22 (6): 741–755.

Rengefors, K. 1998. „Seasonal succession of dinoflagellates coupled to the benthic cyst dynamics in Lake Erken“. *Arch. Hydrobiol. Spec. Issues Advanc. Limnol.* 51:123–141.

Reusch, T. B., and P. W. Boyd. 2013. „Experimental evolution meets marine phytoplankton“. *Evolution* 67 (7): 1849–1859.

Reusch, T. B., J. Dierking, H. C. Andersson, E. Bonsdorff, J. Carstensen, M. Casini, M. Czajkowski, B. Hasler, K. Hinsby, K. Hyytiäinen, et al. 2018. „The Baltic Sea as a time machine for the future coastal ocean“. *Sci. Adv.* 4 (5): eaar8195.

Ribeiro, S., A. Amorim, T. J. Andersen, F. Abrantes, and M. Ellegaard. 2012. „Reconstructing the history of an invasion: the toxic phytoplankton species *Gymnodinium catenatum* in the Northeast Atlantic“. *Biol. Invasions* 14 (5): 969–985.

Ribeiro, S., T. Berge, N. Lundholm, T. J. Andersen, F. Abrantes, and M. Ellegaard. 2011. „Phytoplankton growth after a century of dormancy illuminates past resilience to catastrophic darkness“. *Nat. Commun.* 2:311.

Ribeiro, S., T. Berge, N. Lundholm, and M. Ellegaard. 2013. „Hundred years of environmental change and phytoplankton ecophysiological variability archived in coastal sediments“. *PloS One* 8 (4): e61184.

Rönnberg, C., and E. Bonsdorff. 2004. „Baltic Sea eutrophication: area-specific ecological consequences“. *Hydrobiologia* 514 (1-3): 227–241.

Sackett, O., K. Petrou, B. Reedy, R. Hill, M. Doblin, J. Beardall, P. Ralph, and P. Heraud. 2016. „Snapshot prediction of carbon productivity, carbon and protein content in a Southern Ocean diatom using FTIR spectroscopy“. *ISME J* 10 (2): 416–426.

Sathyendranath, S., A. D. Gouveia, S. R. Shetye, P. Ravindran, and T. Platt. 1991. „Biological control of surface temperature in the Arabian Sea“. *Nature* 349 (6304): 54–56.

Schaum, C.-E., S. Barton, E. Bestion, A. Buckling, B. Garcia-Carreras, P. Lopez, C. Lowe, S. Pawar, N. Smirnoff, M. Trimmer, et al. 2017. „Adaptation of phytoplankton to a decade of experimental warming linked to increased photosynthesis“. *Nat. Ecol. Evol.* 1 (4): 0094.

Schaum, C.-E., A. Buckling, N. Smirnoff, D. Studholme, and G. Yvon-Durocher. 2018. „Environmental fluctuations accelerate molecular evolution of thermal tolerance in a marine diatom“. *Nat. Commun.* 9:2813.

Schaum, C.-E., B. Rost, and S. Collins. 2015. „Environmental stability affects phenotypic evolution in a globally distributed marine picoplankton“. *ISME J* 10 (1): 75–84.

Schaum, C.-E., B. Rost, A. J. Millar, and S. Collins. 2013. „Variation in plastic responses of a globally distributed picoplankton species to ocean acidification“. *Nat. Clim. Chang.* 3 (3): 298–302.

Schenk, F., and E. Zorita. 2012. „Reconstruction of high resolution atmospheric fields for Northern Europe using analog-upscaling“. *Clim. Past* 8 (5): 1681.

Schlüter, L., K. T. Lohbeck, M. A. Gutowska, J. P. Gröger, U. Riebesell, and T. B. Reusch. 2014. „Adaptation of a globally important coccolithophore to ocean warming and acidification“. *Nat. Clim. Chang.* 4 (11): 1024–1030.

Steinacher, M., F. Joos, T. Frölicher, L. Bopp, P. Cadule, V. Cocco, S. C. Doney, M. Gehlen, K. Lindsay, J. K. Moore, et al. 2010. „Projected 21st century decrease in marine productivity: a multi-model analysis“. *Biogeosciences* 7 (3): 979–1005.

Stomp, M., J. Huisman, F. De Jongh, A. J. Veraart, D. Gerla, M. Rijkeboer, B. W. Ibelings, U. I. Wollenzien, and L. J. Stal. 2004. „Adaptive divergence in pigment composition promotes phytoplankton biodiversity“. *Nature* 432 (7013): 104–107.

Stosch, H. v. von. 1973. „Observations on vegetative reproduction and sexual life cycles of two freshwater dinoflagellates, *Gymnodinium pseudopalustre* Schiller and *Woloszynskia apiculata* sp. nov.“ *Brit. Phycol. J* 8 (2): 105–134.

Stramska, M., and A. Zuzewicz. 2013. „Influence of the parametrization of water optical properties on the modelled sea surface temperature in the Baltic Sea“. *Oceanologia* 55 (1): 53–76.

Struck, U., K.-C. Emeis, M. Voss, C. Christiansen, and H. Kunzendorf. 2000. „Records of southern and central Baltic Sea eutrophication in δ13C and δ15N of sedimentary organic matter“. *Mar. Geol.* 164 (3-4): 157–171.

Suikkanen, S., M. Laamanen, and M. Huttunen. 2007. „Long-term changes in summer phytoplankton communities of the open northern Baltic Sea“. *Estuar. Coast. Shelf Sci.* 71 (3-4): 580–592.

Suikkanen, S., S. Pulina, J. Engström-Öst, M. Lehtiniemi, S. Lehtinen, and A. Brutemark. 2013. „Climate change and eutrophication induced shifts in northern summer plankton communities“. *PLoS One* 8 (6): e66475.

Sundström, A. M., A. Kremp, N. Daugbjerg, Ø. Moestrup, M. Ellegaard, R. Hansen, and S. Hajdu. 2009. „*Gymnodinium corollarium* sp. nov. (Dinophyceae) - a new cold-water dinoflagellate responsible for cyst sedimentation events in the Baltic Sea“. *J Phycol.* 45 (4): 938–952.

Tahvanainen, P., T. J. Alpermann, R. I. Figueroa, U. John, P. Hakanen, S. Nagai, J. Blomster, and A. Kremp. 2012. „Patterns of post-glacial genetic differentiation in marginal populations of a marine microalga“. *PloS One* 7 (12): e53602.

Thomas, M. K., C. T. Kremer, C. A. Klausmeier, and E. Litchman. 2012. „A global pattern of thermal adaptation in marine phytoplankton“. *Science* 338 (6110): 1085–1088.

Thomas, W. H., H. L. Scotten, and J. S. Bradshaw. 1963. „Thermal gradient incubators for small aquatic organisms“. *Limnol. Oceanogr.* 8 (3): 357–360.

Tréguer, P. J., and C. L. De La Rocha. 2013. „The world ocean silica cycle“. *Annu. Rev. Mar. Sci.* 5:477–501.

Vallius, H. 2006. „Permanent seafloor anoxia in coastal basins of the northwestern Gulf of Finland, Baltic Sea“. *Ambio* 35 (3): 105–108.

Vernal, A. de, A. Rochon, B. Fréchette, M. Henry, T. Radi, and S. Solignac. 2013. „Reconstructing past sea ice cover of the Northern Hemisphere from dinocyst assemblages: status of the approach“. *Quat. Sci. Rev.* 79:122–134.

Walter, B., J. Peters, J. van Beusekom, and M. S. John. 2015. „Interactive effects of temperature and light during deep convection: a case study on growth and condition of the diatom *Thalassiosira weissflogii*“. *ICES J Mar. Sci.* 72 (6): 2061–2071.

Warns, A., I. Hense, and A. Kremp. 2012. „Modelling the life cycle of dinoflagellates: a case study with *Biecheleria baltica*“. *J Plankton Res.* 35 (2): 379–392.

Watts, P. C., N. Lundholm, S. Ribeiro, and M. Ellegaard. 2013. „A century-long genetic record reveals that protist effective population sizes are comparable to those of macroscopic species“. *Biol. Lett.* 9 (6): 20130849.

Webb, W. L., M. Newton, and D. Starr. 1974. „Carbon dioxide exchange of *Alnus rubra*“. *Oecologia* 17 (4): 281–291.

Worden, A. Z., M. J. Follows, S. J. Giovannoni, S. Wilken, A. E. Zimmerman, and P. J. Keeling. 2015. „Rethinking the marine carbon cycle: factoring in the multifarious lifestyles of microbes“. *Science* 347 (6223): 1257594.

Xu, L., J. A. Cruz, L. J. Savage, D. M. Kramer, and J. Chen. 2015. „Plant photosynthesis phenomics data quality control“. *Bioinformatics* 31 (11): 1796–1804.

Zehr, J. P., and R. M. Kudela. 2011. „Nitrogen cycle of the open ocean: from genes to ecosystems“. *Annu. Rev. Mar. Sci.* 3:197–225.

Zhang, Y., R. Klapper, K. T. Lohbeck, L. T. Bach, K. G. Schulz, T. B. Reusch, and U. Riebesell. 2014. „Between- and within-population variations in thermal reaction norms of the coccolithophore *Emiliania huxleyi*“. *Limnol. Oceanogr.* 59 (5): 1570–1580.

Zonneveld, K. A., G. J. Versteegh, and G. J. de Lange. 1997. „Preservation of organic-walled dinoflagellate cysts in different oxygen regimes: a 10,000 year natural experiment“. *Mar. Micropaleontol.* 29 (3-4): 393–405.

Eidesstattliche Versicherung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

Hamburg,