# Adaptive potential of the Arctic diatom *Thalassiosira hyalina* to climate change: intraspecific diversity, plasticity and population dynamics

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*„Wahrlich, es würde euch bange werden, wenn die ganze Welt, wie ihr es fordert, einmal im Ernst durchaus verständlich würde.“*

*Indeed, you would be frightened if the whole world was once, as you demand, truly and thoroughly understandable.*

*Friederich Schlegel*

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# Contents

# Abbreviations

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| | |
|---|---|
| AIC | Akaike information criterion |
| ANOVA | Analysis of variance |
| asqPCR | allele specific quantitative polymerase chain reaction |
| bp | base pair |
| BSi | Biogenic silica |
| CCM | Carbon concentrating mechanism |
| Chl *a* | Chlorophyll *a* |
| $CO_2$ | Carbon dioxide |
| $CO_3^{2-}$ | Carbonate ion |
| DIC | Dissolved inorganic carbon |
| ESC | East Spitsbergen current |
| ETR | Electron transfer rates through PSII |
| FRRF | Fast repetition rate fluorometry |
| $HCO_3^-$ | Bicarbonate ion |
| MPB | Microsatellite poolSeq barcoding |
| $NO_3^-$ | Nitrate |
| $NH_4^+$ | Ammonium |
| NPP | Net primary production |
| OA | Ocean acidification |
| $pCO_2$ | Carbon dioxide partial pressure |
| PCR | Polymerase chain reaction |
| pH | Negative logarithm of the hydronium ion activity of a solution |
| POC | Particulate organic carbon |
| PON | Particulate organic nitrogen |
| PS | Photosystem |
| RubisCO | Ribulose-1,5-bisphosphate carboxylase/oxygenase |
| TA | Total alkalinity |
| WSC | West Spitsbergen current |

# Summary

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Climate change is and will continue to be affecting marine ecosystems everywhere on the globe. In the Arctic, these changes are progressing faster than in any other region, which makes it an exemplary study area for ecosystem responses to fast environmental alterations. Of paramount interest for ecosystem functioning and biogeochemical cycling is primary productivity, which sustains all higher trophic levels and is in the oceans mainly performed by unicellular phytoplankton. While the projections on physico-chemical drivers like temperature and $pCO_2$ for the next century are widely agreed upon, their effects on phytoplankton are highly uncertain. Seemingly minor alterations of species' characteristics and abundance shifts between them can have major consequences for the ecosystem.

In the context of global predictions, phytoplankton responses are typically scaled up from reaction norms of single strain experiments in the laboratory. While the retrieved response curves depict an individual's physiological plasticity across a treatment range, they do not include the trait diversity within a species, which can substantially broaden its optimum range. They also do not take interactions with a complex biological environment into account, nor the potential to adapt to future conditions. Although interactions and shifts are more visible between species, many adjustments to environmental conditions take place within or among individual cells of the same taxon. Processes on this intraspecific level are methodologically difficult to detect in the laboratory and even more so in natural contexts, especially in unicellular, planktonic organisms. Therefore, our knowledge on intraspecific phytoplankton diversity and selection is very limited.

The aim of this thesis was to investigate the responses of Arctic phytoplankton communities to climate change scenarios and to improve the understanding of underlying intraspecific mechanisms. I focused on populations of the frequently dominant diatom *Thalassiosira hyalina* as a model system in order to elucidate which processes impact their resilience or responsiveness to environmental drivers. A step-wise experimental approach of incubation experiments with natural communities, monocultures and artificial populations combined with observations in the field allowed me to gain insights into otherwise cryptic mechanisms and to investigate whether they apply in contexts of different complexity.

*Publication I* comprises the results of ten phytoplankton community experiments from different Arctic and Subarctic regions. The natural communities were incubated under different $pCO_2$ scenarios in setups with a range of different temperature and light regimes. Contrary to expectations from other regions, effects of elevated $pCO_2$ were largely compensated and primary productivity as well as species composition remained stable in most cases. Only in the experiment conducted at the lowest temperature did high $pCO_2$ induce a substantial decrease in productivity along with a pronounced species shift. I suspected the observed resilience to be caused on the intraspecific level and thus by physiological acclimation or selective sorting among diverse lineages.

Each phytoplankton cell can acclimate its physiology to various conditions within its plastic response range. In *Publication II,* I investigated individuals (i.e. strains) of *T. hyalina* in monoculture, which had been isolated from the former community incubations. None of the

applied conditions of elevated temperature and $pCO_2$ had fundamentally detrimental effects on them. Although originating from the same population, however, the six strains differed strongly not only in a variety of cellular traits but also in their growth rates and response directions under the applied future scenarios. In line with a former study (Wolf et al., 2018), this indicates a high potential for both, the ability for physiological adjustment as well as for selection among diverse phenotypes.

It is commonly assumed that having such different optima, individuals of a population should be differently competitive according to their response range and should be favored by natural selection under certain conditions. This assumption was also tested in *Publication II* by incubating the same six strains together as an artificial population under different temperature and $pCO_2$ scenarios. Applying an extended microsatellite-based method (asqPCR), I was able to resolve the genotypic composition over time within this multi-strain culture, making selective processes among genotypes visible. Opposed to my expectations, the traits of strains measured in monoculture did neither comprehensively correspond to their response in the presence of others, nor did they appear to determine their competitive success in all cases. Furthermore, the selection dynamics differed reproducibly in the two applied treatments while productivity remained very stable. Although these results confirm that even within a simplified setup strain sorting is a realistic mechanism that can buffer a response in productivity, they also reveal that selection may be influenced by intraspecific interactions that we hardly take into consideration so far.

Although selection between conspecific genotypes (i.e. lineage sorting) is commonly assumed to be an important and fast mechanism of adaptation, it is methodologically extremely difficult to measure, especially in natural populations. By establishing a novel approach, microsatellite poolSeq barcoding (MPB), to differentiate population composition over time, in *Publication III* I was able to trace the population development within the community incubations of *Publication I* (i.e. the selection environments of the used strains) at high temporal resolution. Similarly to the observations in species composition, neither a differentiation nor a decrease in diversity could be detected in most of the *T. hyalina* populations. The only exception was again the high $pCO_2$ treatment at the lowest temperature. Therefore, the plastic responses within populations of the community experiments in *Publication I* appear indeed sufficient to compensate for the effects of high $pCO_2$ in the majority of conditions. Under the lowest temperature, however, plasticity as well as intraspecific diversity were unable to stabilize the population performance and overall productivity under elevated $pCO_2$. One of the major advantages of the new MPB method is its applicability in environmental samples. Thus, I could observe in two consecutive years that even in the natural habitat, T. hyalina populations exhibit a surprising allelic stability throughout the spring bloom, but marked shifts between years (*Publication III*). This suggests the occurrence of such stabilizing mechanisms also *in situ* and affirms the general sensitivity of this method for changes between temporally separated natural populations.

In conclusion, large plasticity and sorting between highly diverse genotypes are both mechanisms that enable the investigated Arctic coastal diatom communities to adjust to strong environmental changes. Thus, these communities are very resilient towards a range of

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conditions, but the limits of this resilience appear suddenly, as is known for ecological tipping points. A deeper understanding of the underlying stabilizing mechanisms is therefore essential. While physiological plasticity can be investigated in laboratory monocultures, this work has also shown that such experiments can only be meaningful for a natural context if biological and even intraspecific interactions as well as the enormous diversity within species are accounted for. Furthermore, selection among lineages does not seem to function as linearly as often assumed, but may act in more subtle ways in highly diverse populations from variable environments. With the herein developed methods, these cryptic processes can finally be assessed. The competitive ability of genotypes or species as a whole is not only determined by their response to a small set of environmental drivers, but also strongly dependent on interactions with their surroundings and their ability to adjust. Forecasts on the future functioning of phytoplankton populations at the base of marine ecosystems are therefore bound to include intraspecific diversity and evolutionary adaptation as crucial processes.

# Zusammenfassung

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Der Klimawandel beeinflusst Ökosysteme weltweit und wird dies auch in den kommenden Jahrzehnten verstärkt tun. In der Arktis schreiten diese Veränderungen am schnellsten voran, weshalb diese Region als ein Vorbote dafür gesehen werden kann, wie Ökosysteme auf solch rasche Umweltveränderungen reagieren können. Primärproduktion ist sowohl für die Funktionalität des Ökosystems als auch für biogeochemische Kreisläufe von höchster Bedeutung. Im Ozean wird dies vorrangig von einzelligem Phytoplankton geleistet, von welchem alle höheren trophischen Ebenen abhängig sind. Während aber die Vorhersagen für physikalische und chemische Treiber wie Temperatur und $CO_2$ für das kommende Jahrhundert recht eindeutig sind, bleiben deren Konsequenzen für Phytoplankton noch immer ungewiss. Selbst scheinbar kleinste Veränderungen in der Artzusammensetzung und deren Charakteristika können enorme Konsequenzen für das Ökosystem mit sich bringen.

Wie sich Phytoplankton unter zukünftigen Bedingungen im globalen Kontext entwickeln wird, wird meist von Reaktionsnormen extrapoliert, die in Laborexperimenten an einzelnen Zellkulturen gemessen werden. Solche Optimumskurven stellen zwar die physiologische Plastizität eines Organismus dar, decken aber nicht die Vielfältigkeit dieser Eigenschaften einer Art ab. Auch berücksichtigt diese Herangehensweise weder Interaktionen mit der komplexen biologischen Umgebung, noch das evolutionäre Anpassungspotential an zukünftige Bedingungen. Obwohl Interaktionen und Abundanzverschiebungen zwischen Arten sichtbarer sind, finden viele Anpassungsprozesse zwischen oder innerhalb einzelner Zellen der gleichen Art statt. Prozesse auf dieser intraspezifischen Ebene sind, besonders in einzelligem Plankton, methodisch nur schwer greifbar. Daher ist unser Wissen über intraspezifische Diversität und Selektion in Phytoplankton noch immer sehr limitiert.

Das Ziel der vorliegenden Arbeit war es zu untersuchen, wie arktische Phytoplankton-Gemeinschaften auf Klimawandel-Szenarien reagieren und die zugrunde liegenden intraspezifischen Mechanismen besser zu verstehen. Die Diatomee *Thalassiosira hyalina* diente mir hier als Modell-Organismus, um jene Prozesse zu beleuchten, welche die Reaktionen oder die Resilienz von Populationen beeinflussen. Dabei nutzte ich ein experimentelles Design, das schrittweise die Komplexität von natürlichen Gemeinschaften, Einzelkulturen und künstlichen Populationen mit Feldbeobachtungen kombinierte. Diese Herangehensweise ermöglichte mir Einblicke in ansonsten kryptische intraspezifische Dynamiken und deren Anwendbarkeit auf verschiedenen Komplexitätsebenen.

In *Publikation I* werden die Ergebnisse von 10 Inkubationsexperimenten mit Phytoplankton-Gemeinschaften aus verschiedenen arktischen und subarktischen Regionen präsentiert. Diese Gemeinschaften wurden unter mehreren $pCO_2$-Szenarien in Kombination mit unterschiedlichen Temperatur und Lichtverhältnissen inkubiert. Anders als in anderen Regionen beobachtet, konnten die $pCO_2$-Effekte hier großteils kompensiert werden, und sowohl Primärproduktion als auch Artzusammensetzung blieben meist stabil. Nur bei der niedrigsten Temperatur verursachte erhöhtes $pCO_2$ sowohl eine stark verminderte Produktivität als auch eine Artenverschiebung. Ich vermutete, dass die Gründe der

beobachteten Resilienz innerhalb der Arten liegen mussten, und damit in der physiologischen Anpassungsfähigkeit von Organismen oder der Selektion zwischen diversen Zelllinien.

Jede Zelle kann ihre Physiologie innerhalb ihrer plastischen Möglichkeiten an die Umgebungsbedingungen anpassen. In *Publikation II* isolierte und untersuchte ich verschiedene *T. hyalina* Zelllinien aus den vorhergehenden Inkubationsexperimenten. Keine der experimentellen Bedingungen von erhöhter Temperatur und $pCO_2$ bereiteten den Isolaten fundamentale physiologische Probleme. Doch obwohl sie der gleichen Population entstammten, unterschieden sich die sechs Zelllinien stark in verschiedenen zellulären Eigenschaften und Wachstumsraten, sowie ihren Reaktionen auf die angewendeten Zukunftsszenarien. Gemeinsam mit einer vorhergehenden Studie (Wolf et al., 2018), deutet dies nicht nur darauf hin, dass Individuen dieser Art eine hohe Plastizität aufweisen, sondern auch, dass sie sich diese grundsätzlich voneinander unterscheiden. Dies impliziert eine große physiologische Anpassungsfähigkeit sowie ein hohes Potential für intraspezifische Selektion.

Wenn Individuen der gleichen Population unterschiedliche Optima besitzen, sollte sich auch ihre Konkurrenzfähigkeit unter verschiedenen Bedingungen unterscheiden. Ich überprüfte diese Annahme ebenfalls in *Publikation II*, indem ich die gleichen sechs Zelllinien auch gemeinsam als künstliche Population unter verschiedenen Temperatur- und $pCO_2$-Szenarien inkubierte. Durch die Anwendung einer erweiterten molekularbiologischen Methode (asqPCR) war es mir möglich, die genotypische Zusammensetzung in diesen artifiziellen Populationen zeitlich aufzulösen und so intraspezifische Selektionsprozesse sichtbar zu machen. Anders als erwartet, entsprachen die Charakteristika der Zelllinien in Einzelinkubationen aber nicht denen, die sie im Zusammenspiel mit anderen (d.h. unter erhöhter Diversität) zeigten, noch schienen sie ihre Konkurrenzfähigkeit zu determinieren. Des Weiteren unterschieden sich die gemessenen Selektionsdynamiken deutlich und reproduzierbar unter verschiedenen Bedingungen, obwohl die Produktivität gleich blieb. Diese Ergebnisse bestätigen zum einen, dass selbst in einem stark vereinfachten System intraspezifische Selektion Effekte auf die Produktivität einer Population abpuffern kann. Zum anderen zeigen sie auch, dass Selektion von Zell-Zell Interaktionen beeinflusst zu sein scheint, die bislang häufig nicht berücksichtigt werden.

Obwohl Selektion zwischen Artgenossen als ein wichtiger und schneller Adaptationsmechanismus vermutet wird, ist dies methodisch extrem schwer messbar, besonders in natürlichen Populationen. Durch die Etablierung einer neuen Methode (microsatellite poolSeq barcoding; MPB) in *Publikation III,* konnte ich im Nachhinein die Entwicklung der Populationen innerhalb der Inkubationsexperimente von *Publikation I* zeitlich verfolgen. Ähnlich zu der Entwicklung der Artenzusammensetzung, konnte auch auf der intraspezifischen Ebene weder eine Verschiebung noch eine Reduzierung der genotypischen Diversität festgestellt werden. Die einzige Ausnahme waren wiederum die hoch-$pCO_2$ Bedingungen unter der niedrigsten Temperatur. Dies weist darauf hin, dass die phänotypische Plastizität in den Populationen aus *Publikation I* ausreichend war, um die Effekte von erhöhtem $pCO_2$ in den meisten Fällen zu kompensieren. Unter der niedrigsten Temperatur allerdings konnten weder Plastizität noch eine Verschiebung der genotypischen Zusammensetzung die Population und ihre Produktivität stabilisieren. Einer der großen

# Zusammenfassung

Vorteile der neuen MPB Methode ist die einfache Anwendbarkeit auf Feldproben. Dadurch gelang mir die Beobachtung, dass *T. hyalina* Populationen auch in ihrer natürlichen Umgebung innerhalb einer Frühlingsblüte eine überraschend stabile Allelstruktur aufwiesen, sich aber zwischen zwei Folgejahren deutlich unterschieden (*Publikation III*). Dies weist darauf hin, dass die im Labor beobachteten intraspezifischen stabilisierenden Mechanismen auch im natürlichen Kontext Gültigkeit haben könnten.

Zusammenfassend haben die vorliegenden Studien gezeigt, dass sowohl phänotypische Plastizität als auch Selektion zwischen hochdiversen Genotypen effektive Mechanismen sein können, um die untersuchten Phytoplankton-Gemeinschaften arktischer Küsten vor den Effekten rascher Umweltveränderungen zu schützen. Somit waren die Populationen über einen breiten Bereich von Bedingungen unerwartet resilient, jedoch zeigten sich die Grenzen dieser Resilienz recht plötzlich unter einzelnen Kombinationen von Umweltbedingungen – dies ist von ökologischen ‚Tipping-Points' durchaus bekannt. Ein tieferes Verständnis der zugrundeliegenden Mechanismen ist daher essenziell, um solche Kipppunkte in Zukunft besser einschätzen zu können. Obwohl physiologische Plastizität in Zellkulturen untersucht werden kann, hat diese Arbeit gezeigt, dass solche Experimente nur Geltung für natürliche Systeme haben, wenn biologische Interaktionen sowie die enorme Diversität innerhalb von Arten mit einbezogen werden. Des Weiteren scheint intraspezifische Selektion nicht so linear abzulaufen wie meist angenommen, sondern funktioniert in diversen und variablen Umgebungen wahrscheinlich auf subtilere Weise. Die hier etablierten Methoden könnten dabei helfen, diese verborgenen Prozesse endlich zu ans Licht zu bringen. Die Konkurrenzfähigkeit von Individuen oder Arten wird also nicht nur von einigen dominanten Umwelttreibern bestimmt, sondern auch von ihrem biologischen Umfeld und ihrer Anpassungsfähigkeit all diesen Faktoren gegenüber. Vorhersagen über die zukünftige Funktionalität von Phytoplankton-Populationen an der Basis des marinen Nahrungsnetzes sollten daher intraspezifische Diversität und evolutionäres Potential von Organismen als essentielle Prozesse enthalten.

# Glossary

# Glossary

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|---|---|
| **Acclimation** | phenotypic adjustment to the environment within the physiological scope of an organism at the individual level (sensu Falkowski & LaRoche, 1991). |
| **Amplicon** | short DNA sequence products of polymerase chain reaction (PCR) amplification using taxon- or gene-specific primers to target a particular region of the genome (Porter & Hajibabaei, 2018). |
| **Adaptation** | long-term evolutionary change resulting from natural selection on the population level (sensu Falkowski & LaRoche, 1991). |
| **Allele** | variant of a given gene or gene locus. |
| **Allele frequency** | abundance of one variant of a gene locus relative to all variants present within a sample or population. |
| **Assemblage** | see community |
| **Bulk response** | cumulative response of an entire culture, incubation, population or species community. |
| **Cell quota** | cellular concentration of a substance (e.g. nitrogen, organic carbon, Chl $a$). |
| **Clone** | one organism or cell, produced by mitotic cell division. |
| **Clonal dominance** | occurs when one or few cell lineages dominate a population. |
| **Phytoplankton community ≈ species assemblage** | group of organisms belonging to a number of different species that co-occur in the same area. Although in community ecology, the terms community and assemblage are sometimes defined differently, within the scope of this work I use them synonymously. |
| **Research Community** | group of scientists belonging to the same field of research or investigating related research questions, ideally interacting and communicating (e.g. through conferences, publications and networks). |
| **Genetic drift** | process by which chance alone determines a change in allele frequency. Typically occurs in very small populations or when populations go through evolutionary bottlenecks (sensu Rengefors et al., 2017). |

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| **Growth rate** | rate at which cells of a strain of a unicellular organism multiply; usually exponential and measured as specific growth rate constant ($\mu$) or doublings per day ($k$). |
| **Directional selection** | selection that favors the fixation of one particular allele in a population. In the absence of other factors, the frequency of this allele will increase at a rate proportional to the strength of directional selection (Barrett & Schluter, 2008). |
| **Driver** | environmental change that results in a quantifiable biological response, ranging from stress to enhancement (Boyd & Hutchins, 2012). |
| **Ecosystem function** | processes that occur within an ecosystem that are related to species interactions, energy flow and the cycling of materials, e.g. primary productivity, biogeochemical cycling (Millennium-Ecosystem-Assessment, 2003) |
| **Effective population size** | size of a hypothetical ideal population with random mating that corresponds to population genetic processes within the focal wild population (Harvey et al., 2014). |
| **Environmental history** | the environmental conditions that an organism or lineage has been exposed to throughout its recent existence and that may have shaped its phenotype. |
| **Epigenetics:** | heritable changes in gene regulation processes that are not caused by changes in the DNA sequence (Harvey et al., 2014). |
| **Evolution** | change in genotype frequencies within a population between generations. Genetic variation can originate from de novo mutation, or may already be present as standing variation (Collins et al., 2014). |
| **Adaptive evolution** | evolution where fitness increases as a result of natural selection (Collins et al., 2014). |
| **Evolutionary bottleneck** | reduction in population size due to environmental events, leading to a strong reduction of the variation in the gene pool (Harvey et al., 2014). |
| **Field** | natural environment. |
| **Fitness** | average reproductive success of a genotype (Elena & Lenski, 2003). |

# Glossary

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**F$_{ST}$—Fixation index**  the most common measure of genetic differentiation between two populations, which allows an objective comparison of the effect of population structure in different organisms because it is expressed relative to the total genetic variance of one or many genetic loci (Rengefors et al., 2017).

**Genotype**  set of DNA variants found at one or more loci in an individual.

**Genotype frequency**  proportion of specific genotypes within a population (Rengefors et al., 2017).

**Haplotype**  combination of linked alleles. In asexually reproducing populations, some alleles may be lost if sex does not occur for many generations because haplotypes comprising specific allelic variants at a number of loci out-compete less fit combinations (Rengefors et al., 2017).

**Hardy-Weinberg Equilibrium (HWE)**  the mathematical model describing the relation between allele frequencies and genotype frequencies based on a number of assumptions about an ideal population, such as random mating, large population size, diploid, as well as negligible migration, mutation and selection (Rengefors et al., 2017).

**Heterozygosity**  the likelihood that there are different alleles at one genetic locus. In an ideal population, its fraction is determined by HWE. Deviations in the heterozygosity indicate inbreeding (deficiency) or heterozygote superiority or extensive clonality (excess) (Rengefors et al., 2017).

**Individual**  one genotype within a species; in unicellular organisms, this may describe a single cell or strain.

**Lineage**  a number of clones founded from a single individual that propagates asexually so that all genetic variation within a lineage comes from mutation (sensu Collins et al., 2014). See also strain.

**Linkage disequilibrium (LD)**  amount by which haplotype frequencies in a population deviate from the frequencies they would have had if the genes at each locus were combined at random. Occurs when alleles at different loci are not in random association. LD is one way to detect the predominance of asexual over sexual reproduction, as the multiplication of one certain genotype effectively creates one huge linkage block (Rengefors et al.,2017).

# Glossary

**Monoculture**

Clonal laboratory culture that consists only of one strain (typically established by single-cell isolation from a population)

**Multi-locus genotype (MLG)**

genotype identified by several loci of marker genes (such as microsatellites). Depending on the number and quality of loci investigated, it is more or less likely that individuals identified as an identical MLG are actually different genotypes that vary in their genetic composition elsewhere in the genome.

**Net Primary Productivity (NPP) ≈ productivity**

amount of photosynthetically fixed carbon, i.e. diurnal photosynthesis minus diel respiration of photosynthetic organisms (Behrenfeld et al., 2008).

**Phenology**

annually recurring life cycle events such as the timing of migrations and flowering (Edwards & Richardson, 2004)

**Phenotype**

set of observable characteristics and traits of an individual resulting from the interaction of its genotype with the environment.

**Phenotypic buffering**

type of phenotypic plasticity, in which no difference in the response of a trait to a given environment might be observed because plasticity in a physiological process allows an organism to maintain its performance (sensu Harvey et al., 2014).

**Plasticity**

phenotypic plasticity is the phenomenon of a genotype producing different phenotypes in response to different environmental conditions. While an individual is plastic through acclimation of its physiology, a population is plastic though the phenotypic plasticity of its organisms as well as though lineage sorting.

**Population**

a group of organisms of the same species living in close enough proximity that any member of the group can potentially mate with any other member (Waples & Gaggiotti, 2006).

**Reaction norm**

the expected phenotype of a given genotype as a function of an environmental driver (sensu Chevin et al., 2010)

**Resilience**

the capacity of a system to reorganize and return to a prior state after a disturbance (Connell &Ghedini,2015). Whether a deviation from the original state or an internal reorganization is notable for us may depend on degree of disturbance as well as on the level of observation.

# Glossary

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| **Resistance** | the capacity of a system to absorb the effects of disturbance without changing (Connell & Ghedini, 2015). |
| **Selection** | Non-random reproduction or survival of individuals of a particular phenotype (Harvey et al., 2014). |
| **Selective sweep** | the reduction or elimination of genetic variation at sites that are physically linked to a site under directional selection (Barret 2008). During a hard sweep, a new mutation with a strong selective advantage arises and is quickly driven to complete fixation in the population. In a soft sweep, selected variants are not associated with a single genomic background but occur with several variants in close proximity (Weigand & Leese, 2018). |
| **Stability** | persistence of a system within a specific state (Connell & Ghedini, 2015). |
| **standing genotypic/ phenotypic diversity** | variety or presence of more than one genotype/phenotype in a population. The base for selection to act upon. |
| **Standing genetic diversity** | variety or presence of more than one allele at a locus in a population. |
| **Strain** | ≈ lineage, a number of clones of one genotype within a species. Although 'strain' is used more in the context of physiological experiments, and therefore ideally describes only the original version of one genotype, we do not know how fast mutations occur and diversify a monoculture. In this context I use the terms strain and lineage synonymously. |
| **Strain/lineage sorting** | selection-based abundance shifts between the present diversity ('standing stock') of conspecific genotypes within a population of clonal organisms. |
| **Stressor** | An environmental change that decreases organismal fitness (Boyd & Hutchins, 2012). |
| **Tipping point** | a point at which a relatively small perturbation can cause a large, qualitative change in the future state of a system. This encapsulates the concept of a strongly non-linear response, which is abrupt but not necessarily irreversible (sensu Wassmann & Lenton, 2012). |
| **Trait** | any morphological, physiological, phenological or behavioral feature measurable at the individual level (Violle et al., 2012). |

# Chapter 1   General Introduction

## 1.1    A changing blue planet and its northernmost habitat

The oceans cover more than 70% of our Earth's surface and comprise about 300 times more habitable volume in its water column than the terrestrial environment (Brierley & Kingsford, 2009). Since the aquatic ecosystem is not our natural habitat, however, it is difficult to access and we often do not intuitively understand the dynamics and rules that govern this realm. We have been exploring this submerged world for a few centuries, but are still at the beginning of understanding it, especially in the more remote regions like the deep sea and the polar areas.

While the human population is expanding at an exponential rate, so is its consumption of carbon-rich fuels (IPCC, 2014). The consequences of this are expanding all the way to the remote and icy ends of the world. A large amount of carbon fixed from the atmosphere by photosynthesis over the past millennia has been stored in large natural reservoirs. These repositories are now being unburied, burnt and released back into the atmosphere in the form of $CO_2$, methane and other gases that interact with the climate as we know it. The partial pressure of $CO_2$ in the atmosphere ($pCO_2$; measured in µatm = micro-atmospheres, also expressed in ppm = parts per million) has increased from 280 µatm in pre-industrial times to currently 400 µatm (Tans & Keeling, 2015) and is predicted to exceed 1000 µatm by the year 2100, if human emissions do not drastically decrease (IPCC, 2014; scenario RCP 8.5). While we are just starting to fully grasp the complex interconnectivities of the ocean system, our actions are already causing far-reaching changes in its properties and mechanisms (Wassmann et al., 2011).

The polar regions, and the Arctic especially, are affected far stronger than the global average by the occurring changes (IPCC, 2014). The term *Arctic Amplification* describes the phenomenon of the magnified warming effect of the released greenhouse gases there (Manabe & Stouffer, 1980). This is mainly due to different feedback mechanisms related to reduced surface albedo, i.e. the fraction of incident sunlight that is reflected back into space. The retreating sea ice, for example, uncovers vast areas of open water, which absorbs the sun's energy nine times more effectively than snow (Miller et al., 2010). On land, the properties of northward expanding larger vegetation hinder the preservation of the terrestrial snow cover and thawing permafrost releases even more greenhouse gases into the atmosphere (Schuur et al., 2015). The global average air temperature has been rising by approximately 0.2°C per decade since the 1970ies (Hansen et al., 2006) and even the global ocean, which is heated up much more slowly, has increased its surface water temperatures by 0.11°C per decade in the same timeframe (IPCC, 2014). In the Arctic Svalbard archipelago, a detailed time-series over 20 years near the Kongsfjord, which is also the site of these studies, has revealed an air temperature increase of 1.35°C per decade (Maturilli et al., 2013) – almost 7 times that of the global average (Figure 1.1b). As a result of the rising temperatures, the Arctic has already lost almost half of its summer ice extent since 1970 and may experience ice-free summers within the next 30 years (Wadhams, 2012), which in turn will further accelerate ocean warming.
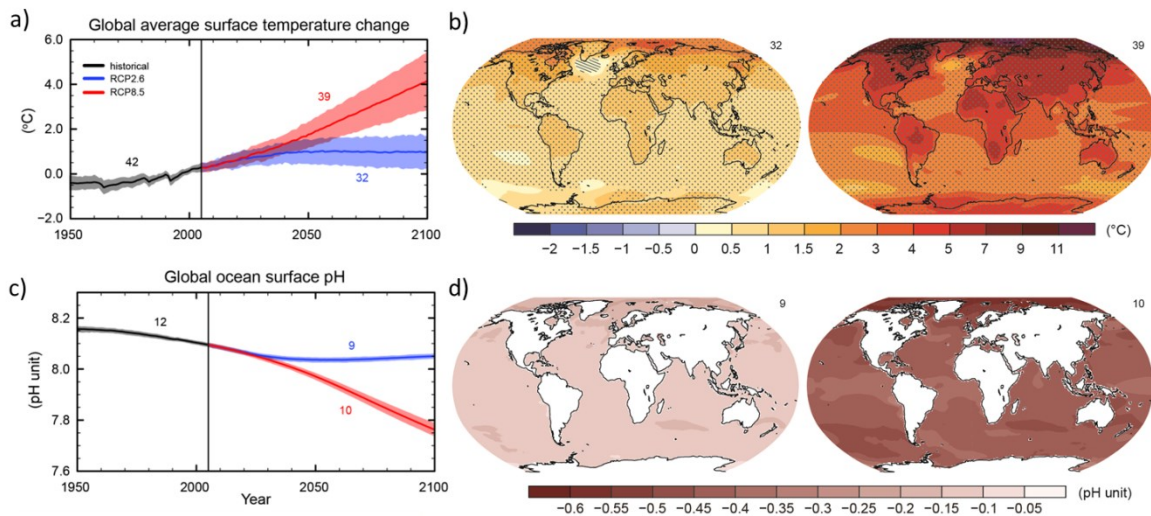
*Figure1.1*: *Projected global change in temperature (a+b) and pH (c+d) as determined by multi-model simulations for 2006 to 2100 under the scenarios RCP2.6 ("stringent mitigation scenario") and RCP8.5 ("very high greenhouse gas emissions", the latter of which current developments are closely following). All changes are relative to 1986–2005. The number of models used to calculate the multi-model mean is indicated in the upper right corner of each panel. a) Global average surface temperature change and c) ocean surface pH from 1950 to 2006 (black) and as projected under scenarios RCP2.6 (blue) and RCP8.5 (red) with measures of uncertainty (shading). Maps of projected late 21st century annual mean (b) surface temperature change and (d) in ocean surface pH. Dramatic changes are expected especially in the Arctic. Adopted from IPCC (2014).*

Next to temperature changes, the rapid increase of greenhouse gases has also a direct physico-chemical consequence for ocean chemistry, which has been termed Ocean Acidification (OA; Caldeira & Wickett, 2003; Wolf-Gladrow et al., 1999). As the ocean surface is constantly equilibrating with the atmosphere above (Schlesinger, 1997), it absorbs large amounts of $CO_2$ as the atmospheric partial pressure rises. The exceptionally large storage capacity of the ocean, which is a crucial part of the global carbon cycle, is caused by the fact that $CO_2$ in seawater is present in the form of different carbon species, collectively called dissolved inorganic carbon (DIC): the majority of $CO_2$ reacts with water molecules to form bicarbonate ($HCO_3^-$) and carbonate ions ($CO_3^{2-}$). These reactions lower the partial pressure of $CO_2$ in the water, permitting further uptake of the gas. Moreover, the reactions cause the release of protons and thus a drop in oceanic pH, i.e. acidification. The degree to which seawater is acidified by $CO_2$ uptake is determined by the total alkalinity (TA). A high TA signifies that the water contains a large amount of proton acceptors (like $HCO_3^-$, $CO_3^{2-}$, $OH^-$ and Boric acid $B(OH)_4^-$), which can act as a buffer system against the acidifying protons (Dickson, 2010; Wolf-Gladrow et al., 2007). This marine carbonate chemistry is the reason why the ocean is considered a major 'carbon sink' (Gruber et al., 2009). To date, it has taken up about one third of the $CO_2$ that we have released into the atmosphere by human activities (Khatiwala et al., 2013) and will continue to do so in the future (Le Quéré et al., 2010). As explained above, these increasing amounts of $CO_2$ in the ocean are shifting the current average ocean pH of 8.1 towards less alkaline conditions (Figure 1.1c+d). Since the industrial revolution, the mean surface pH has dropped by about 0.1 pH units and predictions anticipate

changes of close to 0.3 pH units by 2100, which translates to a more than 100% increase of proton concentration (IPCC, 2014). The Arctic Ocean is again especially affected, since fresh water input from sea ice melt and river inflow decrease its salinity and alkalinity - and therefore its proton buffer capacity. Both of these freshwater sources are intensified as temperatures rise (AMAP, 2013). Combined with higher gas solubility in colder waters, this makes polar systems more susceptible to Ocean Acidification than most other regions (AMAP, 2013).

Another physical consequence of the described extraordinary warming and freshening of the surface waters is the larger stability of the water column, the so-called surface stratification (Steinacher et al., 2010). When stratification is weak, water from the deep is frequently mixed into the euphotic surface layer, bringing valuable nutrients from below into the upper euphotic zone, where photosynthetic organisms can use them together with the penetrating sun light and $CO_2$ to create biomass. A more stratified ocean weakens this nutrient supply, while simultaneously increasing the average amount and intensity of irradiance that organisms at the surface layer receive. In polar areas, the increase of available light is additionally intensified by the sea ice retreating earlier and further each season (Arrigo et al., 2012).

These signals of global change are usually quantified by values that are averaged across large temporal and spatial scales. Especially on an organismal level, however, not only alterations in the long-term trends matter, but also their short- and mid-term variability (Vázquez et al., 2017). Higher frequencies of extreme events including temperature records (Rahmstorf & Coumou, 2011) illustrate that it is not enough to cope with average conditions, but that physiological and biogeographic limits are rather set by extremes, which have historically often been the cause of dramatic evolutionary responses (Grant et al., 2017). The Arctic has seen a series of extreme events in recent years, with several winters in a row setting records of high temperature and precipitation (Boisvert et al., 2016; Cullather et al., 2016). Arctic ice-edges and coasts are and will continue to be especially variable environments also in terms of pH values, irradiance and nutrient conditions (Matsuoka et al., 2011; Popova et al., 2014; Thoisen et al., 2015). The Arctic is therefore often seen as a miner's canary, which is already today strongly affected by changes that still seem minor in other regions of the world (Thomas, 1996). The very base of this fragile ecosystem is the photosynthetic activity of marine phytoplankton and its response to the described new conditions will thus be crucial to the fate of this and other environments.

## 1.2     The role of phytoplankton in the world's oceans

Photosynthesis, the process of transforming inorganic carbon into organic molecules by using the sunlight's energy, water and a few essential nutrients, is a truly exceptional biochemical process, which has not changed in its basic functioning since 2.4 billion years (Fischer et al., 2016). Phytoplankton are the ocean's main primary producers and consist of single-celled protists, in most cases invisible to the naked eye. Since 1.2 billion years, long before multicellular plants arose, they have been populating the oceans (Knoll et al., 2007), and are responsible for the oxygenation of earth's atmosphere that shapes life as we know it (Benoiston et al., 2017). Although they constitute only 1% of the earth's standing biomass, they are responsible for about 50% of today's global primary production (Field et al., 1998). This is made possible by their rapid mitotic, mostly asexual proliferation and because, in contrast to multicellular plants, each cell is photosynthetically active (Benoiston et al., 2017). While this allows for rapid exponential growth, their biomass buildup is usually kept at bay by grazing and immediate remineralization, which causes exceptionally short turnover times in the rage of days (Falkowski et al., 1998).

Because of their photosynthetic activity, phytoplankton have two fundamental global roles: they provide oxygen as well as organic matter for other organisms, and draw carbon into the depth of the ocean (Falkowski et al., 1998). The fact that the ocean carbon cycle stores a massive pool of carbon that equals 50 times that of the atmosphere is thus not only driven by physical but also by biological processes (Passow & Carlson, 2012; Sabine et al., 2004). While the surface ocean is in constant exchange with the atmospheric gases, $CO_2$ is drawn into the deeper layers of the sea by the two carbon pumps (Figure 1.2): As surface waters move towards polar regions they cool down, increase their gas solubility, and concurrently sink down to form deep water masses that contribute to the global overturning circulation (Macdonald & Wunsch, 1996). When surface waters submerge, they draw $CO_2$ (and other gases they have absorbed) from the atmosphere along with them into depth, and store them for centuries. Next to this physical pump, there is the biological carbon pump: Phytoplankton cells that fix carbon in the euphotic zone at the surface eventually die and are remineralized by zooplankton and bacteria which provides nutrients for further primary production. As this recycling process usually cannot keep up with the production and/or sinking speed of particles, carbon-rich biomass is exported into intermediate waters and even into the deep sea. Only minute amounts of the produced biomass ever reach the ocean floor, but still the biological carbon pump contributes three quarters of the DIC gradient from the surface to the deep ocean (Sarmiento & Gruber, 2006; Volk & Hoffert, 2013). Although these processes are powerful enough to shape global climate on geological timescales (Sigman & Hain, 2012), they are slow compared to anthropogenic changes in the carbon cycle. In fact, the amount of carbon we are emitting each year equals impressive one million years of this biological pumping (Falkowski, 2015).

While removing their substrate $CO_2$ from the inorganic pool, the biomass produced by phytoplankton nourishes direct grazers, such as other protists (e.g. ciliates and dinoflagellates) or small zooplankton. At the same time, photosynthesis releases oxygen as a byproduct. Primary producers therefore provide the base for all subsequent heterotrophic

levels of the food chain (Field et al., 1998), all the way up to tasty or charismatic megafauna like fish, whales and polar bears. The before mentioned fast turnover rates illustrate how rapid these trophic dynamics in the surface ocean are running to be near equilibrium (Falkowski et al., 1998). The biomass buildup of phytoplankton is usually controlled by grazing and sinking, but at times such top-down processes are decoupled from the dynamics of primary productivity (e.g. at the beginning of the season). Only then, photosynthetic cells can accumulate in an exponential way (Behrenfeld, 2010), which is commonly referred to as a phytoplankton bloom.
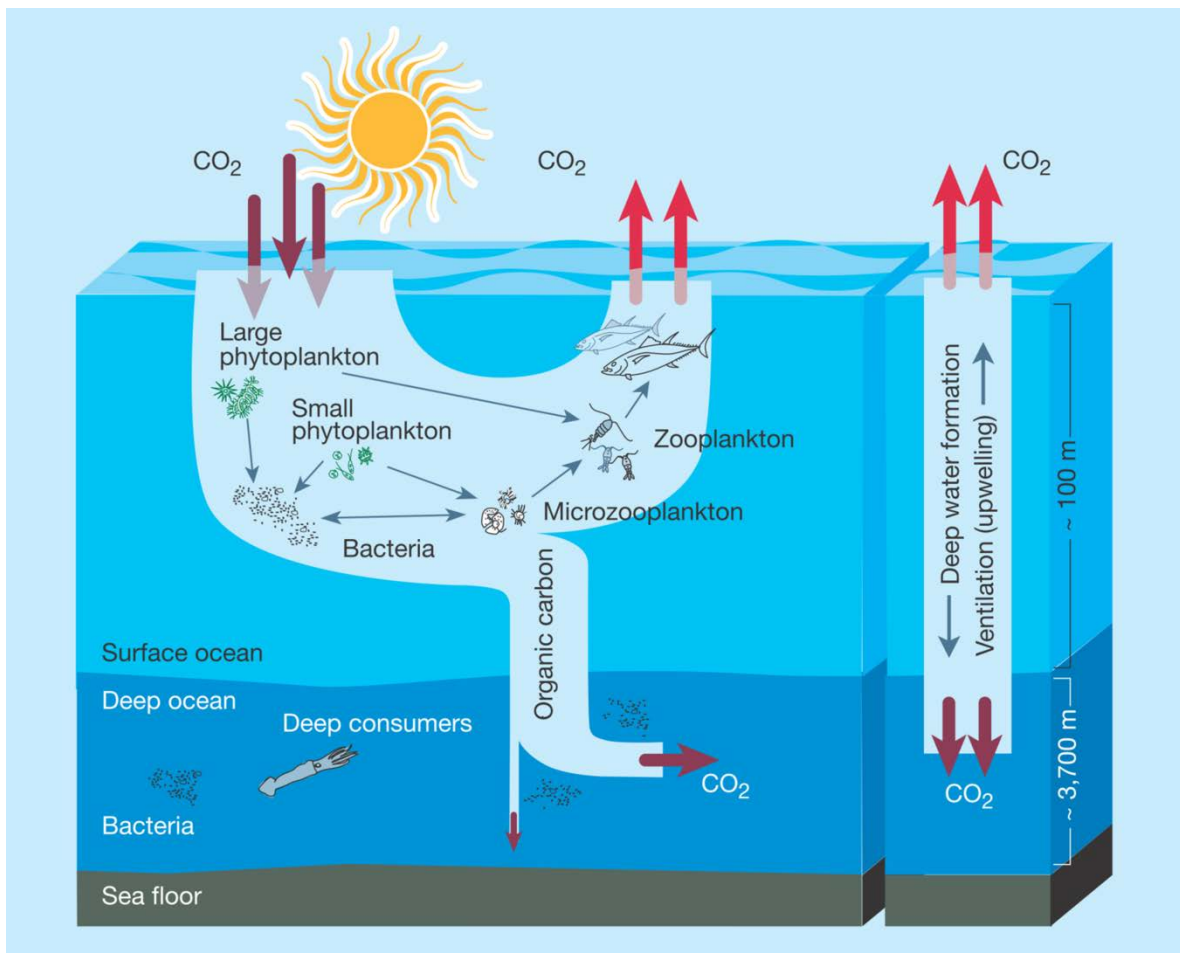


*Figure 1.2: The physical and the biological carbon pump draw $CO_2$ from the atmosphere into the deep oceans, where it accumulates and is stored for centuries. While the physical pump depends on physical and chemical processes, the biological pump is mainly driven by phytoplankton and its consumers. Through photosynthesis, carbon is fixed from the surface ocean and passed up the food chain, where the majority is respired. Some of it, however, sinks into the deeper water layers where it is only slowly remineralized by bacteria. The structure of the foodweb, relative species abundances and nutrient availabilities influence how much $CO_2$ is pumped into the deep ocean. After Chisholm (2000)*

## 1.2.1 Phytoplankton blooms

During phytoplankton blooms, massive amounts of photosynthetic biomass can accumulate, which are sometimes even visible from space (Wassmann et al., 2011). What makes a bloom a bloom, however, is not always easy to define. In a general sense, it is simply a high concentration of phytoplankton, which means that division rates must exceed the loss rates over a certain period of time (Behrenfeld & Boss, 2018). Notably, this concentration is distributed over the three-dimensional water column and can be difficult to detect if measurements focus on a single depth. Specific thresholds in biomass or its accumulation rates, however, are difficult to generalize because bloom characteristics (i.e. their phenology) are very diverse. Bloom phenology is determined by two basic processes: growth and loss. The division rate of phytoplankton cells is mainly driven by the supply of nutrients and light (i.e. 'bottom-up' control due to limitation). The loss rate depends on mortality and therefore on the abundance and preferences of grazers, viruses and bacteria ('top down' controls; Behrenfeld & Boss, 2018). Both, loss and division rate are strongly influenced by the mixing of the water column: loss through dilution and sinking, division rate through the light regime that cells are effectively exposed to (MacIntyre et al., 2000). Therefore, if systems are subject to deeper mixing under nutrient-replete conditions, blooms with similar biomass can develop rather slowly and over a longer timeframe, while they can exhibit shorter, explosion-like dynamics if reduced to a rather shallow surface layer.
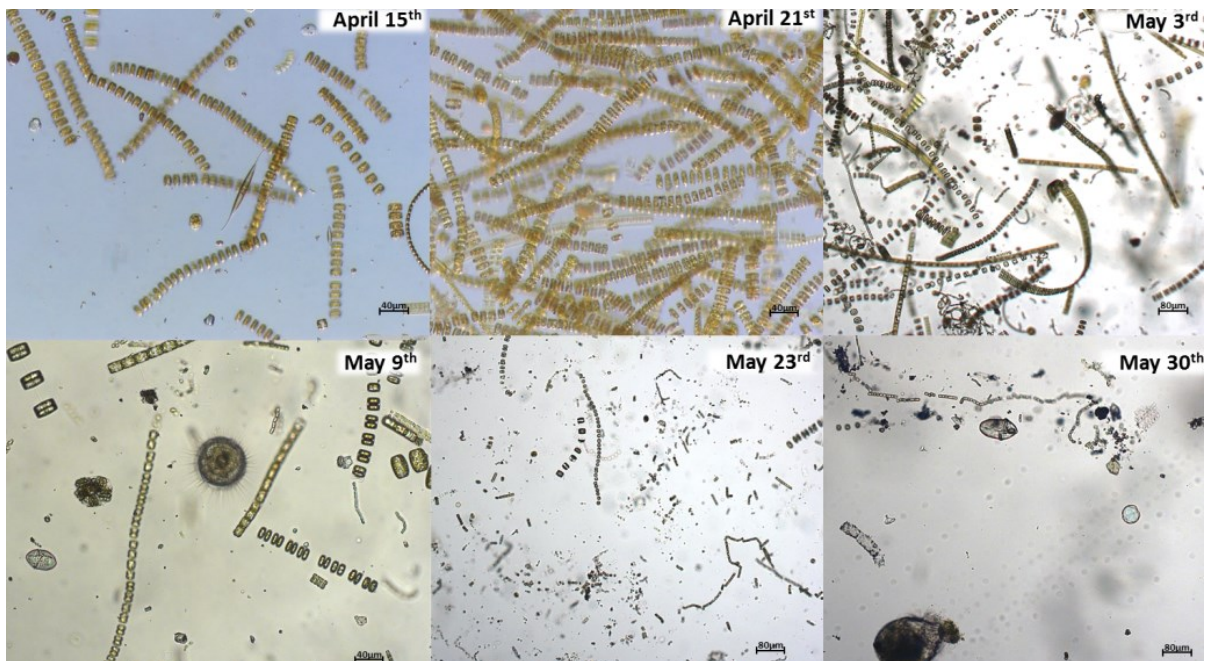


***Figure 1.3:*** *Microscopic snapshots of the development of the spring bloom community of 2016 in Kongsfjorden as observed in a light microscope. Samples were taken between April 15th and May 30th at the mid-fjord station (KB3) and concentrated by the use of a Plankton-net from 25m depth to surface. The dominant chain-forming diatom in April was Thalassiosira hyalina.*
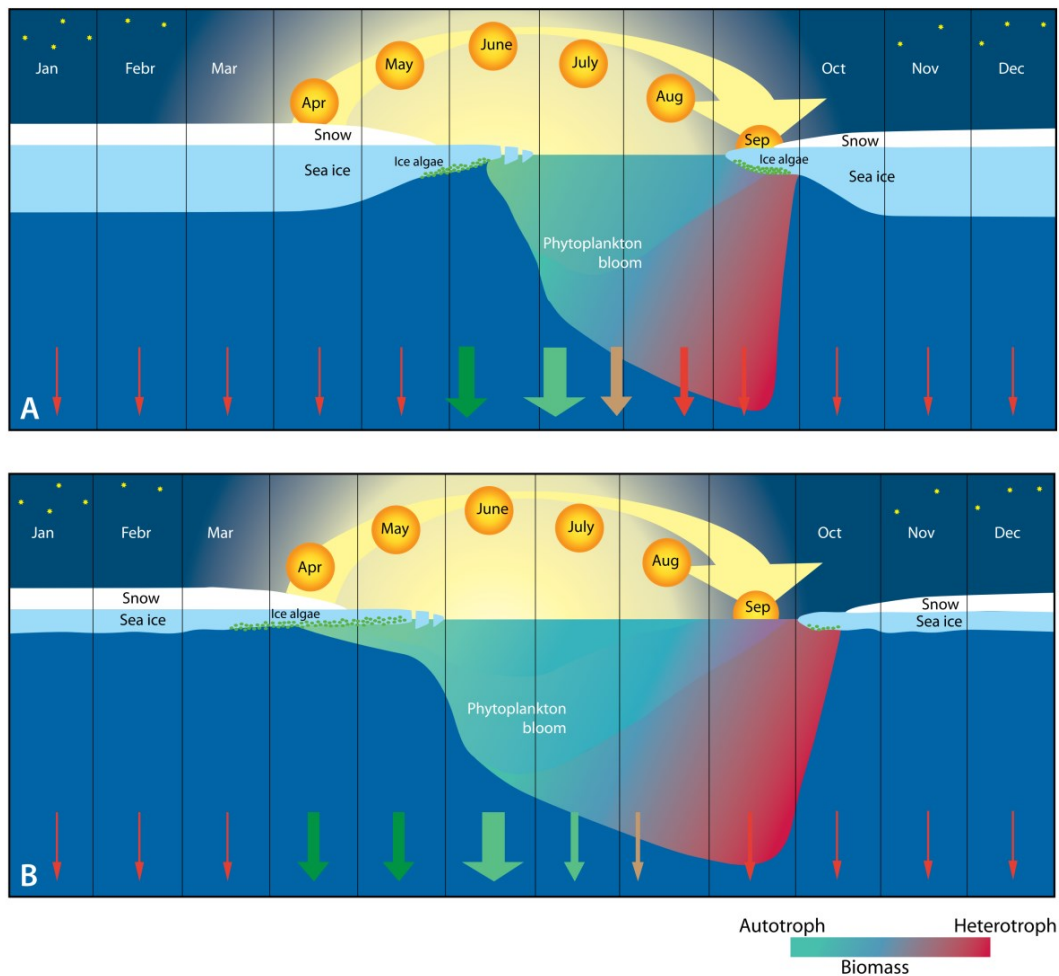
**Figure 1.4:** *Arctic bloom phenology and carbon export today (A) and as expected in the future (B). The green-to-red gradient indicates the transition from predominantly autotrophic to heterotrophic biomass. The width and color of the vertical arrows illustrate the semi-quantitative magnitude and key composition of exported organic matter: dark green = ice algae-derived carbon; light green = phytoplankton-derived carbon; orange/ red arrows = increasing degree of detritus (nonliving particulate organic material). Progressive thinning of the sea ice causes a widening of the seasonal ice zone and a prolongation of the growing season. New and export production in both scenarios remain similar because stratification limits nutrient availability. Adopted from Wassmann and Reigstad (2011)*

In regions with strong seasonal cycles, where nutrients can accumulate in winter while other essential ingredients for photosynthesis are limiting or absent (i.e. light), the strongest and best predictable blooms are typically observed in spring when the sun and more stratified conditions return. If nutrients are abundant, these blooms are often dominated by diatoms, which cause especially strong export to the deep because of their heavy silica shells (Sarthou et al., 2005). Especially when they grow in chains or are otherwise aggregated, they can exhibit surprisingly fast sinking rates (Agusti et al., 2015) and make a substantial contribution to the biological carbon pump (Falkowski et al., 1998). If not remineralized in the surface ocean, large amounts of biomass from such bloom events can sink as aggregates ('marine

snow') or in their grazers' fecal pellets into the deep sea (Turner, 2002). In the Artic, where light is entirely absent for almost half a year, bloom events are especially pulsed (Behrenfeld et al., 2016) and can easily account for half of the annual primary production (Klein et al., 2002). As soon as the snow covering the ice begins to melt, it changes its optical properties, which allows specialized phytoplankton species to form blooms of considerable size, even underneath the sea ice (Hancke et al., 2018; Leu et al., 2015). As soon as the ice weakens and finally breaks up, however, the highly productive pelagic blooms appear at the ice edge, which draw down nutrients in the water column more quickly (Arrigo et al. (2012) and Figure 1.4).

While bloom phenology strongly varies across regions and habitats, some general mechanisms apply in most of them. Early in the season, when grazers are not abundant, bloom dynamics are mainly driven by bottom-up processes, where light and nutrients control a community that is often dominated by diatoms, prymnesiophytes (e.g. coccolithophores and *Phaeocystis sp.*) and green algae (Assmy & Smetacek, 2009) (c.f. Figure 1.3). Under these conditions, the system is often mainly autotrophic, i.e. showing an overall positive net community production (where photosynthesis exceeds respiration of the entire ecosystem). When grazer populations catch up and nutrients become limited, however, these blooms can crash quickly and switch to a more heterotrophic system in summer, including high numbers of dinoflagellates and ciliates (Wassmann & Reigstad, 2011). Concurrently, the system shifts from mainly new production fueled by new nutrients, which accumulated during winter or in deeper waters, to a regime where production is based on 'regenerated' nutrients that are directly recycled by grazers and bacteria (Dugdale and Goering (1967) and Figure 1.3 and 1.4). In temperate regions, where light is still available later in the season, wind-driven mixing can renew the nutrient supply and cause a second autumn bloom. As sea ice freezing is delayed and winds increase, this phenomenon is also increasingly being observed in the Arctic, which may be a further sign for fundamental system shifts to approach (Ardyna et al., 2014).

### 1.2.2   Diatoms

Diatoms are today one of the most dominant phytoplankton taxa in the oceans, and have been exceptionally successful since at least 60 million years (Armbrust, 2009). Because of their high nutrient requirements and fast growth rates, they are traditionally affiliated with highly productive upwelling regions and mid to high latitude blooms in nutrient-rich surface layers (Benoiston et al., 2017). The successful occupation of this niche may be connected to the ability of many species to take up nutrients in amounts that exceed their demands and store them in internal vacuoles for later use ('luxury consumption'; Droop, 1975; Katz et al., 2004; Raven, 1997). As we gain more knowledge about the world's oceans, diatoms are found almost everywhere, including deeper water layers and oligotrophic regions, even though here as a less dominant fraction (Tréguer et al., 2017). They are estimated to perform about 40% of marine and 20% of the global net primary production (NPP), as much as all rainforests combined (Field et al., 1998). Their high abundance and productivity is accompanied by an enormous species diversity, with cell sizes ranging from 2 μm to 2 mm, that often form chain-

like colonies of mitotic clones (Tomas & Hasle, 1997). The probably most prominent and biogeochemically relevant feature of diatoms are their often beautifully ornamented silica frustules, which may serve as grazer and virus protection (Hamm & Smetacek, 2007; Smetacek, 2001) and can, depending on their architecture and active buoyancy mechanisms, influence their sinking rates considerably (Raven & Waite, 2004; Waite et al., 1992). This makes diatoms major drivers of carbon export into the deeper ocean (Henson et al., 2012; Jin et al., 2006).

The transparent frustules around diatom cells also have strong implications for their life cycle: Upon each mitotic cell division, the daughter cell inherits one half of the frustule and synthesizes the other half while still enclosed in the cell wall of the mother cell. This implies a progressive size reduction with each generation and makes a restoration of the cell size necessary in certain intervals by sexual reproduction and auxospore formation (Chepurnov & Mann, 2004; Moeys et al., 2016). Unlike many protists, diatoms are therefore obligate to have sex. Since the majority of their life takes place in a diploid form and through clonal division, however, our knowledge on diatom sex in natural populations is still very limited. Suggestions on the frequency of recombination, for example, range from 2 to 40 years (Collins et al., 2014). Genetic tools slowly reveal the physiological mechanisms involved and we do know now that sexual strategies depend on surrounding conditions and differ between species (Montresor et al., 2016): while some are homothallic and genotypes can even mate with themselves, others are divided into different mating types (Basu et al., 2017). Modelled sexual phases based on cell size distribution patterns in *Pseudo-Nitzschia multistrata*, for instance, hint towards periodic sexual phases occurring at least every 4 years (D'Alelio et al., 2010). Similarly, Ruggiero et al. (2017) recently found indications of pulsed cycles of asexual and sexual phases in the same ecosystem by genetic fingerprinting. Population genetics (see *chapter 1.4.2.*) are thus providing new tools to indirectly resolve dynamics of recombination in natural populations.

Another peculiarity that many diatoms have in common with only some other phytoplankton groups, is their ability to form resting spores (hypnospores). Although such resting stages can be produced at any time, their presence increases under adverse conditions, e.g. at the end of a bloom when nutrients are depleted (Smetacek, 1985). Being especially heavily silicified, these spores quickly sink into deep water layers or the sediment (Rynearson et al., 2013). There, they can persevere for several years or decades in cold and dark conditions until they are mixed up and find more favorable conditions (Ellegaard & Ribeiro, 2018). Especially in coastal areas, diatoms can thus have a reservoir of genotypes stored in 'seed banks' with important implications for their population structure and evolution (Hargraves & French, 1983; Härnström et al., 2011).

### 1.2.3    Projections on future phytoplankton productivity

As explicated above, the fate of phytoplankton is strongly interlinked with the oceans' biosphere and elemental cycles. As global environmental changes are becoming increasingly tangible, it is getting more and more urgent to understand and predict the responses of phytoplankton to the expected changes. Only then can we make knowledge-based assumptions on the further consequences for ecosystem functioning and biogeochemical processes. While global models still contain only very simplistic features of phytoplankton, projections of their primary productivity are highly variable and work better in some regions than in others (Bopp et al., 2013; Laufkötter et al., 2015). Nevertheless, under expected climate developments the majority of them forecast a mean decrease in NPP globally. For the Arctic Ocean, however, they predict and observe already a strong increase of productivity, but the magnitude even of the present developments is highly debated (Arrigo & van Dijken, 2015; Kahru et al., 2016; Laufkötter et al., 2015).

Such global or regional projections are typically realized by Earth System Models. They describe the physical properties of the atmosphere, ocean and cryosphere as well as biological processes through biogeochemical cycling of organic matter (Asch et al., 2016). The standard ocean ecosystem modules within these models have changed very little in their fundamental design in the past 60 years (Record 2014). Their representation of phytoplankton is based on size classes and some key traits (Le Quéré et al., 2005; Tréguer et al., 2017), which are usually defined by a set of rigid parameters and their functions towards temperature, light and nutrients as measured in laboratory studies (Laufkötter et al., 2015). There is a continuous call for this empirical base (Tagliabue et al., 2011), not necessarily because experiments are lacking but rather because they fail to yield simplistic enough patterns for mathematical models. The traditional approach for such experiments is to define species' responses in simplified experimental scenarios and project this knowledge onto future conditions in order to describe how traits and species composition will change (e.g. Beardall & Raven, 2004). The final consequences of global changes on phytoplankton, however, are unlikely to be grasped by linear physiological responses in laboratory cultures.

This introduction aims to provide some fundamental knowledge on three important aspects of phytoplankton biology. First, cellular physiology in a complex matrix of environmental drivers lays the basis for the response range of a species or organism (1.3). Second, the diversity of these phenotypic responses and the underlying genotypes shape a species' persistence and success by making its response scope more flexible (1.4). Third, evolutionary adaptation is continuously altering the characteristics of individuals, populations and species within them. Since the basic mechanisms behind them act on the individual rather than on taxonomic groups, I will focus here on processes on the intraspecific level (1.5). I intend to demonstrate that all three of these elements are paramount for understanding and anticipating how changes in the environment may translate to responses or stability of phytoplankton and the marine ecosystem.

## 1.3 Phytoplankton physiology in a changing environment

Phytoplankton have sustained the marine biosphere for millennia and will continue to do so in spite of human perturbations of the earth system. However, even minor changes in competitive abilities, cell stoichiometry, grazer or virus defense and bloom timing may have large effects on species composition and productivity, which can have consequences for higher trophic levels, carbon export and elemental fluxes on a global scale (Edwards & Richardson, 2004; Rost et al., 2008). It is therefore the objective of a large research community to describe and understand the responses of marine phytoplankton towards those environmental parameters that are expected to change most profoundly in the coming decades: Temperature, $pCO_2$ and pH, as well as light and nutrient availability.

### 1.3.1 Response curves and interacting drivers

When considering physiological responses, two basic concepts should be kept in mind. One is the generally applicable knowledge that organisms' responses are typically not linear positive or negative developments but are much better defined in curves (Kingsolver, 2014): when tested along a gradient of environmental drivers, most organismal traits can be described in an optimum curve with an increasing slope (i.e. stimulatory effects of an environmental driver), an optimum range (i.e. no effects) and a decreasing slope (i.e. negative effects). Describing the phenotype of a given genotype as a function of an environmental parameter is also called a reaction norm (Chevin et al., 2010). The exact shape of such a reaction norm describes an organism's plasticity, i.e. the acclimation range of an organism. Despite this general optimum shape, the range and position on the parameter scale can vary considerably between differently adapted organisms (Thomas et al., 2012). A certain change in an environmental parameter may therefore not at all have the same consequences for different populations or species. Even the same genotype may show a different direction of response depending on its state of acclimation and the tested parameter range. While this appears fairly intuitive, it is not as trivial when experiments resolve only a part of the entire curve (grey shaded areas Figure 1.5). Deduced implications can therefore diverge depending on which part of the curve is covered by the experimental design (e.g. Boyd et al., 2018).

Furthermore, a second aspect needs to be considered: Interactive effects between different environmental drivers can modulate such reaction norms even within the same genotype. This can be caused by direct physiological links or more indirect energy reallocation among cellular processes. In ocean acidification research, for example, such effects have caused considerable confusion about the general biological implications of rising $pCO_2$ for calcifiers (Rost et al., 2008). While a reaction norm towards a range of $pCO_2$ treatments may be observed at one temperature, it can be shifted by e.g. warmer conditions and thus suggest a fundamentally different trend (Sett et al., 2014), as illustrated in Figure 1.5. This is one of the reasons why the results of detailed physiological investigations that usually do not cover the full reaction norm, can seem contradictive and why it is not a simple task to identify overarching patterns or retrieve generally applicable relationships (which is what models require).
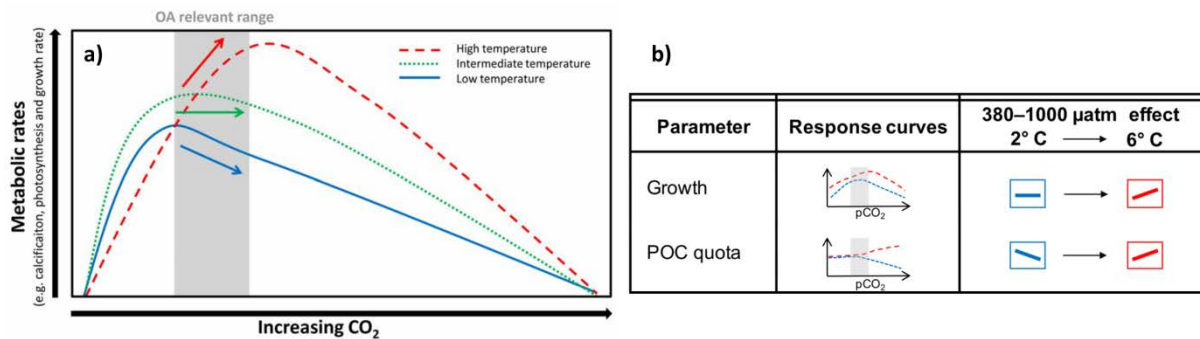
***Figure 1.5:*** *Conceptual response curves and how they can be influenced by interactive effects of drivers. The examples illustrate metabolic responses to elevated $pCO_2$ in a) coccolithophores (after Sett et al. 2014) and b) Micromonas pusilla (after Hoppe et al. 2018a). The implications of measured $CO_2$ responses when interpreted as a linear trend (arrows in a), boxes in b) can seem contradictive within the same organism: within the same response curve, they depend on the tested range of $CO_2$ (grey shaded areas). Furthermore, interactive effects with the applied temperature levels (blue, green and red) can substantially shift the curve. These ambiguities can only be resolved by interpreting the results in consideration of the underlying full reaction norms.*

The ambiguity of experimental studies on phytoplankton responses may therefore often be found in the subtleties of the experimental setup applied, because they also depend on interactive effects of the investigated drivers with specific laboratory conditions that are not intended to be tested. Side effects of cultivation, such as population density or the growth phase (exponential or stationary), can also cause considerable differences in response (e.g. limitation by nutrients or light through self-shading etc.). Consequently, initiatives to establish best-practice guides and standardization procedures have been launched (Boyd, 2013; Dickson et al., 2007; Riebesell et al., 2010). It is an important criterion, for instance, whether experiments include a thorough acclimation phase of the cultures to the experimental conditions, since otherwise they display a shock or adjustment response rather than a stable phenotype that represents the full plastic potential (Barcelos e Ramos et al., 2010). Another factor that is often neglected in physiological studies is the history of the cultures used. The origin of strains as well as the time since isolation can have a large impact on their response (Lakeman et al., 2009). Cultures that have been subject to detailed investigations are usually grown as monocultures in the laboratory since years, i.e. thousands of generations and thus evolutionarily very relevant timescales (Collins & Bell, 2004; Lenski, 2017). This implies that they may have evolved in a way that is beneficial in the laboratory but not representative in an ecological context (Bach et al., 2018; Lakeman et al., 2009).

### 1.3.2    Eco-physiological responses of phytoplankton in a nutshell

A useful mechanistic approach that helps to identify and explain general patterns in physiological responses to environmental drivers is the consideration of supply and demand of energetic resources. Since every cellular process requires energy and metabolites, which are never available in unlimited amounts, this often encompasses the balancing allocation of energy budgets within the cell (Behrenfeld et al., 2008). For instance, if more resources are required to acquire nutrients (e.g. use of $NO_3^-$ vs $NH_4^+$), less photosynthetic energy can be allocated to the fixation of carbon, i.e. the creation of organic biomass.

All the most fundamental environmental factors that determine phytoplankton growth and productivity are expected to change in the future. While temperature influences the dynamics of nearly all cellular processes and $CO_2$ input alters the cells' chemical environment, light and a range of nutrients, including inorganic carbon, are required as resources for photosynthesis and other obligatory cellular processes (Falkowski & Raven, 1997). The anticipated changes in temperatures and carbonate chemistry are relatively well defined, while predictions on nutrient and light availability vary more strongly due the more complex controls on these drivers as explained above. Therefore, much of the research on global ocean change biology has focused on the effects of warming and elevated $pCO_2$. In spite of much controversy on general trends in phytoplankton responses to these drivers, the conducted studies yielded some frequently reoccurring patterns, which I will summarize shortly in the following.

Responses to warming are the most prominent and extensively researched ones. Since warming generally accelerates chemical reactions, it affects a broad range of physiological processes on basic and molecular levels (e.g. enzyme kinetics). In laboratory experiments, maximum growth rates and productivity generally increase at higher temperatures, ideally following a temperature coefficient ($Q_{10}$ law; (Bissinger et al., 2008; Boyd et al., 2013; Eppley, 1972; Kremer et al., 2017a)). Therefore, the possible maximum growth rate increases with rising temperature ('Eppley-curve', Figure 1.6a). The range of plasticity, however, varies greatly between single species and even genotypes, and the rule only applies as long as the respective optimum temperature range is not breached, which depends strongly on regional adaptation (Thomas et al., 2012). Since temperature optimum curves are typically skewed and show a steeper fitness decline at higher temperatures than an incline at colder ones, thriving at temperatures below the optimum is generally a safer ecological strategy (Martin & Huey, 2008). In Arctic diatoms, optima seem to be especially far above the conditions they commonly experience, which suggests that most of them are growing below their preferred temperature (e.g. Pančić et al., 2015; Schlie & Karsten, 2016). Up to which point this implies a stimulation of their productivity in a warming environment is being debated and likely depends on more than just latitude (Hare et al., 2007; Holding et al., 2013). In fact, within an ecological framework, where top-down processes such as grazing are taken into account, total warming effects on phytoplankton biomass are often predicted as negative because heterotrophic grazing activities may be stimulated more strongly by warming than photosynthetic ones (Sommer et al., 2015).
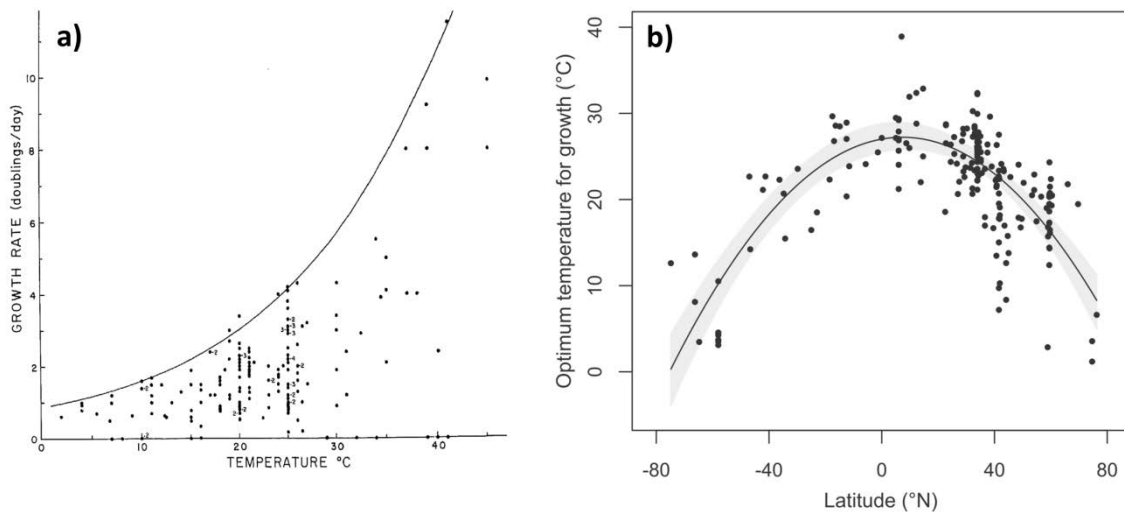
*Figure 1.6: Temperature responses across species from two points of view. a) possible maximum growth rates increase with temperature when pooled across a range of taxa (Eppley, 1972). b)The origin of the tested organism strongly influences its optimum growth temperature due to regional adaptation (after Thomas et al., 2012). Within a single organism, however, the reaction norm towards temperature typically resembles a skewed curve around the specific optimum value.*

Inorganic carbon is the basic substrate for photosynthesis and phototrophic biomass buildup, but also a substantial part of ocean chemistry (*chapter 1.1*). This causes some complexity in phytoplankton responses to rising $pCO_2$, as it affects cellular physiology in two opposing ways, i.e. by carbonation and acidification (Bach et al., 2011). The term 'carbonation' describes the increased availability of inorganic carbon in the form of $HCO_3^-$ and more importantly $CO_2$, which ultimately is the substrate needed by photosynthetic organisms. RuBisCO (Ribulose-1,5-bisphosphate carboxylase/oxygenase), the central enzyme of carbon fixation, is dependent on $CO_2$ only, but is also characterized by very low affinities for it. To overcome the ineffective nature of the enzyme's active site, cells require very high intracellular $CO_2$ concentrations and typically employ active carbon concentrating mechanisms to avoid carbon-limitation (Raven et al., 2008; Riebesell et al., 1993). Therefore, they can profit energetically from downregulating this process under elevated $pCO_2$ levels (Rost et al., 2008). Concomitantly, however, acidification of the seawater (*chapter 1.1*) can pose a challenge to the homeostasis of cells, for example for intracellular pH levels and their regulation (Flynn et al., 2012; Suffrian et al., 2011). This applies especially for organisms dependent on calcification (Kottmeier et al., 2016; Rost et al., 2003; Taylor et al., 2011). One could generally assume that within the $pCO_2$ range anticipated for the next 100 years, the positive effects of carbonation dominate the response in non-calcifying organisms, like diatoms, while acidification proves more problematic for calcifiers (Raven, 2011). Diatom responses in laboratory experiments, however, have been shown to vary strongly, ranging from growth rate inhibition to stimulation or an absence of response (reviewed in Gao & Campbell, 2014; Schulz et al., 2017). Although compared to the effects of other environmental drivers, $CO_2$ responses in non-calcifying organisms are often rather subtle and lack universal

patterns across genera and species, even small changes in competitive fitness have the potential to strongly influence species composition (Dutkiewicz et al., 2015). In Arctic areas, more integrative approaches like mesocosm studies suggest varying, but in their majority rather stimulating effects of $CO_2$ on phytoplankton growth and primary productivity (Engel et al., 2013; Holding et al., 2015; Hussherr et al., 2017; Sommer et al., 2015; Thoisen et al., 2015).

Light energy is one of the basic requirements of phytoplankton because it is the irreplaceable energy source of photosynthesis. An excess of this energy, however, can be highly damaging for the cell (e.g. due to the formation of reactive oxygen species). Therefore, balancing between sufficient acquisition of and protection from excess irradiance requires constant regulation within plant cells – especially in a highly fluctuating environment like a mixing surface ocean. Light is harvested for photosynthesis by adjustable antenna complexes that make this process more or less efficient, and a range of downstream processes can be modified to optimize the transformation of light into biologically available energy depending on specific cellular demands (e.g. ratio of ATP vs. NADPH; Behrenfeld et al. (2008)). The resulting responses to light levels are usually depicted as photosynthesis-irradiance curves. Here, a given proxy of photosynthesis (e.g. electron transport rate at Photosystem II (ETR), carbon fixation or oxygen production) typically increases linearly at low irradiance ($\alpha$), saturates within an individual optimal irradiance range where photosynthesis reaches its maximum value ($V_{max}$, or $ETR_{max}$), and then declines due to photoinhibition ($\beta$)(Platt & Jassby, 1976). Depending on its long- and short-time acclimation, a single genotype can vary dramatically in its light requirements. In a meta-study of laboratory experiments, Edwards et al. (2015) found, for instance, that independently of the taxon, isolates of coastal phytoplankton were often better adapted to high irradiances and maximizing productivity than open ocean ones. Furthermore, at light levels below the optimum, diatoms and *Phaeocystis* species generally showed a steeper ascent in growth than other taxa and comparably high maximum growth rates above.

Nutrients are often subdivided into macronutrients, of which larger amounts are required by each cell (carbon, nitrate, phosphate, for diatoms also silica) and micronutrients, that are essential only in small concentrations (such as iron, molybdenum, manganese, boron, cobalt and zinc). The impact of nutrient concentrations follows a saturation response curve. All phytoplankton profit from nutrients until they reach saturation, but they differ in their demand and uptake kinetics. Hence, the ability to cope with limiting situations is a crucial competitive characteristic of phytoplankton cells. Although co-limitations can make requirements more complex (Saito et al., 2008), as a simplified rule, the scarcest nutrient is the decisive one (Liebig's law of the minimum, Liebig 1840). Therefore, productivity may be limited by different nutrients in different regions. In the Arctic environment, like in many others, the macronutrients nitrogen and phosphate, that are necessary for basic protein and DNA synthesis, are the limiting factors and typically become depleted in summer after the spring bloom (Tremblay et al. (2015) and Appendix 2a). In low-nutrient conditions, the high nutrient demands of diatoms are a substantial disadvantage, although they can compensate with high luxury consumption and nutrient storage for several division cycles (Katz et al., 2004). If limiting conditions persist, however, they eventually lose their dominance to smaller

species with higher surface-to volume ratios, nutrient uptake affinities or mixotrophic abilities (Bopp et al., 2005; McKie-Krisberg & Sanders, 2014; Schulz et al., 2013)

Interactive effects among the mentioned drivers or an entire scope of others can take place in synergistic or antagonistic ways (Boyd & Hutchins, 2012). As a general pattern, detrimental influences of any stressor are more easily compensated when other drivers are kept at a non-stressful level and resources are not limiting. High $pCO_2$ levels, for instance, have often more positive effects on photosynthetic productivity if optimal temperatures allow efficient metabolic processes (Tatters et al., 2013) and nutrients are replete (Li et al., 2018). Low or stressfully high irradiance often intensifies responses to elevated $pCO_2$ because cells are differently susceptible depending on their energetic status, which is primarily determined by light (Gao et al., 2012; Kranz et al., 2011; Rokitta & Rost, 2012). On the other hand, high irradiance levels and damaging UV-B radiation are more detrimental to cells at colder temperatures, where repair of a light-damaged photosystem takes longer (Gao et al., 2012), and under OA conditions (Hoppe et al., 2015; Kottmeier et al., 2016; Li & Campbell, 2013; McCarthy et al., 2012). The usually stimulating effect of warming is decreased under conditions where other resources are limiting, e.g. shifting temperature optima to much colder conditions when nutrients or light are becoming more scarce (Bestion et al., 2018b; Edwards et al., 2016). The few selected examples illustrate that impacts of one factor can only be understood in the context of others by designing multiple stressor experiments that cover at least a realistic spread of values at which the environmental factor is experienced by cells in nature.

Nevertheless, reviewing the extensive literature on the numerous physiological responses that have been observed in phytoplankton can easily create the impression that the more species, conditions and interactions we study, the harder common patterns become to identify (Boyd et al., 2018; Häder & Gao, 2015). One reason for such ambiguities may be the mentioned interactions of drivers as well as methodological problems. In manipulations of more complex systems, like community and mesocosm experiments, we frequently observe that responses expected from the knowledge of monocultures in the lab can be buffered or amplified in an ecologically more realistic context (e.g. Hoppe et al., 2015; Hoppe et al., 2018; Irwin et al., 2015; Li et al., 2007; Peña et al., 2018; Schulz et al., 2013). As described above, this may be attributed to the higher complexity of interacting stressors or the inclusion of more trophic levels (e.g. Riebesell & Gattuso, 2014; Tatters et al., 2018). An increase in productivity, for instance, may be countervailed by an according increase in grazing (Ghedini et al., 2015). However, for a realistic system-understanding it seems furthermore inevitable to take another type of fundamental biological processes in account: the adaptive potential of organisms and populations.

## 1.4    Ecology and diversity within species: the basis for adaptation

Ecology, as defined by the Oxford dictionary, deals "with the relations of organisms to one another and to their physical surroundings." Research on global change biology is often focused on the influence of the physico-chemical surroundings on organisms' physiology, but investigations on their biological interactions are increasingly recognized. Reaction norms established from a small set of drivers are often not in accordance with experiments that include a larger ecological context, such as mesocosms (Collins et al., 2014). The classical field of ecology has dealt with the interaction of species on a theoretical and applied level for a long time and many concepts established there will be highly relevant to consider when assessing the future of ocean biota. In both disciplines, however, physiology and ecology, the entity considered to be of relevance for the ecosystem is usually the taxonomic species.

When focusing on physiological interactions with the environment, one can easily forget that a species is not one uniform existence, but is comprised of a multitude of diverse and interacting individuals. Therefore, eco-evolutionary interactions take not only place between species but also within them. A phytoplankton community can adjust to changing conditions at three ecological levels: the community, the population and the individual. Abundance shifts among species of a community may be the most visible ones and occur if organisms of one species are fitter than those of another. Such selection-based abundance shifts can, however, also take place on the intraspecific level between genotypes of the same population, and are commonly referred to as strain or lineage sorting. Such selection requires diversity to choose from and implies alterations in the genotypic composition, which is why it is directly linked to adaptive evolution. On a third ecological level, each organism can adjust to its environment by acclimation within its physiological capacities (reflected by its reaction norms sensu *chapter 1.2.1*). Since the reaction norms of individuals can strongly diverge, so may their competitive abilities.

### 1.4.1    Resolving diverse individuals in a sea of clones

The plasticity on the individual level (i.e. clonal strains or cell lineages) can be studied in monoculture experiments, and shifts on the species level can be investigated in mesocosm experiments (Riebesell et al., 2008) and long-term field observations (Nöthig et al., 2015). The role of sorting between lineages on the population level, however, is difficult to assess and has been neglected in the majority of investigations (Bolnick et al., 2011). One reason may be that an individual in clonal populations is not trivial to define, and even more complicated to identify and characterize.

Being multicellular organisms, we are used to cells of the same genotype to cling together and form an individual. In unicellular, asexually reproducing microbes, however, clones of the same genotype may be distributed across large distances and even compete for the same resources. From our human perspective, this raises the question if a single cell is to be defined as an individual or if all clones of the same genotype should be considered as one. The latter is referred to as a strain, and describes a group of cells (usually in culture) of the same genotype. Strains are also the common unit used for physiological investigations. As

long as all cells are derived from one single genotype, this is also called a lineage (Collins et al., 2014). Since mutations do occur, however, after a number of generations a lineage may comprise more than one version of the original genotype. Across longer timescales, a lineage may thus rather resemble a population again (Lakeman et al., 2009). The definition of an individual in phytoplankton is thus not bound to a number of organisms (i.e. cells or clones), but purely to their genetic composition (Figure 1.6).



*Figure 1.6:* Cells of Thalassiosira hyalina under a light microscope. While this picture was taken, they were likely attempting to adjust their physiology to the conditions of a microscopy slide at room temperature and under bright light. As the clones still form a chain, they must have recently originated from the same genotype and could be considered an individual. As soon as the chain breaks, however, we can identify this strain or lineage only by its genetic composition.

One of the challenges in studying the dynamics of populations in single-celled organisms is directly connected to this: the identity of conspecifics is not distinguishable for us except by their genes. The only way to resolve the composition of a population to date is the use of genetic markers, the currently most frequently used ones being amplified fragment length polymorphisms (AFLP) (e.g. John et al., 2004; Kremp et al., 2012) and microsatellites (e.g. Evans & Hayes, 2004; Rynearson & Armbrust, 2000). Because microsatellites resolve genotypes with different allele versions of a locus (heterozygotes) more reliably, they are more useful for diploid organisms. Microsatellites are non-coding DNA regions with tandem repeats of 1-6 basepairs (e.g. trimers such as [...]TCATCATCATCA[...]), which are scattered randomly throughout the genome of all organisms. Their causes are not fully understood, but may lie in an elevated error rate of the DNA-polymerase during genomic replication and proofreading (slippage), because the enzyme cannot easily recognize mistakes in a sequence that consists of the same bases repeated for dozens of times (Selkoe & Toonen, 2006). Therefore, microsatellites are considered to be hotspots of mutation and highly polymorphic, even in closely related organisms. After having identified the location of such a sequence in a given species' genome, the more conserved flanking sequence regions can be used to design primers, which can then be used to detect the allele (or more simply only its length) of this locus in any organism of that species by the use of PCR. By combining several of these loci, multi-locus genotypes (MLGs) can be identified, each of which exhibits a unique combination of alleles (Alpermann et al., 2010).

While this is an elegant method to discern genotypes from a seemingly homogenous population, it has two technical drawbacks: Since the repeat sequences can be found anywhere in the genome, they have to be identified and established anew for each species and sometimes even subpopulation, a highly cost- and time-consuming process with realistic chances of failure (Leese & Held, 2011). In phytoplankton, furthermore, the sampling of individuals is even more intricate than in multicellular organisms because extracting DNA from a single genotype in sufficient quantities requires a first step of single-cell isolation (which usually needs to be repeated in order to prevent contamination by other genotypes), followed by the establishment of a single-clone culture, which can then produce sufficient biomass needed for the analysis (Medlin et al., 2000). Because of this tedious process, studies rely on sample sizes of a few dozen to a few hundred cells, which is an extremely small fraction of the actual population size of thousands to millions of cells within a single drop of water. Considering these shortcomings and a mere 20 years of investigation, it is not surprising that our knowledge on intraspecific variation in general and evolutionary processes, such as lineage sorting in particular, is still very limited in microeukaryotes (Rengefors et al., 2017).

### 1.4.2 Intraspecific diversity and population structure

The realization that phytoplankton populations contain a large diversity is not new. It was described phenotypically as early as 1962 (Guillard & Ryther) and on a genome-based level using allozymes by Gallagher (1980). Investigations from many regions and species, often even isolated from the same water sample, have revealed a large phenotypic variability between isolated strains in many ecologically and biogeochemically relevant traits, like growth, biogenic silicate or Chl *a* quota (Brand, 1989; Gallagher, 1982; Krawiec, 1982; Nelson et al., 1976; Wood & Leatham, 1992). In hindsight, it is surprising that this knowledge seems to have disappeared from common opinion until it was virtually rediscovered two decades later with the appearance of molecular fingerprinting methods (Gsell et al., 2012; Kremp et al., 2012; Langer et al., 2009; Pančić et al., 2015; Rynearson & Armbrust, 2004; Saravanan & Godhe, 2010).

The underlying reasons for the existence of this enormous intraspecific diversity are not easy to identify. While the coexistence of various species in an unstructured environment is long known as the 'paradox of the plankton' (Hutchinson, 1961), intraspecific diversity is today sometimes termed the 'second paradox of the plankton' (Hebert & Crease, 1980). Since this second paradox suggests species to have a distribution of trait values among their genotypes rather than rigidly fixed characteristics, it may indeed be part of the explanation of the first paradox (Menden-Deuer & Rowlett, 2014). Still, it only shifts the question to another ecological level. The founder of the paradox himself (Hutchinson, 1961; Record et al., 2014) realized that although the marine environment is unstructured, it is certainly not uniform (because highly fluctuating) and may thus allow for both inter- and intraspecific diversity through high temporal and spatial variability.

In phytoplankton, the use of microsatellites, which allowed more advanced investigations on genetic population structure, has only been established in the early 2000s (Evans & Hayes, 2004; Iglesias-Rodríguez et al., 2002; Rynearson & Armbrust, 2000). Since then, all marine studies report vast levels of intraspecific genotypic diversity (Godhe & Rynearson, 2017), meaning that the same genotype is only rarely found twice within the investigated water sample. Even in entirely enclosed lake systems, such high levels of diversity are found (Vanormelingen et al., 2015). If close to 100% of the sampled genotypes differ, this conceals population dynamics and selection patterns. Therefore, the identification of genotypes in phytoplankton can in many cases merely illustrate an enormous diversity. However, traditional population genetics contain a suite of techniques that can be used for inferences about populations based on the pooled allele frequencies measured. Accordance to ecological concepts (like the Hardy-Weinberg-equilibrium) and genetic linkage between alleles can be tested based on allele distributions and may yield indirect inferences about the population structure (such as recombination dynamics). Conventional F-statistics are usually applied to analyze and cluster allele patterns into more or less distinct populations (Balloux & Lugon-Moulin, 2002) although the relativity of such measures of relatedness should be kept in mind. The most common measure is the fixation index ($F_{ST}$), which correlates two random alleles within a subpopulation relative to alleles sampled at random from the total population (Wright, 1965). Most of the theories behind such tools were originally established for predominantly sexually reproducing multicellular organisms. Thus, our lack of basic knowledge concerning frequency of sexual recombination as well as mutation and dispersal rates in investigated taxa (Rengefors et al., 2017) challenges the inferences we can make.

For a long time, it has been assumed that marine microbes should have very weak population structure because of their enormous diversity, fast generation time, rapid mutation rates and ubiquitous dispersal with currents (Finlay, 2002). In the past 15 years, however, an increasing number of records have revealed distinct structures within phytoplankton populations (Medlin, 2007); not everywhere (Casteleyn et al., 2009; Evans et al., 2005; Rynearson & Armbrust, 2005) but in many, even clearly intermixed oceanographic regions (Nagai et al., 2007; Rynearson & Armbrust, 2004).

Various mechanisms for the differentiation of phytoplankton populations have been suggested in a range of studies: The most traditional one is isolation by distance. It describes a decrease in relatedness with physical distance (Casteleyn et al., 2010; Nagai et al., 2007) or along oceanographic currents or gradients (Godhe et al., 2013; Sjöqvist et al., 2015). A few studies have sampled population composition at several points in time (e.g. throughout a bloom) and are proposing a similar concept on a temporal scale ('isolation by time'; Tammilehto et al., 2017). In such contexts, succession of several, clearly distinct subpopulations have been found, although patterns are clearly influenced by non-linear events such as mixing (Erdner et al., 2011; Ruggiero et al., 2017; Rynearson et al., 2006). Many records, however, explain the observed differentiation by diverging environmental conditions, e.g. salinity gradients or coastal proximity (Härnström et al., 2011; Sefbom et al., 2018; Sjöqvist & Kremp, 2016). Indeed, it has been shown experimentally that local populations can phenotypically differ from each other and outcompete foreign lineages

(Kremp et al., 2012; Rynearson & Armbrust, 2004; Schaum et al., 2012; Sildever et al., 2016; Zhang et al., 2014). Godhe et al. (2016) pointed out, that both, the spatial as well as the temporal population genetic patterns throughout an intensely studied bloom along a transect were confounded by salinity gradients and fluctuations in nutrient limitations. Such an 'isolation by conditions' (driven by selection rather than drift) may remain concealed if the relevant environmental parameters (e.g. nutrient concentrations) are not recorded. This may be valid even on a global scale: Distinct gene pools have been described in studies comparing European and North American waters in two species (Casteleyn et al., 2010; Evans et al., 2004; Iglesias-Rodriguez et al., 2006). However, a global meta-study found relatedness of populations to be generally more dependent on regional conditions than on geographical distance (Whittaker & Rynearson, 2017). The same pattern has also been observed in the fossil record for diatom species assemblages (Cermeño & Falkowski, 2009).

Such findings support the longstanding paradigm that 'everything is everywhere but environment selects' (Baas-Becking, 1934), although this concept of 'universal distribution and local selection' (Iglesias-Rodríguez et al., 2002) has often been challenged by biogeographical patterns (e.g. Kim et al., 2004; Medlin, 2007). This debate illustrates that the basis of genetic distribution and diversity is still unclear and likely there is some truth to both views. Since diversity seems so large even within populations, both distribution theories comprise the possibility that local population structure is driven by temporary strain dominances, which are selected from an extensive and dynamic diversity pool. Indeed, this is indicated by the observation of coexistence or succession of different subpopulations as environmental conditions diverge in the same place (Chen & Rynearson, 2016; Erdner et al., 2011; Godhe et al., 2016; Rynearson & Armbrust, 2005).

Most probably, the mechanisms that govern population structure and dynamics are neither simple nor universal, but strongly dependent on environmental settings and species-specific characteristics. For species with resting stages (such as many diatoms and dinoflagellates), for instance, it has been found that distinct populations can persist for decades or centuries (Härnström et al., 2011). 'Seed banks' in the sediment can reinforce local adaptation and thus differentiation between populations in the long term (Sundqvist et al., 2018). At the same time, germinating resting stages can effectively sustain or add genotypic diversity on the short term to an exponentially growing population during a bloom (Alpermann, 2009; Lebret et al., 2012). The different mechanisms of diversification within a bloom can be difficult to disentangle within the current methodological scope. This is because increases in genotypic diversity (i.e. richness) can likewise be caused by mutation, migration and recombination (i.e. sex, see *chapter 1.2.2*) – three processes whose frequency and rates are largely unknown (Collins et al., 2014).

## 1.5 Eco-evolutionary dynamics within species

Across timescales of generations, the characteristics of biological systems and of the organisms within are never fixed, but are constantly shaped by the influence of their environment. Although in some contexts stronger than in others, organisms are subject to constant selection and therefore evolution, allowing them to be best suited to the requirements of their surroundings. Evolutionary change can be defined as a change in genotype frequencies within a population between generations (Collins et al., 2014). In phytoplankton, generation times are often very short and evolution is therefore faster than in multicellular organisms with sexual reproduction. Regardless whether diverse genotypes originate from *de novo* mutation, recombination, migration, seed banks or from a present standing stock of genetic variants in the water column, the acting mechanism behind a shift between their frequencies is always selection. Although selection is often referred to as a process taking place *between* species, it is probably even more relevant *within* them, since it acts among individuals or phenotypes independently of their taxonomic affiliation (Rynearson & Menden-Deuer, 2016). As a rule of thumb, the combination of mutation rate and genotypic diversity is thought to set the adaptive potential of a species. While our knowledge on mutation rates of phytoplankton is insufficient, we do know that phytoplankton genotypic diversity is extremely high (*chapter 1.4.1*) and that selection among this standing stock (lineage sorting) is usually faster than mutations (Barrett & Schluter, 2008). On the timescales of classical incubation experiments, lineage sorting is therefore likely to be the more relevant process, but it is difficult to detect within the high diversity context it naturally occurs in.

Fitness is the ultimate parameter that selection acts upon and is generally described as the average reproductive success of a genotype (Elena & Lenski, 2003). In mostly asexual phytoplankton populations and simplified environments (as in the laboratory), growth rate or competitive success as measured in cell abundance can serve as good proxies of inclusive fitness, because they integrate most other influential parameters, such as nutrient uptake or mortality (Wood et al., 2005). In more complex or natural systems, however, fitness may diverge strongly from intrinsic growth rates, since factors like life cycle and grazer defense strategies, stress responses and sex can play a decisive role (Schaum & Collins, 2014).

Much of the theoretical framework in ecology to understand evolution and selection was developed to describe the interactions of different species. To which extent such considerations may also apply for the intraspecific level is not yet well understood. One debated issue is the influence of phenotypic plasticity on evolutionary adaptation (Ghalambor et al., 2007). On the one hand, the ability to cope with a wide range of conditions may impede adaptive evolution of the population because it shields genotypes from natural selection. As long as plasticity does not come at a high cost, it could prevent or at least delay shifts in the population's genotypic composition and thus in the average reaction norm. On the other hand, plasticity is argued to favor evolution by keeping population sizes large enough for adaptive selection to work effectively on the existing phenotypes (Chevin et al., 2010). The Baldwin effect (Baldwin, 1896) describes that during a sudden change in the environment, plasticity can produce partly adaptive phenotypes, which are then genetically fixed and can be further improved by mutation and selection over time. The latter view has recently been supported

by evolution experiments (Schaum & Collins, 2014) but is still strongly debated (Fox et al., 2019). Magnitude, time-scale and predictability of environmental fluctuations are thought to determine the optimal amount of plasticity for evolutionary adaptation (Lande, 2009; Schaum et al., 2015). Epigenetics are also likely to play a significant role at the interface of plasticity and evolution. This recently discovered mechanism describes reversible modifications, such as DNA-methylation, that are potentially transferrable to the offspring and may therefore enable organisms to transfer plastic adjustments to following generations. Extensive methylation patterns have been found throughout diatom genomes, but investigations on this aspect are still in their infancy (Schmidt et al., 2016; Veluchamy et al., 2013).

The question, to which degree selection allows only the very fittest to survive opposed to allowing coexistence has a long history of controversy as well. The before mentioned 'paradox of the plankton' is based on the classical notion of competitive exclusion and states that a number of limiting resources allows only the same number of niches and therefore species, because in each niche there must be a winner (Hutchinson, 1961; Levin, 1970; Record et al., 2014). In contrast, neutral theory argues that arbitrary processes and dispersal play a much larger role than niche speciation (Bell, 2001; Hubbell, 2001) and explains a multitude of coexisting species with random dispersal (i.e. ecological drift). None of these two extremes can fully explain the observed dynamics, as a number of examples reveal, and variability as well as co-limitation of several resources has been shown to reconcile the two theories to a degree (Burson et al., 2018; Keymer et al., 2012). Nevertheless, how to explain the equilibrium coexistence of organisms in some systems and not in others is far from being resolved. Still, it is a fairly accepted rule that fluctuating conditions (such as strongly varying oceanic irradiance fields or small-scale nutrient variability) select for both, more plastic and more diverse individuals (Bell, 2010; Schaum & Collins, 2014).

In natural or other complex systems, it is often difficult to discern the different mechanisms that can cause an adaptive response. Climate change, for instance, can act as an evolutionary driver on phytoplankton through direct effects of the physico-chemical environment on physiology, but also through indirect effects of biological interactions, like predation pressure or competition (Collins et al., 2014). The impact of such indirect effects is much more difficult to simulate and assess, because in contrast to the physico-chemical surroundings, they cannot be tested in monoculture. Furthermore, there is a baffling suite of biological interactions to be tested, while our knowledge on their specific characteristics left alone future development is similarly limited for most species. One theoretical framework for discerning the individual from the population response has been proposed in form of an equation by Price (1972). His idea aims to partition the change in a trait of a population into the effect of selection of its components (i.e. the traits of individuals with more offspring driving the population traits) and the effect of transmission (i.e. the physiological trait difference between parents and their offspring). Theoretically, in clonal microorganisms within a stable environment, this transmission effect should equal zero unless mutations occur (Gardner, 2008). The concept of this equation was applied to assess the effects of biodiversity on ecosystem function by Loreau and Hector (2001). Here, the effect of diversity on the productivity of an ecosystem is separated into those of selection and those of

complementarity, such as nutrient facilitation or other species interactions. A further extension to Price's idea, that includes the consideration of adaptation on the three levels elaborated in the beginning of this chapter (individual, population and community, i.e. species composition) was proposed by Collins and Gardner (2009). They suggest separating responses to change into three mechanisms: physiological (i.e. acclimation), evolutionary (i.e. lineage sorting) and ecological change (i.e. species shift). While this is a much more inclusive approach than currently adopted, obtaining the necessary parameters for such a calculation in a realistic way remains a major challenge.
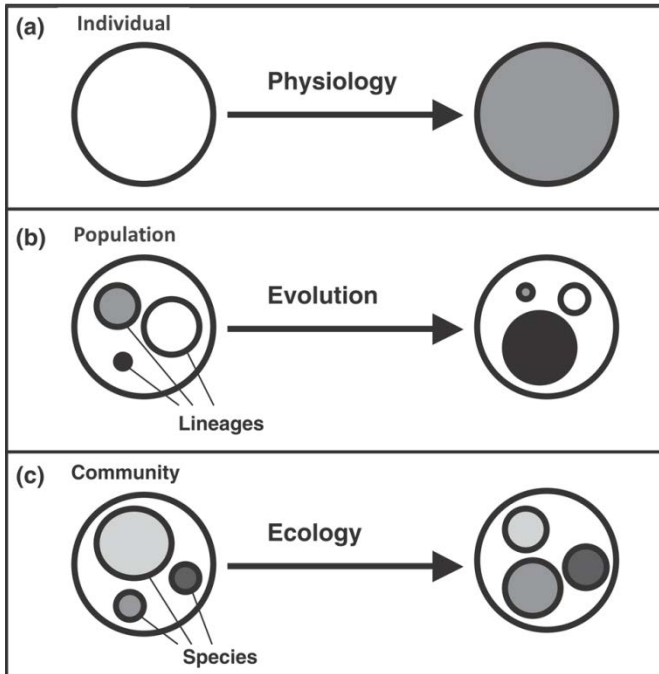


*Figure 1.7: Change in a trait of interest can take place by physiological, evolutionary or ecological reorganization. a) Each lineage or individual can adjust its physiology by acclimation within its plastic range. b) Each population or species can adapt by selection among lineages with different trait values. c) The entire community can adapt by selection among species with different characteristics. Modified after Collins and Gardner (2009).*

The existence of selection in form of lineage sorting in highly diverse populations like phytoplankton seems obvious, but it is still problematic to resolve (*chapter 1.4.2*). Therefore, the majority of investigations rely on inferences about selection and mutation from artificial evolution experiments or from observed changes in subsamples of genotypic or allelic diversity. In experimental evolution research, replicate populations are typically exposed to defined environmental scenarios for a number of generations. Subsequently, the change in fitness or specific traits of interest is assessed in comparison to control populations. Such studies have shown that populations originating from a single genotype can adapt to changed conditions beyond acclimation within 100-1000 generations (Hutchins et al., 2015; Lohbeck et al., 2014; Schaum et al., 2018; Tong et al., 2018), and that the resulting reaction norms are measurably shifted (Listmann et al., 2016). Such a long-term adaptation can only be caused by mutation and subsequent selection or by epigenetics. However, winners from the lab seem to fail to dominate in a more complex environment (Bach et al., 2018), and adaptive evolution is often only observed after an initial drop in fitness (Collins et al., 2014). In a community context, Tatters et al. (2013) showed that a selection period of two weeks under warming and

high pCO$_2$ was sufficient to select for isolates that did not further increase their fitness after another year of exposure as monoculture. While both, mutation and standing stock selection seem to be capable of facilitating adaptation in phytoplankton, this shows how much more efficient lineage sorting can be and how much influence the initial population diversity may have.

Reorganization on each of the ecological levels mentioned in *chapter 1.4* (community, population and individual) can stabilize a community and may therefore be understood as buffers against impacts on the scale of the ecosystem. Because they typically act at different timescales, a failure in the buffering capacity on one level may lead to an alteration on the next: Acclimation is by definition a rather rapid process (i.e. hours to days, Kremer et al. (2018)). Although such a plastic response may imply significant physiological reorganization, it does not include a change on the genetic level and is therefore usually reversible in unicellular organism ('phenotypic buffering'; Botero et al., 2014; Reusch & Boyd, 2013). If conditions change beyond the plastic scope of individuals, a dominance shift among genotypes (lineage sorting) may occur within species, which may adjust the average population reaction norm enough to maintain the original performance. If the standing stock of intraspecific genotypic diversity is large enough, this can happen within a few generations, while the rise and fixation of new mutations can take substantially longer (hundreds of generations). If the degree of intraspecific diversity is not sufficient to buffer against the environmental alterations, a species shift can occur, which may alter or buffer ecosystem characteristics depending on the degree of functional redundancy between the dominant species (Hoppe et al., 2017a). One assumption is valid for all three levels: even if the investigated ecosystem function can be sustained, a larger difference between the former and the new dominant organisms make concurrent alterations of other traits more likely, with respective implications for other trophic levels and biogeochemical cycling.

## 1.6    Model organism and study site

To investigate the importance of physiology, diversity and evolutionary dynamics on an intraspecific level in relation to climate change, I required an organism and a site that I could focus on as a model system. The organism needed to be a phytoplankton species of ecological relevance, which could be studied in its natural environment and should also be compliant with laboratory conditions. I therefore chose *Thalassiosira hyalina*, a widely distributed pelagic centric diatom species frequently dominant in Arctic and Sub-Arctic spring blooms of northern Norway, Svalbard and Greenland (von Quillfeldt, 2000). It is known to form resting spores, which can persist over several years in the sediment. *T. hyalina* is often found in waters at the ice-edge and is the only *Thalassiosira* species with high abundances north of 75° (von Quillfeldt, 2001). Although *T. hyalina* has never been studied in the laboratory before, it has proven to be a very convenient model organism. Cells vary in size from 16 to 45 μm, grow fast and often form chains (Figure 1.6) that are easy to isolate in the field and widely enough spaced for automated counting.

The perfect study site was to be found in a region sensitive to and already affected by the ongoing environmental transitions, such as the Arctic. Furthermore, I required it to be sufficiently accessible and logistically equipped for on-site experiments as well as regular field sampling routines. Environmental meta-data on such a site provides valuable knowledge about the organisms' environmental history as well as influences and variability throughout the study period. The Kongsfjord, where this project was based, is a well-studied coastal Arctic glacial fjord system in Svalbard with extensive time-series data since several decades available and well-equipped laboratories close by (Bischof et al., in press; Hop et al., 2002). Situated on the west coast of Spitsbergen (79°00 N 11°39 E, Figure 1.8), the fjord is influenced by two currents that pass by its opening: the West Spitsbergen Current (WSC), which carries warmer, more saline Atlantic water, and the East Spitzbergen Current (ESC), which brings Arctic-type coastal water (Svendsen et al., 2002b). It has already experienced many of the changes projected for the higher Arctic in the coming decades. Especially later in the season and in recent years, Atlantic water is more dominantly advected into the fjord in irregular events and mixes with the colder water masses influenced by the glaciers around (Cottier et al., 2007; Tverberg & Nøst, 2009). The intrusion of such waters is accompanied by North Atlantic species, which is known as 'Atlantification' in this region, and intensifies as conditions in the Arctic become increasingly similar to the ones such migrants are used to (Hegseth & Sundfjord, 2008; Rat'kova & Wassmann, 2002). In terms of oceanography as well phytoplankton dynamics, the Kongsfjord is a seasonally and interannually highly variable system (Cottier et al., 2005; Hodal et al., 2012; Hop et al., 2002; Leu et al., 2006). One of the most dramatic transformation has probably occurred in the past two decades, since the Atlantic influence has increased and there are no reports of closed winter sea ice cover since the early 2000s (Cottier et al., 2007; Hegseth & Tverberg, 2013; Svendsen et al., 2002a).
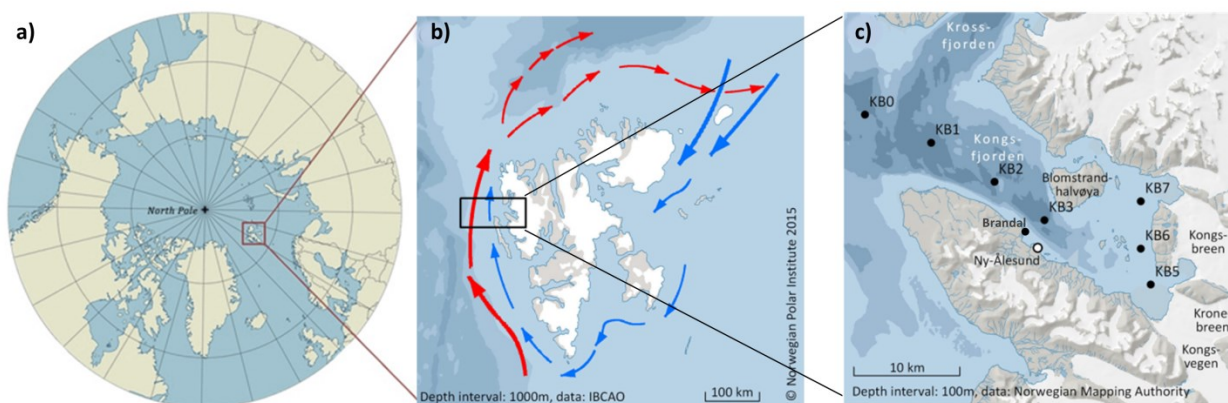


***Figure 1.8****: Map of Svalbard and the Kongsfjord showing its position on the globe (a), surrounding ocean currents (b) and sampling stations (c). Red arrows depict the Atlantic West Spitsbergen current, blue errors the Arctic East Spitsbergen current. Samples of this study were taken at stations KB2, 3 and 5, sensor data and field species composition originated from a mooring at 'Brandal'. Modified after Fransson et al. (2016) and (Nandan et al., 2016)*

## 1.7      Aims of this thesis

In spite of extensive research on phytoplankton responses to climate change and much progress made, a coherent picture with general mechanisms that would allow better models and more accurate projections into the future still seems out of reach. While investigations aiming at process-understanding are usually bound to use single-strain cultures of representative organisms, my objective was to find out how well such knowledge is transferrable to the population level and in turn, whether it can explain what we observe within an increasingly realistic ecological framework of natural communities. Temperature and $pCO_2$ manipulations were chosen as drivers, because they are already changing drastically and projections on their future development are relatively well defined. The underlying ecological mechanisms should be applicable to other components of environmental change. I applied a stepwise approach that considers several ecological levels in order to identify those where important acclimation or adaptation can take place: from a natural species community to the individual, to a simplified population and all the way back up to the fully diverse population.

In a first step (*Publication I*), we exposed natural Arctic phytoplankton communities to temperature and $pCO_2$ treatments in microcosms in order to investigate how Arctic phytoplankton respond to anticipated change within their natural diversity context. Next (*Publication II*), I incubated monocultures of single isolates from these experiments to investigate, how much of the observed responses could theoretically be attributable to the plasticity of individuals (i.e. acclimation) or that of the population (i.e. selection on standing stock). In *Publication II*, I also took a closer look at the mechanisms of intraspecific interactions and selection in artificial populations in order to test whether we understand it well enough to base projections on it. Finally (*Publication III*), I wanted to know whether the results from those experiments with simplified diversity would translate back to the population in our original community experiment and which role intraspecific sorting may play in natural diatom populations

### 1.7.1 How do Arctic phytoplankton communities respond to ocean acidification?

Although the Arctic is one of the most rapidly changing regions in the world, there is very little data on what the ongoing and future alterations of this extreme habitat imply for phytoplankton communities and their productivity. *Publication I* presents a compilation of ten incubation experiments of phytoplankton communities from three Arctic and subarctic regions that were exposed to elevated $pCO_2$ concentrations under a range of temperature and light conditions. The response of the incubated communities was evaluated based on net primary productivity (NPP) and species composition. The observed effects of ocean acidification are discussed with regard to the interactive influence of temperature, as well as by applying conceptual frameworks on plasticity and diversity of individuals and populations. Some of the incubations lay the base for the following studies of this thesis and represent the initial selection environments of the isolated strains used in and *Publication II* (insolated in 2016 from experiment KFb).

### 1.7.2 Can diversity increase resilience? The phenotypic view

While it has been shown before that individuals of the same species can differ in their phenotype, the implications of this fact depend crucially on the extent of variability between the present strains. In a former study (Wolf et al., 2018), I have shown that genotypes of *Thalassiosira hyalina* from the Kongsfjord in 2014 (KFa in *Publication I*) differed substantially in a range of cell properties and their optima within reaction norms towards $pCO_2$ under different temperature conditions. In *Publication II*, a larger number of strains from experiment KFb in *Publication I*, which had been initiated with a community of the same location two years later, were screened for their physiological characteristics in response to elevated temperature, $pCO_2$ and the combination of both drivers. The question to be answered was whether the differences between individuals may be large enough to make intraspecific sorting a potential mechanism for rapid adaptation on the population level and whether I would find indications of such a selection within the community incubations.

### 1.7.3 Does selection play by our rules?

The physiological properties we assume for a species under a set of conditions are often extrapolated from responses observed in a single or at best in a few strains of this taxon. If the standing stock of phenotypes is taken into account for this, projections are based on the assumption that selection will favor the best adapted strain to the current conditions. Assuming this strain will become dominant, the population is hence expected to adopt or approximate its traits. In *Publication II*, I wanted to test the validity of this assumption. To do so, I first characterized the response of six strains of *T. hyalina* in monoculture, which had been isolated from different treatments of the community experiment KFb. Subsequently, I incubated those strains as a mix in multi-strain cultures under the same conditions, creating a simplified artificial population, and followed their properties and composition over time. This was accomplished by the development of an innovative extension of a method using microsatellite markers that makes it possible to easily resolve the genotypic composition within a mix of known strains. By comparing the expected with the measured traits of the artificial population, I could quantify how much single-strain-based predictions differed from observations, while the temporal resolution of the population composition gave hints towards underlying reasons.

### 1.7.4 Does resilience change diversity? The genotypic view

Having studied intraspecific selection in a simplified but more mechanistic way, the next step was to find out how the observations would translate to more realistic or even natural populations (*Publication III*). At the end of the community incubation KFb 2016, I have therefore isolated several hundred individual *T. hyalina* strains, which were used with the previously established microsatellites to search for hints of intraspecific selection within the entire population under different treatments. Despite substantial efforts being associated with this approach, a few hundred sampled individuals are still an unreasonably small representation of the entire population and thus may be subject to subsampling bias. I

therefore developed a new microsatellite-based method, microsatellite poolSeq barcoding (MPB), which allowed me to depict the entire allelic range of genotypes within our incubated populations. Subsequently, I applied this new tool also to resolve the intraspecific development of the natural spring bloom of the same and the following year. Since the new method is incomparably less time-consuming than the traditional one, I could follow the dynamics of the natural system at unprecedented allelic and temporal resolution. The intention was to reveal how intraspecific selection may function at different levels of genotypic diversity and environmental complexity.

### 1.7.5    Towards a more integrative view on phytoplankton responses

In a final synthesis (*chapter 5*), I summarize the major findings of the presented publications and set them into the context of the current research. The results provide new insights into the potential stability in and adaptive mechanisms of Arctic phytoplankton communities and the role of interactions among individual cell lineages. I also discuss the implications for predictions of phytoplankton responses from simplified experimental setups into ecological contexts. Finally, I argue for the integration of laboratory and field studies, and how such integrative approaches may help to incorporate the role of complex interactions and adaptation into large-scale projections of phytoplankton ecology.

## 1.7.6 List of Publications and declaration of own contribution

**Publication I**  C. J. M. Hoppe, K. K. E. Wolf, N. Schuback, P. D. Tortell, and B. Rost (2018): Compensation of ocean acidification effects in Arctic phytoplankton assemblages. Nature Climate Change 8: 529–533.

*Six of ten experimental datasets were conducted by CJMH and myself. The data was analyzed and the manuscript was written by CJMH, and revised with the help of myself and the other co-authors.*

**Publication II**  K. K. E. Wolf, E. Romanelli, B. Rost, U. John, S. Collins, H. Weigand and C. J. M. Hoppe: Company matters: The presence of other genotypes alters traits and intraspecific selection in an Arctic diatom under climate change. Under revision in Global Change Biology.

*The experiments were planned together with the co-authors. The experiments were conducted and their results analyzed by myself with the help of ER and CJMH. The manuscript was written by myself, and revised with the help of the co-authors.*

**Publication III**  K. K. E. Wolf, C. J. M. Hoppe, F. Leese, B. Rost, S. Neuhaus, N. Kühne, and U. John: Revealing population dynamics of the Arctic diatom *Thalassiosira hyalina* using a novel microsatellite poolseq barcoding (MPB) approach. To be submitted in Evolutionary Applications.

*The experiments were planned together with the co-authors. The experiments were conducted by myself with the help of CJMH. Sample analyses were conducted by me and NK, SN, and UJ. The manuscripts was written by myself, and revised with the help of the co-authors.*

# Chapter 2   Publication I

**Compensation of ocean acidification effects in Arctic phytoplankton assemblages**

# Compensation of ocean acidification effects in Arctic phytoplankton assemblages

Clara Jule Marie Hoppe [1,2*], Klara K. E. Wolf[1], Nina Schuback[2,3], Philippe D. Tortell[2,4,5] and Björn Rost[1]

The Arctic and subarctic shelf seas, which sustain large fisheries and contribute to global biogeochemical cycling, are particularly sensitive to ongoing ocean acidification (that is, decreasing seawater pH due to anthropogenic $CO_2$ emissions). Yet, little information is available on the effects of ocean acidification on natural phytoplankton assemblages, which are the main primary producers in high-latitude waters. Here we show that coastal Arctic and subarctic primary production is largely insensitive to ocean acidification over a large range of light and temperature levels in different experimental designs. Out of ten $CO_2$-manipulation treatments, significant ocean acidification effects on primary productivity were observed only once (at temperatures below 2 °C), and shifts in the species composition occurred only three times (without correlation to specific experimental conditions). These results imply a high capacity to compensate for environmental variability, which can be understood in light of the environmental history, tolerance ranges and intraspecific diversity of the dominant phytoplankton species.

Global change will affect marine ecosystems in many ways, inevitably altering the complex balance of biogeochemical cycles and climate feedback mechanisms[1]. As phytoplankton provide the carbon and energy for higher trophic levels in marine food webs, changes in phytoplankton productivity and species composition will affect entire ecosystems[2,3]. The assessment of phytoplankton responses to environmental change is thus of high scientific importance. To date, the scientific community has tended to focus on significant ocean acidification (OA) effects on primary productivity, with less attention given to those cases where responses are subtle or absent. Only recently has there been increased interest in understanding the mechanisms that buffer and thus prevent physiological and ecosystem-level change[4,5].

The effects of climate change are particularly pronounced in the subarctic and Arctic oceans, with Arctic temperatures rising at more than twice the global average rate[6]. The concurrent rapid reduction in ice cover allows for more light penetration and longer growing seasons, which can change the dynamics of phytoplankton primary production[7,8]. Furthermore, the increase in dissolved $CO_2$ and the concurrent decrease in seawater pH is most pronounced in the low-temperature and low-alkalinity waters of the Arctic Ocean[9,10]. Processes such as river and glacial runoff, ice melt and photosynthetic $CO_2$ uptake cause high variability of Arctic coastal pH (up to 0.8 units), which is expected to intensify in the future[9,11,12]. Hence, models predict a strong decline in average Arctic seawater pH by 0.3 to 0.5 units from pre-industrial levels until the end of the century[13,14], together with higher seasonal pH fluctuations particularly in coastal waters[9,11].

The effects of OA on primary producers have been studied extensively over the past decade, with results showing both positive and negative OA effects[15,16]. While increased $CO_2$ concentrations may facilitate inorganic carbon acquisition of phytoplankton and thus enhance primary productivity[3], changes in seawater pH are thought to have predominantly negative effects[17,18]. The interactive effects of OA and other environmental drivers have gained much attention, with varying responses observed across different investigated species and ecosystems[19–21]. Compared to temperate and tropical regions, OA responses of Arctic Ocean phytoplankton have been significantly understudied[9], due in part to harsh environmental conditions and logistical constraints. The few existing studies in Arctic and subarctic waters indicate moderate stimulation of phytoplankton growth under realistic OA scenarios[11,22–25]. However, these observations have thus far been limited to the nutrient-limited, low-productivity summer months, and it is unclear how phytoplankton biomass accumulation in nutrient-rich and highly productive waters may be influenced by changes in the partial pressure of $CO_2$ ($p_{CO_2}$).

## No OA response in Arctic primary productivity

Here, we present a comprehensive data set of ten individual 1 to 3-week-long OA manipulations (Table 1) with Arctic and subarctic phytoplankton assemblages under non-limiting nutrient concentrations. We used phytoplankton assemblages from three different coastal locations (Davis Strait, Baffin Bay and Svalbard fjords), representing areas of high primary production that sustain economically important Arctic and subarctic ecosystems[9]. Despite large differences between the applied experimental set-ups (for example, variable temperature and light in on-deck incubations versus constant temperature and 24 h light in the laboratory; Table 1), we found strong evidence that Arctic primary productivity and phytoplankton community structure are largely non-responsive to OA. As shown in Fig. 1, phytoplankton assemblages responded to elevated $p_{CO_2}$ levels in only one out of ten manipulations. Overall, no significant correlation of 24 h net primary production (NPP) with $p_{CO_2}$ was observed when rates were normalized for differences in chlorophyll $a$ (Fig. 1a,b and Supplementary Table 5; four-way analysis of variance; $n = 58$) or particulate organic carbon concentrations (data not shown). These results suggest that coastal Arctic and subarctic phytoplankton assemblages employ efficient mechanisms to compensate for the effects of differences in $CO_2$ availability and proton levels on NPP. This response contrasts strongly with

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**Table 1 | Overview of the experimental conditions in ten individual OA manipulations in terms of incubation type, irradiance and temperature regimes as well as the respective responses in NPP and species composition**

| Incubation | Year | Type | Temperature | Irradiance regime | NPP change | Species shift | Dominant taxa and shifts |
|---|---|---|---|---|---|---|---|
| DS_1 | 2015 | On-deck | 8 °C | Low and variable | No | Yes | *Fragilariopsis* to *Pseudo-nitzschia* under OA |
| DS_2 | 2015 | On-deck | 8 °C | High and variable | No | Yes | *Fragilariopsis* to *Pseudo-nitzschia* under OA |
| BB_1 | 2015 | On-deck | 7 °C | Low and variable | No | No | *Chaetoceros* |
| BB_2 | 2015 | On-deck | 7 °C | High and variable | No | No | *Chaetoceros* |
| KFa_1 | 2014 | Laboratory | 3 °C | Low and 24 h constant | No | No | *Thalassiosira, Chaetoceros, Micromonas* |
| KFa_2 | 2014 | Laboratory | 3 °C | Low and 24 h constant | No | No | *Thalassiosira, Chaetoceros, Micromonas* |
| KFa_3 | 2014 | Laboratory | 6 °C | Low and 24 h constant | No | No | *Thalassiosira, Chaetoceros, Micromonas* |
| KFa_4 | 2014 | Laboratory | 6 °C | Low and 24 h constant | No | No | *Thalassiosira, Chaetoceros, Micromonas* |
| KFb_1 | 2016 | Laboratory | 2 °C | Low and 24 h constant | Yes | Minor | *Navicula, Nitzschia, Thalassiosira* (*T.* decreasing under OA) |
| KFb_2 | 2016 | Laboratory | 7 °C | Low and 24 h constant | No | No | *Navicula, Nitzschia, Thalassiosira* |

The incubation names indicate the sampling location (DS: Davis Strait, BB: Baffin Bay, KF: Kongsfjord, Svalbard). More details on the experimental conditions can be found in Supplementary Tables 1 and 2.

observations from the high-latitude Southern Ocean, where similar experiments with comparable sample sizes have indicated a potential for OA-dependent stimulation of NPP[26,27]. In these previous experiments, the largest $p_{CO_2}$-dependent increase in NPP was found between 180 and 380 µatm $p_{CO_2}$. In our case, however, no stimulation of NPP was observed even when comparing rates obtained under 970 µatm to control levels as low as 240 µatm $p_{CO_2}$ (DS_2, Fig. 1 and Supplementary Fig. 1b).

Our ten manipulation treatments varied significantly in terms of both temperature and irradiance (Supplementary Table 1). While temperature had the potential to modulate OA responses (see the section below), irradiance levels did not significantly alter OA responses in any of our experiments (DS_1 versus DS_2, BB_1 versus BB_2, KFa_1 versus KFa_2; Fig. 1 and Supplementary Tables 1, 3 and 5). This result stands in contrast to previous observations from temperate regions, which have shown antagonistic and synergistic interactions between $p_{CO_2}$ and irradiances under high and limiting light, respectively[28–30]. The absence of measurable light effects raises further questions about the common assumption that enhanced irradiances associated with sea-ice decline could increase coastal Arctic primary productivity[8,31]. In conclusion, our observations from different regions and various environmental settings suggest that coastal Arctic phytoplankton assemblages are able to compensate for OA and change in irradiance regimes across a wide range of conditions (Table 1 and Supplementary Table 1).

**Potential temperature modulation of OA responses**

Phytoplankton assemblages significantly responded to OA in only one of our manipulations (KFb_1, Fig. 1 and Supplementary Fig. 1I), showing a decrease in NPP with increasing $p_{CO_2}$ (two-tailed $t$-test, $t = 10.9$, $P < 0.0004$) and a significant change in species composition (Table 1 and Supplementary Table 1). This experiment was conducted under the lowest applied temperature (that is, 1.8 °C, as compared to values ranging from 3 to 8 °C for the other experiments; Fig. 1c). On the basis of the concurrent significant interaction term between $p_{CO_2}$ and temperature in our statistical analysis (four-way analysis of variance, $F = 9.62$, $P = 0.003$), we thus speculate that OA effects may emerge with decreasing temperatures.

This seems plausible, as $p_{CO_2}$-dependent decreases in pH are larger in cold waters[32]. In our case, however, these thermodynamic effects are unlikely to explain a temperature dependence of $CO_2$ sensitivities. At a $p_{CO_2}$ of 1,000 µatm, the pH at 2 °C would be lower by only 0.014 units compared to 8 °C, which corresponds to a 3.3% increase in proton concentration in the colder incubation.

Temperature changes do not only affect seawater pH, but also the ability of organisms to cope with changes in proton concentrations and to maintain pH homeostasis for cellular functionality[33]. Under low temperature, the underlying machinery for pH homeostasis (for example, proton pumps) may be too slow to compensate for the effects of low-pH environments, giving rise to detrimental OA effects. Under high temperatures, pH homeostasis may be facilitated, thereby minimizing negative OA effects. As phytoplankton responses to increasing temperature follow an optimum curve[34,35] and data from nutrient-limited summer situations indicate a potential for negative OA effects only under extended warming (at 10 °C)[23], we speculate that negative OA effects may also emerge once temperatures exceed the optimum and act as an additional stressor (>8 °C; Fig. 1c). For the temperatures predicted until the end of this century[36], however, we conclude that nutrient-replete coastal Arctic phytoplankton could remain resistant to OA under various irradiance regimes.

In laboratory experiments, increasing temperatures have been shown to shift $p_{CO_2}$ optima of phytoplankton to higher values[18,37], which could explain the lack of responses to OA in our higher-temperature incubations (manipulation KFb_2 compared to KFb_1; Fig. 1, Supplementary Fig. 1I,J and Table 1). Previous field studies have also shown a lack of OA responses during summer experiments with warmer in situ temperatures[24]. If the proposed interaction between warming and OA holds true, OA might have a negative effect on NPP under current early-spring temperatures (<2 °C), while coastal Arctic phytoplankton assemblages may be able to compensate for these changes under the higher temperatures predicted for future spring conditions. The ecologically important ice-edge blooms, however, are predicted to shift their distribution further north[7,38]. Under such low-temperature conditions, we may expect negative OA effects on pelagic NPP. More work is needed to
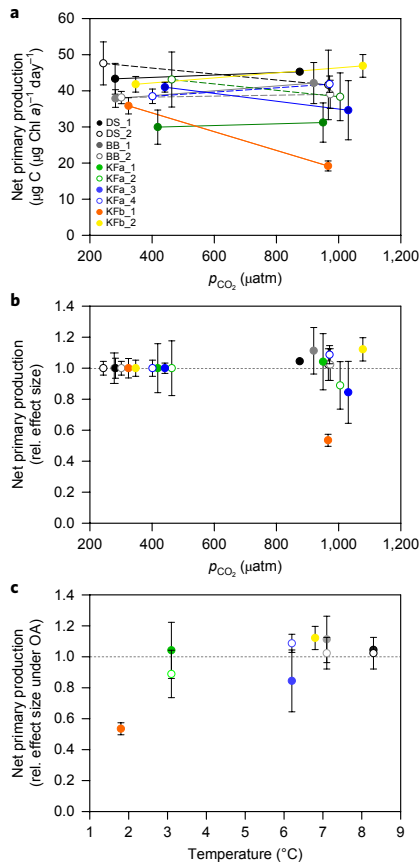
**Fig. 1 | Responses of chlorophyll *a*-normalized NPP to OA under different environmental conditions. a**, NPP for each individual $CO_2$ manipulation as a function of $p_{CO_2}$. **b**, Effect size of $CO_2$-dependent changes in NPP for each incubation bottle relative to the ambient $p_{CO_2}$ treatment of the respective manipulation. **c**, Relative effect size under future (approximately 1,000 µatm) $p_{CO_2}$ levels as a function of incubation temperature. Data are shown as mean ± 1 s.d. ($n = 3$). The grey dotted line indicates zero effect. Specific $p_{CO_2}$ temperature and light levels are given in Supplementary Tables 1 and 2.

elucidate the interactions of temperature and OA on Arctic primary production in these different habitats.

**Limited response in species composition**

Next to the observed OA insensitivity of NPP, we also observed surprisingly little changes in the phytoplankton species composition as a response to changes in $p_{CO_2}$, irradiance and temperature (Table 1). Even though Arctic phytoplankton have been shown to respond to OA in single-species experiments[18,39], and during a mesocosm study[40], species composition in our investigations responded to changes in $p_{CO_2}$ in only three out of ten manipulations (Table 1 and Supplementary Tables 3 and 6). This stands in contrast to results from similar experimental approaches applied to Southern Ocean assemblages, where strong shifts in species composition were consistently observed[26,41–43].

When changes in the species composition were observed in our study, their effects on bulk properties such as NPP differed (Table 1). In two manipulation treatments conducted in Davis Strait (69° N) under different irradiance regimes (DS_1 and DS_2; Fig. 1 and

Supplementary Fig. 1A,B), an OA-dependent shift in the most dominant species (>50%) between the diatoms *Pseudo-nitzschia* sp. and *Fragilariopsis* sp. was not associated with changes in NPP[44]. Such functional redundancy was, however, not observed in one of the manipulations conducted in Svalbard (KFb_1, Supplementary Fig. 1I), where a change in species composition (that is, decreasing abundances of *Thalassiosira* sp.; two-tailed *t*-test, $t = 4.2$, $P = 0.013$) co-occurred with a decline in NPP at low temperatures (but not at high temperatures, KFb_2, Supplementary Fig. 1J; two-tailed *t*-test, $t = 1.0$, $P = 0.389$). Interestingly, the species shift observed in the incubations from Davis Strait (DS_1 and DS_2, Supplementary Fig. 1A,B) was very similar to that of a similar experiment conducted in the Southern Ocean[26]. In the latter, the shift in species composition was associated with stronger changes in community NPP than would have been predicted from single-species experiments[45]. It thus seems that the buffering or amplifying nature of species shifts does not depend on the overarching characteristics of the competing species involved, but rather on the specific sensitivities of the respective populations (that is, their physiological plasticities and the diversity therein). As in the present study, a shift between species was observed in only two out of nine incubations with constant NPP (Table 1), functional redundancy (Fig. 2c) does not seem to be the main mechanism responsible for the observed non-responsiveness of this trait.

**Mechanistic basis for compensatory effects**

To estimate the effects of climate change on marine ecosystems and ecosystem services, a thorough understanding of the mechanisms and limits of physiological and ecological plasticity needs to be developed[4]. It is being increasingly acknowledged that the responses of organisms to climate change are influenced by their environmental history, which drives adaptation of species and ecosystems[46]. This is particularly important in our study, as all sampling was conducted in coastal areas located on Arctic inflow shelves. These highly productive systems[47,48] are characterized by particularly strong variability in environmental conditions. For instance, phytoplankton experience strongly varying irradiance fields, associated with frequent formation and melt of land-fast ice, and riverine and glacial inputs of suspended material causing strong attenuation of light[49,50]. Furthermore, coastal systems are characterized by higher variability in carbonate chemistry compared to the open ocean[51]. Evolutionary adaptation experiments have indeed shown that variable conditions select for phytoplankton cell lineages that benefit more strongly from future conditions than those evolved under stable carbonate chemistry[52]. Arctic coastal areas are often advective systems under a strong influence of northward-flowing Atlantic water masses[53], which carry planktonic organisms from lower latitudes that are adapted to different environmental conditions. In fact, many important bloom-forming organisms in the Atlantic sector of the Arctic, where our experiments were conducted, are also abundant in upstream Atlantic waters (for example, *Chaetoceros socialis/gelidus, Phaeocystis pouchetii*[54–56]). These organisms might have a wide tolerance towards environmental conditions that are expected to be more common in the future Arctic Ocean.

In addition to the potential importance of functionally redundant species (Fig. 2c; discussed above), we thus propose two other processes that allow Arctic phytoplankton to thrive in highly variable environments and may function as underlying mechanisms for the observed compensatory capabilities. Firstly, ecosystem services such as NPP can stay unaltered over a range of environmental conditions, such as $p_{CO_2}$ levels, if individual organisms exhibit high physiological plasticity and perform well over a large range of conditions (Fig. 2a). This ability to cope with changes in the environment is inevitably accompanied by regulation on the subcellular level. In incubation treatments from Davis Strait (DS_2), for example, we observed a strong OA-dependent increase in the photosynthetic
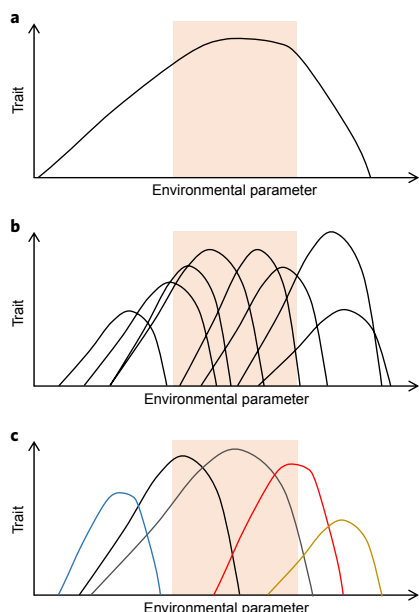
**Fig. 2 | Schematic illustration of the potential mechanisms underlying the observed resistance. a–c**, Unaltered traits (for example, NPP rates) over the investigated range (red-shaded area) of an environmental parameter (for example, $p_{CO_2}$ levels) can be caused by high physiological plasticity (**a**), strain sorting in combination with high intraspecific diversity (**b**) or shifts between functionally redundant species (**c**).

energy generation at photosystem II without changes in carbon fixation, potentially providing extra resources that allowed the phytoplankton to compensate for altered irradiance and pH levels[44]. Two Arctic diatom species (*Fragilariopsis cylindrus* and *Thalassiosira hyalina*) have been shown to possess wide $p_{CO_2}$ optima, exhibiting similar growth rates over the range of $p_{CO_2}$ levels investigated here[18,39]. Notably, these very same species were dominant in some of our incubations (Table 1). It is therefore likely that high physiological plasticity of dominant species contributes to the compensation of OA effects observed here.

Secondly, these dominant species have also been observed to exhibit high levels of intraspecific diversity (that is, isolates of the same species having different $p_{CO_2}$ optima and tolerance ranges)[18,39]. On the basis of these differences between isolates, a sorting between cell lineages of the same species may yield different population structures under diverging conditions, providing an additional mechanism to buffer changes in both NPP and species composition[18,57] (Fig. 2b). Regarding the origin of such high intraspecific diversity, the vast shelf areas and the proximity of coastline in our study areas could provide a sufficiently large and diverse seed bank of resting spores that can hatch and initiate blooms[58]. Such seed banks have been shown to significantly increase intraspecific diversity[59] and concurrent resilience[60,61]. Indeed, the centric diatom genera *Chaetoceros* and *Thalassiosira*, which are known to form high numbers of resting spores[58], often dominated our assemblages (Table 1 and Supplementary Table 3).

### From compensatory effects to tipping points

The field of OA and multiple-stressor research aims to describe and predict the effects of climate change on marine biota and their ecosystem services. This aim may lead to a bias against data sets showing

little or no effects of OA and multiple stressors[62]. While it is critical to explain the responses toward multiple stressors, it is equally important to understand the mechanisms employed by individual phytoplankton species and mixed assemblages to compensate for changes in environmental parameters, and to identify the limits of such compensatory mechanisms[4,63]. Regarding predictions for the future Arctic, our results suggest that OA may impact NPP or species composition to a far lesser degree than indicated by single-strain experiments[18,39]. Thus, while detailed physiological laboratory studies are indispensable for understanding cellular mechanisms of both resistance and responses toward OA, they are not, in isolation, suited for predictions of ecosystem services such as NPP[63].

The scientific community increasingly acknowledges the importance of ecological mechanisms that can either amplify[26,64] or buffer change[4,44,65]. As apparently resistant or resilient ecosystems are often observed to respond nonlinearly by abrupt regime shifts beyond a specific tipping point[66], the underlying ecological mechanisms need to be understood[63,67]. Moreover, the underlying changes in physiology with consequences on food availability and quality for higher trophic levels need to be studied. For climate change within current predictions, however, our observations suggest that OA is unlikely to cause large alterations in NPP and severe shifts in coastal Arctic phytoplankton composition under typical bloom conditions.

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## Author contributions

C.J.M.H., N.S., P.D.T. and B.R. designed the study; C.J.M.H., K.K.E.W., N.S. and P.D.T. carried out the experiments; C.J.M.H. carried out the sample and data analyses; C.J.M.H. wrote the manuscript with contributions from K.K.E.W., N.S., P.D.T. and B.R.

## Competing interests

The authors declare no competing interests.

## Additional information

## Methods

**Initial sampling.** Two experiments were initiated during the Arctic-GEOTRACES 2015 summer campaign on-board the CCGS *Amundsen*. In July 2015, an initial phytoplankton assemblage for the DS experiment was sampled in the subarctic southern part of Davis Strait (63° 57.857' N, 60° 7.552' W), while experiment BB was initiated in August 2015 in Baffin Bay near Clyde River (71° 24.327' N, 68° 36.057' W). For both experiments, seawater was collected from the bottom of the sub-surface chlorophyll maximum (54–50 m depth) using a trace-metal clean rosette system equipped with 12 l Teflon-coated GO-FLO bottles (General Oceanics). Water was first dispensed into acid-cleaned 50 l carboys, and then transferred into experimental bottles. This approach was taken to minimize variability in the initial phytoplankton assemblages represented among the experimental treatments. Full details on methods and eco-physiological responses of the phytoplankton assemblages in these experiments can be found in ref. [44] (DS experiment) and ref. [64] (BB experiment).

The initial phytoplankton assemblages for incubations KFa and KFb were sampled from the Kongsfjorden (station KB3, 78° 55' N, 11° 56' E; Svalbard) on-board the *Jean-Floch*. Seawater was collected from a depth of 23 to 25 m using a monsoon pump (Mega-Thyphoon, Proactive Environmental Products; flow rate approximately 4 l min⁻¹). Experiment KFa was initiated in April 2014, while experiment KFb was conducted in April 2016. Results on the species composition of phytoplankton assemblages in experiment KFa can be found in the Supplementary Information of ref. [18].

**Experimental set-up.** In DS and BB experiments, phytoplankton assemblages were cultured in 8 l polycarbonate bottles in on-deck incubators on the CCGS *Amundsen*. Incubators were temperature-controlled by flowing surface seawater. $p_{CO_2}$ levels in the bottles were maintained by continuous bubbling with commercially prepared air–$CO_2$ mixtures (380 and 1,000 µatm $p_{CO_2}$; Supplementary Table 2) at two light levels, 15% and 35% surface irradiance (photosynthetically active radiation; 400–700 nm). The KF experiments were conducted in the facilities of the Kings Bay AS Marine Laboratory, Svalbard. Phytoplankton assemblages were incubated in polycarbonate 4 l bottles in laboratory growth chambers at a range of constant temperature between 1.8 and 6.8 °C, with constant 24 h exposure to irradiances ranging between 2.6 and 13.0 mol quanta m⁻¹ day⁻¹ (corresponding to 30 and 150 µmol photons m⁻² s⁻¹, see Supplementary Table 1 for details). Irradiance levels were provided by daylight lamps (Master TL-D 18 W, Philips), adjusted with neutral-density screens. The incubation bottles were continuously sparged with air of different $p_{CO_2}$ delivered through sterile 0.2 µm air filters (Midisart 2000, Sartorius Stedim). Gas mixtures were generated using a gas flow controller (CGM 2000 MCZ Umwelttechnik), which blended $CO_2$–free air (<1 ppmv $CO_2$; Dominick Hunter) with pure $CO_2$ (Air Liquide Deutschland). In all experiments, seawater was pre-screened through 100 µm nylon mesh to exclude large grazers. Treatments were conducted in triplicate bottles.

To provide sufficient time for changes in the phytoplankton assemblages to occur, and to achieve ecologically relevant information, all experiments lasted between 8 and 22 days (Supplementary Table 1). To prevent significant changes in chemical conditions due to phytoplankton growth, incubations were diluted with filtered seawater once to three times over the course of the experiment (dilution ratios varied between 1:15 and 1:19 between experiments). The dilution water was obtained simultaneously to the sampling of initial phytoplankton assemblages, filtered through rinsed 0.2 µm filter cartridges (AcroPak 1500, PALL) and stored at approximately 4 °C in the dark until use. The experiments were run avoiding nutrient limitation (Supplementary Table 1), as this would have been an additional driver affecting the performance of the exposed phytoplankton assemblages. In the DS and BB as well as the KFb experiments, nutrients were added in Redfield proportions at the start and during the dilutions of the experiments to achieve concentrations slightly above typical winter values (for example, 20–25 µM nitrate). In experiment KFa, in situ nutrient concentrations were used (for example, 12 µM nitrate). In this experiment, incubations were diluted and terminated at lower biomass to prevent nutrient limitation (Supplementary Table 1).

**Carbonate chemistry.** In the DS and BB experiments, samples for the determination of dissolved inorganic carbon (DIC) and total alkalinity (TA) were collected in 250 ml borosilicate glass bottles with gas-tight stoppers. Samples for initial values were taken from the 50 l carboys, while samples during dilutions and final sampling were taken directly from the experimental bottles. Samples were analysed within 6 h after collection to minimize alteration by biological activity. DIC and TA were analysed in the field by coulometric and potentiometric titration, respectively, using a VINDTA 3C (Marianda) and following the methods described in a previous study[68]. Routine analyses of certified reference materials provided by A. G. Dickson (Scripps Institute of Oceanography) ensured that the uncertainty in DIC and TA measurements was less than 2 µmol kg⁻¹ and 3 µmol kg⁻¹, respectively.

In KF experiments, samples for TA were GF/F-filtered, fixed with 0.03% HgCl₂ and stored in 150 ml borosilicate bottles at 4 °C in darkness. TA was estimated at the Alfred Wegener Institute (AWI, Germany) from duplicate potentiometric titration[69] using a TitroLine alpha plus (Schott Instruments). DIC samples were gently filtered through 0.2 µm cellulose-acetate syringe filters (Sartorius Stedim), fixed with 0.03% HgCl₂ and stored in 5 ml gas-tight borosilicate bottles at 4 °C.

Triplicate DIC samples were measured colorimetrically at the AWI with a QuAAtro autoanalyser (Seal)[70]. The analyser was calibrated with NaHCO₃ solutions (with a salinity of 35, achieved by addition of NaCl) with concentrations ranging from 1,800 to 2,300 µmol DIC kg⁻¹. Certified reference materials were used for corrections of changes in instrument performance (for example, baseline drift) and ensured that the uncertainty of DIC and TA measurements was less than 2 µmol kg⁻¹ and 5 µmol kg⁻¹, respectively.

In all experiments, seawater pH on the total scale ($pH_{total}$) was determined potentiometrically with a two-point calibrated glass reference electrode (IOline, Schott Instruments). A TRIS-based reference standard[68] was used to convert from the NBS to the total scale and to correct for variability in electrode performance (uncertainty ±0.02 units).

Seawater carbonate chemistry (including $p_{CO_2}$) was calculated from TA and DIC in the DS and BB experiments, and from TA and pH in the KF experiments. Calculations were performed using CO2SYS[71] with the refitted dissociation constants of carbonic acid in refs [72,73]. Dissociation constants for KHSO₄ were taken from ref. [74].

**Chlorophyll *a*.** Samples for the determination of total chlorophyll *a* (Chl *a*) were gently filtered onto pre-combusted glass-fibre filters (GF/F, Whatman; <200 mmHg), taking care to keep the filters out of direct light. Filters were immediately flash-frozen in liquid nitrogen and stored at −20 °C until analysis. Chl *a* was extracted overnight at −20 °C in 8 ml 90% acetone. Chl *a* concentrations were determined on a fluorometer (Turner Designs), using an acidification step (1 M HCl) to determine phaeopigments[75]. The fluorometers were calibrated by measuring known amounts of Chl *a* (*Anacystis nidulans* extract, Sigma) during each expedition.

**Net primary production.** Chl *a*-specific net primary production (NPP) rates were determined in duplicate by incubation with a NaH¹⁴CO₃ spike (53.1 mCi mmol⁻¹ or 2.109 MBq mol⁻¹ stock; Perkin Elmer) for 24 h under the respective treatment conditions. In the DS and BB experiments, three 60 ml sub-samples were incubated after the addition of 60 µCi NaH¹⁴CO₃ (specific activity of 0.1 µCi ml⁻¹). In the KF experiments, three 20 ml aliquots were incubated after the addition of 10 µCi NaH¹⁴CO₃ (specific activity of 0.5 µC ml⁻¹). From these incubations, 0.5 ml aliquots were immediately added to 0.5 ml of 1 M NaOH, mixed with 15 ml of scintillation cocktail and counted as disintegrations per minute (DPM) after 2 h with a liquid scintillation counter (Tri-Carb 246 or Tri-Carb 2900TR, PerkinElmer) to determine the total amount of added NaH¹⁴CO₃ ($DPM_{100\%}$). For blank determination ($DPM_{0\%}$), one replicate was immediately acidified with 0.5 ml 6 M HCl and treated in a manner similar to samples. After 24 h, incubated samples were filtered onto GF/F filters, acidified with 0.5 ml 1 M HCl and left to degas overnight. After the addition of 10 ml of scintillation cocktail (ECOLUMTM, MP Biomedicals for the DS and BB experiments, Ultima Gold AB, PerkinElmer for the KF experiments), samples were vortexed and left to stand in the dark for approximately 12 h before counting on the liquid scintillation counter ($DPM_{sample}$), using automatic quench correction and a counting time of 5 min. NPP (µg C (µg Chl *a*)⁻¹ d⁻¹) was calculated as

$$NPP = ([DIC] \times (DPM_{sample} - DPM_{0\%}) \times 1.05)) / (DPM_{100\%} \times t \times [Chl\ a]) \tag{1}$$

where [DIC] and [Chl *a*] denote the concentrations of dissolved inorganic carbon and Chl *a* in the sample, respectively. $DPM_{sample}$ denotes the DPM in the samples, $DPM_{0\%}$ reflects the blank value, $DPM_{100\%}$ denotes the DPM of the total amount of NaH¹⁴CO₃ added to the samples and $t$ is the duration of the incubation. The value of 1.05 is used to correct for fractionation against ¹⁴C relative to ¹²C following ref. [76].

**Phytoplankton species composition.** To determine the taxonomic composition of phytoplankton assemblages at the end of the experiments, aliquots of 240 ml unfiltered sample were preserved and stored at 4 °C in the dark until further analysis. In the DS and BB experiments, samples for cell counts at the initial and final time points were fixed with a combination of buffered-formalin (2% final concentration) and glutaraldehyde (0.1% final concentration). In the KF experiments, samples were preserved with Lugols solution (1% final concentration). Preserved samples were analysed by inverted light microscopy (Zeiss) after 24 h sedimentation time in 10 ml Utermöhl chambers (Hydro-Bios). In addition, several dominant diatom species were identified using scanning electron microscopy (Philips XL30) according to taxonomic literature[77].

**Statistics.** All data (*n* = 58) are presented as the mean of three replicates with ± 1 standard deviation. To test for significant differences between the treatments, a four-way analysis of variance with quadratic interaction terms and additional Kolmogorov–Smirnov normality and Tukey post hoc tests was performed for the relative effect sizes of the NPP measurements as a function of initial phytoplankton assemblage $p_{CO_2}$ level, temperature and irradiance using the software package R (script written by D. Wolf-Gladrow). To test for $p_{CO_2}$ effects in each individual manipulation experiment, two-tailed *t*-tests were performed with the program SigmaPlot (SysStat Software). The significance level for all analysis was set to 0.05.

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# Compensation of ocean acidification effects in Arctic phytoplankton assemblages

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**Supplementary information to 'Compensation of Ocean Acidification effects in Arctic phytoplankton assemblages'**

by Clara J. M. Hoppe, Klara K. E. Wolf, Nina Schuback, Philippe D. Tortell, Björn Rost

**Table SI1:** Details of experimental conditions during the 10 different incubations.

| Incubation | Location | Type | Duration [days] | No of dilutions | Temp [°C] | PAR (400-700 nm) [mol quanta m$^{-2}$ day$^{-1}$] | Sampling depth [m] | Initial Chl a [µg L$^{-1}$] | final Chl a [µg L$^{-1}$] | final NO$_3$ [µM] | final SiOH$_4$ [µM] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DS_1 | Davis Strait | on-deck | 14 | 1 | 8.3 ± 1.5 | 5.7 ± 1.7 (15% E$_0$) | 45-50 | 0.41 | 12 ± 3 | 7 ± 2 | 7 ± 1 |
| DS_2 | Davis Strait | on-deck | 14 | 1 | 8.3 ± 1.5 | 13.4 ± 4.0 (35% E$_0$) | 45-50 | 0.41 | 17 ± 3 | 1 ± 1 | 2 ± 1 |
| BB_1 | Baffin Bay | on-deck | 8 | 1 | 7.1 ± 2.1 | 6.3 ± 1.4 (15% E$_0$) | 40-45 | 0.58 | 11 ± 1 | 19 ± 1 | 26 ± 1 |
| BB_2 | Baffin Bay | on-deck | 8 | 1 | 7.1 ± 2.1 | 14.7 ± 3.2 (35% E$_0$) | 40-45 | 0.58 | 10 ± 2 | 19 ± 1 | 24 ± 1 |
| KFa_1 | Svalbard | laboratory | 19 | 2 | 3.1 ± 1.2 | 2.6 ± 0.7 | 23 | 0.13 | 8 ± 2 | 5 ± 2 | 3 ± 1 |
| KFa_2 | Svalbard | laboratory | 14 | 2 | 3.1 ± 1.2 | 13.0 ± 1.2 | 23 | 0.13 | 8 ± 2 | 3 ± 2 | 2 ± 1 |
| KFa_3 | Svalbard | laboratory | 12 | 2 | 6.2 ± 1.1 | 2.6 ± 0.7 | 23 | 0.13 | 4 ± 1 | 7 ± 1 | 3 ± 1 |
| KFa_4 | Svalbard | laboratory | 11 | 2 | 6.2 ± 1.1 | 13.0 ± 1.2 | 23 | 0.13 | 12 ± 1 | 2 ± 1 | 2 ± 1 |
| KFb_1 | Svalbard | laboratory | 22 | 3 | 1.8 ± 0.1 | 4.3 ± 0.2 | 24 | 0.12 | 30 ± 5 | 10 ± 4 | 6 ± 2 |
| KFb_2 | Svalbard | laboratory | 16 | 3 | 6.8 ± 0.4 | 4.3 ± 0.2 | 24 | 0.12 | 25 ± 6 | 10 ± 2 | 9 ± 2 |

**Table SI2:** Carbonate Chemistry at the end of the 10 different experimental incubations. In DS and BB experiments, $pCO_2$ was calculated from total alkalinity (TA) and dissolved inorganic carbon (DIC), while in KF experiments pH and TA were used as input parameters for $CO_{2SYS}$ (Pierrot et al. 2006). Low and high $pCO_2$ refer to the ambient and future $pCO_2$ levels applied to the various treatments ($n$=3 per treatment; mean ±1 S.D.).

| Incubation | $pH_{total}$ | | TA [µmol kg$^{-1}$] | | $pCO_2$ [µatm] | |
|---|---|---|---|---|---|---|
| | low $pCO_2$ | high $pCO_2$ | low $pCO_2$ | high $pCO_2$ | low $pCO_2$ | high $pCO_2$ |
| DS_1 | 8.17 ± 0.03 | 7.73 ± 0.02 | 2283 ± 5 | 2288 ± 6 | 281 ± 20 | 875 ± 37 |
| DS_2 | 8.22 ± 0.02 | 7.69 ± 0.03 | 2283 ± 1 | 2284 ± 7 | 243 ± 16 | 967 ± 76 |
| BB_1 | 8.17 ± 0.02 | 7.71 ± 0.01 | 2308 ± 6 | 2308 ± 2 | 282 ± 14 | 920 ± 29 |
| BB_2 | 8.15 ± 0.03 | 7.68 ± 0.02 | 2303 ± 9 | 2293 ± 14 | 301 ± 25 | 973 ± 41 |
| KFa_1 | 8.06 ± 0.01 | 7.73 ± 0.01 | 2422 ± 11 | 2440 ± 3 | 418 ± 13 | 950 ± 33 |
| KFa_2 | 8.02 ± 0.02 | 7.70 ± 0.01 | 2426 ± 6 | 2442 ± 6 | 463 ± 17 | 1005 ± 28 |
| KFa_3 | 8.02 ± 0.01 | 7.68 ± 0.01 | 2431 ± 11 | 2434 ± 1 | 441 ± 15 | 1031 ± 7 |
| KFa_4 | 8.06 ± 0.01 | 7.71 ± 0.01 | 2424 ± 2 | 2427 ± 1 | 401 ± 12 | 971 ± 34 |
| KFb_1 | 8.12 ± 0.01 | 7.68 ± 0.02 | 2330 ± 9 | 2318 ± 6 | 324 ± 12 | 966 ± 50 |
| KFb_2 | 8.10 ± 0.04 | 7.65 ± 0.01 | 2314 ± 17 | 2321 ± 10 | 347 ± 32 | 1078 ± 16 |

**Table SI3:** Dominant species in final phytoplankton assemblages under different temperature as well as low and high irradiance regimes (LL and HL, respectively). Please note that for DS and BB incubations, no quantitative sample analysis was possible and only estimates on the minimum contribution is provided. The percentage contribution to the final cell count are given in brackets ($n$=3 per treatment; mean ±1 S.D).

| Incubation | Temp [°C] | Irradiance regime | pCO$_2$ levels [µatm] low | high |
|---|---|---|---|---|
| DS_1 | 8 | LL variable | *Pseudo-nitzschia sp.* (>50%), *Fragillariopsis cylindrus* (>15%) | *Fragillariopsis cylindrus* (>50%), *Pseudo-nitzschia sp.* (>10%) |
| DS_2 | 8 | HL variable | *Pseudo-nitzschia sp.* (>50%), *Fragillariopsis cylindrus* (>15%) | *Fragillariopsis cylindrus* (>50%), *Pseudo-nitzschia sp.* (>10%) |
| BB_1 | 6 | LL variable | *Chaetoceros socialis* (>50%), *Thalassiosira nordenskoeldii* (>10%) | *Chaetoceros socialis* (>50%), *Thalassiosira nordenskoeldii* (>10%) |
| BB_2 | 6 | HL variable | *Chaetoceros socialis* (>50%), *Thalassiosira nordenskoeldii* (>10%) | *Chaetoceros socialis* (d>50%), *Thalassiosira nordenskoeldii* (>10%) |
| KFa_1 | 3 | LL constant | *Micromonas pusilla* (53 ± 2%), *Chaetoceros socialis* (20 ± 4%), *Thalassiosira hyalina* (5 ± 1%) | *Micromonas pusilla* (65 ± 10%), *Chaetoceros socialis* (21 ± 3%), *Thalassiosira hyalina* (3 ± 3%) |
| KFa_2 | 3 | HL constant | *Micromonas pusilla* (35 ± 10%), *Chaetoceros socialis* (20 ± 2%), *Thalassiosira hyalina* (11 ± 3%) | *Micromonas pusilla* (34 ± 11%), *Chaetoceros socialis* (25 ± 7%), *Thalassiosira hyalina* (14 ± 4%) |
| KFa_3 | 6 | LL constant | *Micromonas pusilla* (43 ± 6%), *Chaetoceros socialis* (16 ± 1%), *Thalassiosira hyalina* (1 ± 1%) | *Micromonas pusilla* (50 ± 4%), *Chaetoceros socialis* (18 ± 6%), *Thalassiosira hyalina* (3 ± 3%) |
| KFa_4 | 6 | HL constant | *Micromonas pusilla* (37 ± 2%), *Chaetoceros socialis* (20 ± 7%), *Thalassiosira hyalina* (9 ± 1%) | *Micromonas pusilla* (37 ± 11%), *Chaetoceros socialis* (15 ± 3%), *Thalassiosira hyalina* (13 ± 5%) |
| KFb_1 | 2 | LL constant | pennates <20µm (43 ± 5%), pennates >20µm (29 ± 8%), *Thalassiosira hyalina* (5 ± 2%) | pennates <20µm (71 ± 2%), pennates >20µm (16 ± 4%), *Thalassiosira hyalina* (0 ± 0%) |
| KFb_2 | 7 | LL constant | pennates <20µm (59 ± 4%), pennates >20µm (19 ± 3%), *Thalassiosira hyalina* (3 ± 2%) | pennates <20µm (57 ± 3%), pennates >20µm (17 ± 3%), *Thalassiosira hyalina* (4 ± 2%) |

**Table SI 4:** Results from two-tailed t-tests investigating pCO$_2$ effects on NPP at the end of incubations under different temperature as well as low and high irradiance regimes (LL and HL, respectively). Bold p-values indicate statistically-significant differences (p<0.05).

| Incubation # | Location | Type | Temp [°C] | Irradiance regime | Sample size $n$ | t-value | p-value |
|---|---|---|---|---|---|---|---|
| DS_1 | Davis Strait | on-deck | 8 | LL variable | 5 | 0.6 | 0.59 |
| DS_2 | Davis Strait | on-deck | 8 | HL variable | 6 | 0.4 | 0.74 |
| BB_1 | Baffin Bay | on-deck | 6 | LL variable | 5 | 1.0 | 0.41 |
| BB_2 | Baffin Bay | on-deck | 6 | HL variable | 6 | 0.3 | 0.74 |
| KFa_1 | Svalbard | laboratory | 3 | LL constant | 6 | 0.3 | 0.78 |
| KFa_2 | Svalbard | laboratory | 3 | HL constant | 6 | 0.8 | 0.46 |
| KFa_3 | Svalbard | laboratory | 6 | LL constant | 6 | 1.9 | 0.13 |
| KFa_4 | Svalbard | laboratory | 6 | HL constant | 6 | 1.3 | 0.25 |
| KFb_1 | Svalbard | laboratory | 2 | LL constant | 6 | 10.9 | **<0.0004** |
| KFb_2 | Svalbard | laboratory | 7 | LL constant | 6 | 2.3 | 0.08 |

**Table SI 5:** Results from four-way ANOVA with quadratic interaction terms investigating the effects of the initial phytoplankton assemblage, as well as the applied temperature, $pCO_2$ and irradiance level on the relative effect size of NPP changes (n = 58). Bold p-values indicate statistically-significant differences ($p<0.05$).

| Factor | Degrees of freedom | Mean square estimate | F-value | p-value |
|---|---|---|---|---|
| Initial assemblage | 1 | 0.01 | 0.34 | 0.57 |
| Temperature | 1 | 0.06 | 2.48 | 0.12 |
| $pCO_2$ | 1 | 0.07 | 2.87 | 0.10 |
| Irradiance | 1 | 0.02 | 0.59 | 0.45 |
| Initial assemblage * Temperature | 1 | 0.06 | 2.41 | 0.13 |
| Initial assemblage * $pCO_2$ | 1 | 0.01 | 0.35 | 0.55 |
| Initial assemblage *Irradiance | 1 | 0.01 | 0.32 | 0.57 |
| Temperature * $pCO_2$ | 1 | 0.25 | 9.62 | **0.003** |
| Temperature * Irradiance | 1 | 0.01 | 0.27 | 0.60 |
| $pCO_2$ * Irradiance | 1 | 0.02 | 0.59 | 0.45 |
| Residuals | 47 | 0.03 | | |

**Table SI 6:** Results from two-tailed t-tests investigating pCO$_2$ effects on the % cell counts of the most dominant species at the end of incubations under different temperature as well as low and high irradiance regimes for the experiments conducted in Svalbard. Please note that also two- and three-way ANOVAs yielded no significant effects of the drivers.

| Incubation # | Location | Type | Temp [°C] | Irradiance regime | most dominant species | Sample size $n$ | t-value | p-value |
|---|---|---|---|---|---|---|---|---|
| KFa_1 | Svalbard | laboratory | 3 | LL constant | *Micromonas pusilla* | 6 | 1.89 | 0.131 |
| KFa_2 | Svalbard | laboratory | 3 | HL constant | *Micromonas pusilla* | 6 | 0.06 | 0.952 |
| KFa_3 | Svalbard | laboratory | 6 | LL constant | *Micromonas pusilla* | 6 | 1.6 | 0.196 |
| KFa_4 | Svalbard | laboratory | 6 | HL constant | *Micromonas pusilla* | 6 | 0.3 | 0.964 |
| KFb_1 | Svalbard | laboratory | 2 | LL constant | *Nitzschia* spp. & *Navicula* sp. | 6 | 1.54 | 0.199 |
| KFb_2 | Svalbard | laboratory | 7 | LL constant | *Nitzschia* spp. & *Navicula* sp. | 6 | 0.46 | 0.668 |

**Figure SI1:** Net primary production (NPP; [µg C (µg Chl)$^{-1}$ d$^{-1}$]; *n* = 2-3 per treatment) as a function of pCO$_2$ for each individual CO$_2$-manipulation. For details on each incubation, see Table SI1.
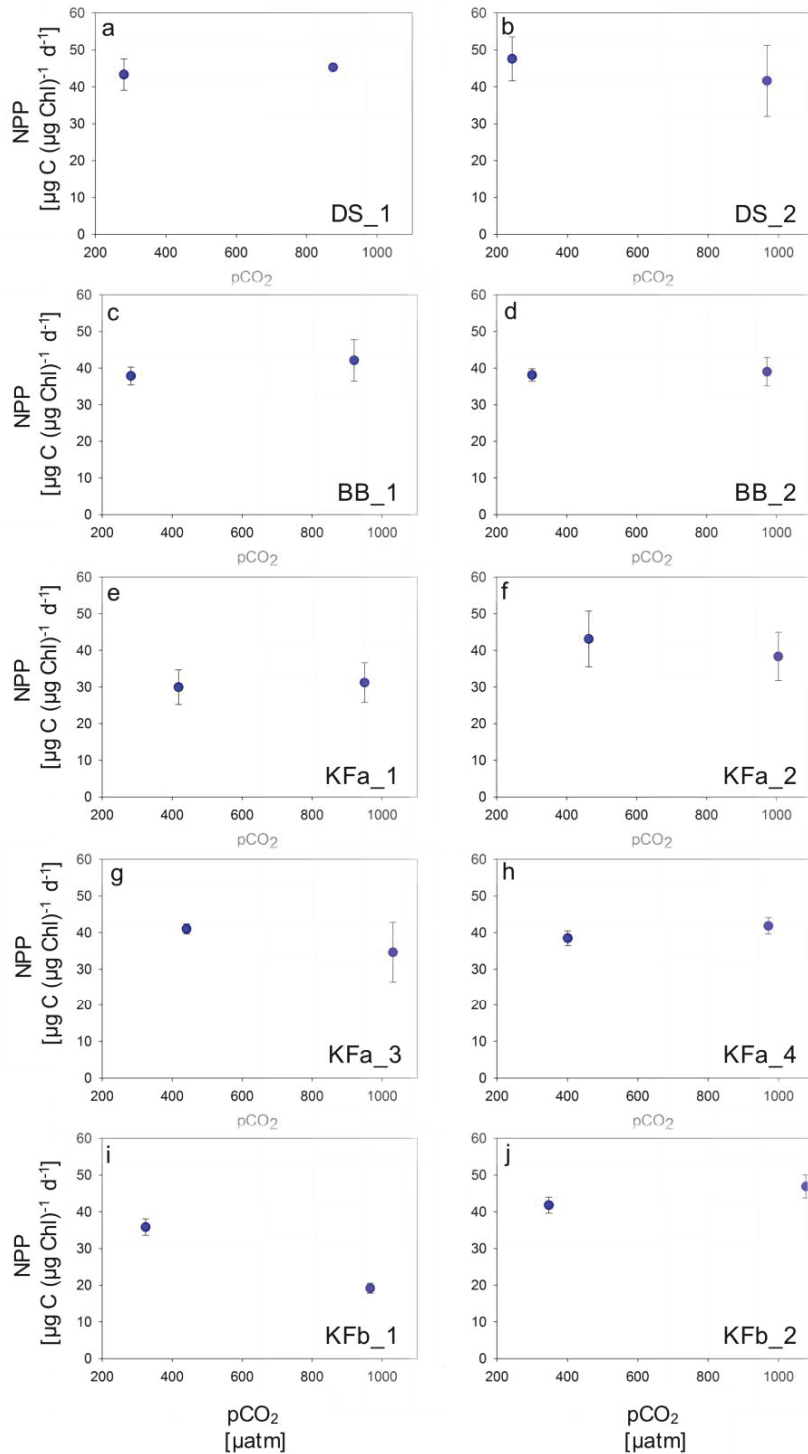
# Chapter 3   Publication II

**Company matters:**
**The presence of other genotypes alters traits**
**and intraspecific selection in an Arctic diatom**
**under climate change**

# Company matters: The presence of other genotypes alters traits and intraspecific selection in an Arctic diatom under climate change

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# Chapter 3 Publication II

**ABSTRACT**

Arctic phytoplankton and their response to future conditions shape one of the most rapidly changing ecosystems on the planet. We tested how much the phenotypic responses of strains from an Arctic diatom population diverge and whether the physiology and intraspecific composition of multi-strain populations differ from expectations based on single strain responses. To this end, we conducted incubation experiments with the diatom *Thalassiosira hyalina* under present-day and future temperature and $pCO_2$ scenarios. Six fresh isolates from the same Svalbard population were incubated as mono- and multi-strain cultures. For the first time, we were able to closely follow intraspecific selection within an artificial population using microsatellites and allele-specific quantitative PCR. Our results show not only that there is substantial variation in how strains of the same species cope with the tested environments, but also that changes in genotype composition, production rates and cellular quotas in the multi-strain cultures are not predictable from monoculture performance. Despite this, the physiological responses as well as strain composition of the artificial populations were highly reproducible within each environment. Interestingly, we only detected significant strain sorting in those populations exposed to the future scenario. This study illustrates that the genetic composition of populations can change on very short timescales through selection from the intraspecific standing stock, indicating the potential for rapid population level adaptation to climate change. We further show that individuals adjust their phenotype not only in response to their physico-chemical, but also to their biological surroundings. Such intraspecific interactions need to be understood in order to realistically predict ecosystem responses to global change.

# Chapter 3 Publication II

## INTRODUCTION

Marine phytoplankton are not only the base of the oceanic foodweb, but also the main driver of the biological carbon pump, which strongly influences the biogeochemical cycles in the oceans (Geider et al., 2001). Diatoms play a central role in these processes as they are the most important primary producers in the present-day oceans and contribute disproportionally to the vertical carbon flux, especially during highly productive bloom events (Sarthou et al., 2005). Therefore, their responses to rising temperatures and exponentially increasing $CO_2$ concentrations are of great relevance for ecosystems as well as for climate feedbacks. The Arctic environment, changing far more rapidly than the global average (Miller et al., 2010), can provide a prime example for the ability or failure of organisms to adapt at a fast pace.

Our attempts to understand and predict future phytoplankton productivity and species composition often rely on the upscaling of single strain responses to environmental drivers as measured in laboratory experiments (e.g. Dutkiewicz et al., 2015). Such laboratory setups, however, have yielded varying results (Gao & Campbell, 2014), especially when compared with observations from studies using more complex communities (Sommer et al., 2015; Tatters et al., 2018). Awareness of genotypic as well as phenotypic diversity within phytoplankton species has grown considerably in recent years (Alpermann et al., 2010; Brandenburg et al., 2018; Godhe & Rynearson, 2017; Hattich et al., 2017; Kremp et al., 2012; Pančić et al., 2015; Wolf et al., 2018) and may partly explain such differing outcomes. With the recognition of trait diversity within species, we have to ask the question how knowledge about single strains can be applied in an ecological context, which is also increasingly realized in environmental models (Follows & Dutkiewicz, 2011; Fontana et al., 2017; Kiørboe et al., 2018).

Understanding the relationships between responses of cultures containing a single genotype (hereafter referred to as monocultures) and populations is an important step towards predicting the responses of species or entire communities. Effects of a rapidly changing environment may be amplified or buffered on any of these ecological levels. Thus far, knowledge about such interactions in phytoplankton mainly stems from research on different species in artificial communities, which are typically composed of very few long-term established laboratory strains. Using a single representative of each selected species, monoculture responses appear to predict the community outcomes fairly well (Low-Décarie et al., 2011; Pardew et al., 2018). In biodiversity research, however, it is a common notion that a species' persistence is not only determined by the physico-chemical conditions (i.e. the fundamental niche), but is also influenced by biological interactions (i.e. the realized niche, Elton 1927), such as competition or facilitation (Bruno et al., 2003; John et al., 2015). Biodiversity effects are often partitioned into 'selection effects', which apply if the community traits are driven by the dominance of a certain species, and 'complementary effects', which describe the (often positive) influence of species interactions (Loreau & Hector, 2001; Cardinale et al., 2006). From early agricultural research we know for example, that a mix of species can have a different, often even higher yield than the best performing species grown

in monoculture ('transgressive overyielding'; Trenbath, 1974). It has also been argued that biodiversity can have a buffering effect on both, species persistence as well as community productivity, called the 'insurance effect' (e.g. Yachi & Loreau, 1999; Loreau et al., 2003).

To which extent such concepts also apply to intraspecific diversity is only beginning to be discussed (e.g. Roger et al., 2012; Aguirre & Marshall, 2012; Reusch et al., 2005). Genotypic diversity has been shown to affect responses of phytoplankton populations in different ways: Some studies find that a diverse population performs as the mean of all strains in isolation (Hattich et al., 2017), while others indicate that they perform like the best performing component of the mix (Bell, 1991), which is then assumed to be the dominant one. It has also been observed that a mixture of strains of the same species performs even better than the best one of its components in monoculture (John et al., 2015; Sjöqvist & Kremp, 2016; Vanelslander et al., 2009), which suggests that mechanisms other than selection are at work. Mixtures of strains, however, were also found to underperform relative to monocultures (Collins, 2010). These inconsistencies suggest that general mechanisms of intraspecific interaction are still poorly understood. Discerning these interactions is limited methodologically as it is difficult to resolve the intraspecific genotypic composition of microbial populations: they are typically inferred from subsamples of a number of re-isolated genotypes present at the end of an experiment, thus not resolving the temporal dynamics of genotype sorting.

In this study, we focus on this knowledge gap by following the intraspecific strain composition during a competition experiment quantitatively and temporally resolved. Our objective was to characterize and compare the responses of different isolates of an Arctic diatom, not only as single-genotype monocultures, but also when combined in an artificial multi-strain population, whose genotypic composition and properties could be measured. The experimental set-up described here was preceded by a natural community incubation of an Arctic phytoplankton assemblage. Aiming at resolving genotypes that may show the broad response range present within this population, we isolated several individual cells of our model species *Thalassiosira hyalina* from the final time-point of two different treatments (i.e. selection environments) of the community incubation. We characterized six of these freshly established strains as monocultures under three scenarios of temperature and $pCO_2$ conditions ('present-day', 'warming' and 'future') to investigate the extent of their plasticity as well as intraspecific variation in responses to climate change. From former experiments with this species (Wolf et al., 2018), we expected responses often found in diatoms: increased growth and productivity under higher temperature and variable, strain-specific effects in the interaction with elevated $pCO_2$. Subsequently, we combined these six strains into artificial populations and used microsatellite markers to measure their genotypic composition over time. This enabled us to compare the sorting dynamics that actually occurred with predictions of population composition and productivity based on monoculture responses.

## MATERIALS AND METHODS

### Strain origin and isolation

The six monocultures of *Thalassiosira hyalina* investigated here were isolated from the final time-point of an experiment with a natural Arctic phytoplankton spring community from the Kongsfjord, in Svalbard (mid-fjord station KB3, 78°55'N, 11°56'E). The species was chosen due to its frequent dominance in Svalbard spring blooms (von Quillfeldt, 2000). The community incubation was conducted in April 2016, applying combined $CO_2$ and temperature treatments under controlled light and nutrient conditions in a laboratory. The details of this experiment can be found in Hoppe et al. (2018b), where the experiment is referred to as KFb.

After 16-22 days of the community incubation (duration depended on nutrient drawdown of the cultures), single cells of the diatom *T. hyalina* were isolated manually under a light microscope and washed three times in sterile seawater. Strains CPa24, CPa49, CPb44 (in the following called strain A, B and C) were isolated from bottles grown under 'present-day' conditions at 1.8°C and ~320 µatm $pCO_2$ (see Hoppe et al., 2018b for details). Strains WFa43, WFb25, WFb51 (in the following called strain X, Y and Z) were isolated from bottles under 'future' conditions at 6.8°C and ~1080 µatm $pCO_2$. Single-cell isolation was repeated after 10-14 days of growth in 48-well-plates at 6.8°C in 1–3 mL sterile nutrient-enriched seawater. Each of the resulting monocultures was checked microscopically for contamination with other algal species and via microsatellites for other genotypes. The resulting stock cultures were maintained at 3°C and 5-10 µmol photons $m^{-2}$ $s^{-1}$ for about 9 months before the start of the experiment.

### Experimental conditions

The six strains were incubated in spring 2017 in 1 L glass bottles in semi-continuous dilute-batch cultures (150-10,000 cells $ml^{-1}$, diluted every 2-5 days depending on cell density). Each strain was tested in a collapsed design matrix of three treatments: at low temperature and $pCO_2$ (2°C, 400 µatm) called 'present-day scenario'; high temperature and low $pCO_2$ (7°C, 400 µatm) called 'warming scenario'; and both high temperature and high $pCO_2$ (7°C, 1200 µatm) called 'future scenario'. Prior to the experimental phase, cultures were acclimated to treatment conditions for at least one week (>7 generations). Each treatment was conducted in biological triplicates, except for strain A (n=2). All sampling and dilutions were conducted under sterile conditions using a laminar flow hood. Cells were cultivated in 0.2 µm sterile-filtered Arctic seawater (salinity: 32) enriched with macronutrients (100 µmol $L^{-1}$ $NO_3^-$, 6.2 µmol $L^{-1}$ $HPO_4^{2-}$, 100 µmol $L^{-1}$ $SiOH_4$), vitamins and trace metals according to f/2 R media (Guillard & Ryther, 1962). Cells were grown under continuous light with 51± 3 µmol photons $m^{-2}$ $s^{-1}$ using daylight lamps (Biolux T8, 6500K, Osram, Germany). Irradiance was adjusted with a black mesh fabric and measured in filled culturing bottles using a 4π sensor (Walz, Germany).

For the temperature treatments, target values of 2°C and 7°C were chosen to simulate the temperatures cells are presently experiencing during spring and summer in the Kongsfjord (Hegseth et al., 2018) as well as current and expected future mean spring bloom

temperatures (AMAP, 2013; Beszczynska-Möller et al., 2012). Experiments were performed in a temperature-controlled 2°C room, with bottles immersed in water-filled aquaria for additional temperature stability. 7°C treatments were established by additional heating of the aquaria by immersion thermostats (Corio CD, Julabo, Germany). Continuous surveillance with a temperature logger (Almemo 2890, Ahlborn, Germany) ensured temperature stability at 2 ±0.17°C and 7 ±0.06°C.

Monocultures of the six single strains were acclimated to the respective treatment and ensured to be growing exponentially prior to the multi-strain incubation. The two treatments ('present-day': 2°C and 400 µatm; 'future': 7°C and 1200 µatm) were applied to n=3 and n=4 replicate bottles, respectively, which were initiated with identical cell numbers of each acclimated single-strain culture. The multi-strain cultures were run in the same experimental setup as the single-strain incubations with cell numbers ranging from 300-9000 cells ml$^{-1}$. All replicates were grown in parallel for 12 days ($\sim$ 13-14 generations) and diluted twice to 300 cells ml$^{-1}$ (day four and eight) in order to guarantee stability of carbonate chemistry and nutrients.

**Carbonate Chemistry**

Target $pCO_2$ levels were established by continuous aeration with a gas flow rate of $\sim$170 ml min$^{-1}$. The appropriately mixed air was delivered through sterile 0.2 µm air-filters (Midisart 2000, Sartorius stedim, Germany) provided by a custom-built gas mixing system (see Hoppe et al., 2015). Before inoculation and dilutions, seawater was equilibrated ($\geq$ 24 h) to the respective $pCO_2$ at treatment temperature.

Total alkalinity (TA) samples of each replicate as well as of control bottles containing sterile medium were taken during the final sampling. TA samples were 0.7 µm-filtered (GF/F, Whatman, UK) and stored in 250 ml borosilicate bottles at 3°C until analysis. TA was determined by duplicate potentiometric titrations (Brewer et al., 1986) using a TitroLine alpha plus autosampler (Schott Instruments, Germany) and corrected using Certified Reference Materials supplied by A. Dickson (Scripps Institution of Oceanography, USA). Stability of carbonate chemistry was ensured by regular measurements of pH throughout the incubations using a three-point calibrated potentiometric glass reference electrode (Aquatrode plus Pt1000, Metrohm, Switzerland). Values were corrected for temperature variation using the program $CO_2$sys (Pierrot et al., 2006) with dissociation constants of carbonic acid by Mehrbach et al. (1973), refitted by Dickson and Millero (1987). Following Hoppe et al. (2012), calculations of the full carbonate system on the final day of incubation were performed in the same program based on measurements of TA and pH (Table S2). Deviations in calculated $pCO_2$ of the incubations compared to abiotic control bottles were $\leq$ 7% in all treatments (except for strain C in present-day conditions with -18%, data not shown). Carbonate chemistry data can be found in the Supplement Table 2.

**Growth, production rates and cellular composition**

Cell densities were counted daily using a Coulter Multisizer III (Beckman-Coulter, USA), where *T. hyalina* cells were quantified within a clear peak in the size range of 11-21 μm. Specific growth rate constants μ ($d^{-1}$) were calculated by an exponential fit through measured cell numbers for each time point according to the formula:

$$\mu = (lnN_t - lnN_0)/\Delta t \qquad (1)$$

where $N_t$ refers to cell density at time $t$, $N_0$ to the initial cell density and $\Delta t$ to the passed time (in days) since the start of the incubation. Growth rate constants were based on at least two dilution cycles for each culture. Specific growth rate constant $\mu$ was converted into division rate $k$ (i.e. divisions $d^{-1}$) by dividing $\mu$ by ln(2). All single strain cultures yielded stable growth rates over time after the acclimation period (three-way ANOVA of factors strain, treatment and time with factor time having no significant impact).

For particulate organic carbon (POC) and nitrogen (PON), cells were filtered onto precombusted (15h, 500°C) glass fiber filters (GF/F, 0.7 μm nominal pore size; Whatman, UK) and stored at −20°C. Filters were soaked with HCl (200 μL, 0.2 M) to remove inorganic carbon and dried over night at 60°C before POC analysis was performed, using a gas chromatograph CHNS-O elemental analyzer (Euro EA 3000, HEKAtech). POC values were blank-corrected by measurements of filters taken from pure medium. Daily production rates of POC were obtained by multiplication of the respective elemental quota with corresponding division rates $k$.

Chlorophyll *a* samples were filtered on GF/F filters, shock-frozen in liquid nitrogen and stored at -80°C. For analysis, filters were shredded in acetone (70%) with glass beads (0.5-1mm diameter) in a homogenizer (Precellys Evolution, Bertin Technologies, France). After overnight extraction at 4°C, chlorophyll *a* was measured fluorometrically (TD-700, Turner Designs), including an acidification step (1 M HCl) to determine phaeopigments (Knap et al., 1996).

**Variable Chl *a* fluorescence**

Variable Chl *a* fluorescence of Photosystem II was measured of the mixed-culture experiment as well as the 'present-day' (2°C 400μtm) and 'future' (7°C 1200μatm) treatment of the single strain incubations using a fast repetition rate fluorometer (FRRf, FastOcean PTX; Chelsea Technologies, UK) in combination with a FastAct Laboratory system (Chelsea Technologies). Photosynthesis-Irradiance (PI) curves were fitted according to Webb et al. (1974) and yielded estimates of maximum light-use efficiency (α) and maximum absolute electron transport rate through photosystem II ($ETR_{max}$) as well as at the irradiance of growth conditions (in-situ ETR). All measurements (n=3-4) were conducted at the respective treatment temperature. Instrument settings as well as data processing and fitting was performed as described in Hoppe et al. (2018a).

**DNA sampling and extraction of multi-strain cultures for microsatellite analysis**

For a relative quantitative determination of genotype composition in the multi-strain experiment, DNA samples were taken from each replicate at the time of every dilution and the final time point. Cultures were well mixed before 160-250ml samples of each bottle were filtered on PC filters (Whatman Nucleopore), which were immediately added to vials containing extraction buffer and stored at -80°C. All multi-strain DNA was extracted using the Nucleospin soil extraction kit (Macharey-Nagel GmbH, Germany) while monocultures for microsatellite characterization were extracted with the NucleoSpin Plant II kit (Macharey-Nagel GmbH, Germany), both according to manufacturers' instructions with an additional cell disruption step in a cell homogenizer (Fast Prep FP120,Thermo Fisher, USA).

**Allele-specific quantitative PCR**

The here described experiment was preceded by the development of six new microsatellite primers for *Thalassiosira hyalina,* Technical details can be found the Supplements of this article. In order to follow the genotype composition throughout the multi-strain experiment, we modified a method described by Meyer et al. (2006) as allele-specific quantitative PCR (asqPCR). Five of the six strains of *T. hyalina* used in the multi-strain experiment had at least one allele of unique size in one of the microsatellite loci ThKF3 or ThKF7. The only strain without a unique allele was strain A, which shared its homozygous allele of locus ThKF3 only with strain B (homozygous as well). However, this could be easily resolved since the abundance of strain B could be reliably determined from its two unique alleles in locus ThKF7. Accordingly, strain-specific amplicons derived by PCR from multi-strain DNA templates of filter samples as described above, could be distinguished and relatively quantified by asqPCR.

Relative abundances of the different strains were calculated from the peak area of the specific allele, i.e. the sum of fluorescence signal from a strain specific allele, relative to total peak area measured. Total peak area was calculated for each sample as the sum of all peak area values minus the values of all stutter factors (see below) taking results from linearity tests (see below) into account. For those genotypes that were homozygous in their specific allele, the according value was multiplied by 0.5. For the calculation of relative contributions of each allele, two additional factors were taken into account.

Stutter factor (sf): Alleles of primer ThKF3 produced reliable stutter peaks at -1 and -3 basepair lengths from the main peak, which were correlated to the main peak area by a factor dependent on allele-size. The stutter factor was established for each allele of locus ThKF3 based on the mean ratio of stutter vs. allele peak of 120 monoculture DNA samples analyzed beforehand. In order to correct for the contribution of the stutter peaks of a larger allele to the area of a shorter allele, an allele-specific 'stutter factor' was multiplied with the peak area of the intruding larger allele. This value was then subtracted from the peak area value of the shorter allele. The amount of area 'lost' was then added to the larger allele. Since primer ThKF7 did not produce any stutter peaks, the stutter factor was here set to 0 for all its alleles.

Linearity factor (lf): the linear relationship between frequencies calculated from asqPCR assays and actual genotype frequencies was validated with standard curves derived from manual DNA mixes for both primer ThKF3 and primer ThKF7. We analyzed samples with

relative contributions of each of the six strains at 0, 5, 10, 16, 25, 33, 50, and 100%, which were added to a master-mix of the remaining five at equal contributions. By linear regression we could show that the relative contribution of an allele's peak area was directly proportional to the actual contribution of the respective cells' DNA in the mixture (Figure S1). Regression coefficients were measured in all cases with $r^2 > 0.99$. The regression slope of each allele multiplied by 2 (to account for heterozygosity) was then used as the linearity factor for correction (0.8-1.0). In order to assess possible aberrations in extraction efficiencies of the different strains or alleles, we also tested the entire process from extraction to final relative contribution on artificial mixtures containing an equal number of cells of the 6 strains (as determined by a Coulter Counter). Since the calculated contributions only deviated between 1 and 3% from the predicted values for each strain, we judged this error to be negligible. Accordingly, each allele frequency was calculated by:

$$rF_x = \frac{(A_x*(1+sf_x)-(A_{x+3bp}*sf_{x+3bp}))*lf_x}{tA}$$

(2)

where $rF_x$ is the relative allele frequency of allele x, $A$ is the measured peak area of allele x (or x+3bp, i.e. the allele 3 basepairs upstream of x). $sf_x$ and $lf_x$ refer to the specific stutter and linearity factor for each allele, respectively. $tA$ is the total peak area of a sample and was calculated as the sum of all corrected allele peaks.

**Calculations and statistical analysis**

Predicted genotypic composition of the multi-strain culture was calculated based on the specific growth rates of each strain in monoculture assuming the time frame and dilution conditions of the actual multi-strain incubations. Standard errors of growth rates for each strain in monoculture were used to calculate uncertainties in these predictions according to the law of propagation of uncertainties. Predicted and observed contribution of each strain to the final genotypic composition of the multi-strain cultures was compared by Pearson's correlation coefficient (R). All predicted bulk values of the multi-strain cultures were calculated according to each strain's relative contribution to the final genotypic composition, its cell properties as measured in monoculture and the total cell counts of the multi-strain culture. Observed growth rates in multi-strain cultures were calculated for each strain based on its relative allele contribution (converted to cell number as fraction of total cell count) between the last dilution and the final time point of the experiment. Pielou's evenness index (Pielou, 1966) was calculated as a measure of diversity for each replicate bottle of the multi-strain cultures from the observed relative contributions of each strain to the final genotypic composition as well as for the predicted contributions derived from monoculture growth rates. Mean differences and standard errors in the growth response and POC production under the future compared to the present-day scenario were calculated after Borenstein et al. (2009). For each strain, mean response differences between mono- and multi-strain cultures were calculated in the same way for the present-day and future scenario.

In order to compare the differences in phenotypic response in monoculture (i.e. growth rates, cellular quota and the derived ratios) caused by the physico-chemical treatments (temperature and $pCO_2$) with the response difference among the genotypes independently of the treatments, we applied linear models with strain or treatment as the only explanatory variable or with both including an interactive term, using the software R (vers. 3.1.1 (2014), R Foundation for Statistical Computing, Austria). Models with different explanatory variables that yielded a lower AIC value (Akaike Information Criterion; Akaike 1974) were judged to be a better choice. Residuals were optically tested for normality and homoscedasticity. Because of deviations from normal distribution, POC, POC production and Chl *a*:POC ratio, alpha and $ETR_{max}$ were log-transformed. For growth rate and POC production, we also tested the effect of elevated temperature alone - pooled across all strains - by comparing only the 'present-day' and 'warming' treatment in a linear model. Similarly, we also tested only data of the treatments 'present' versus 'future'. Differences between responses to the two scenarios in the multi-strain culture were tested for each measured parameter by one-way ANOVAs after testing normality (Shapiro-Wilk-test) and homogeneity (Levene-test). The same tests were applied to compare predicted and observed responses in the multi-strain cultures for each parameter. For the comparison of growth rates, only the final experimental period of the multi-strain incubation (second dilution until final time-point) was considered. Number of generations (in the multi-strain cultures) were calculated from the number of days of incubation and the bulk division rate (k [$day^{-1}$]) of the cultures.

Since it is challenging to identify common patters in all measured parameters across two treatments in 6 strains (Table SI1), an additional principle component analysis (PCA) was run with measured growth rates, cellular quotas and ratios of each strain in monoculture as well as the multi-strain culture as the input variables for the present-day and the future scenario in the software R.

## RESULTS

### Physiological responses of monocultures and multi-strain cultures

All responses of monocultures were repeatable within strains but highly variable between them (Figure 1, Table S1). Although treatment effects were often pronounced within one strain, they had divergent directions and magnitudes amongst different ones (c.f. strain X and Y, Figure 1a-d). Therefore, when pooled across all strains, the mean of most parameters did not change considerably with the applied treatment (e.g. $\mu$ [day$^{-1}$]: present: $0.77 \pm 0.03$, future: $0.75 \pm 0.03$, warming: $0.77 \pm 0.01$, Table S1). Due to the differences in treatment responses of strains, the applied linear models with an interactive term of treatment and strain always explained the measured responses best (c.f. lowest AIC values Table S3a). Models with both predictors without their interaction were also tested, but always yielded higher AIC values (data not shown). While neither strain identity nor treatment alone explained the observed growth patterns as well as the full model, models with strain identity still yielded lower AIC values than those with treatment as explanatory variable in all parameters (Table S3a). All data and linear models for each parameter are summed up in Table S1 and S3a+b, respectively.
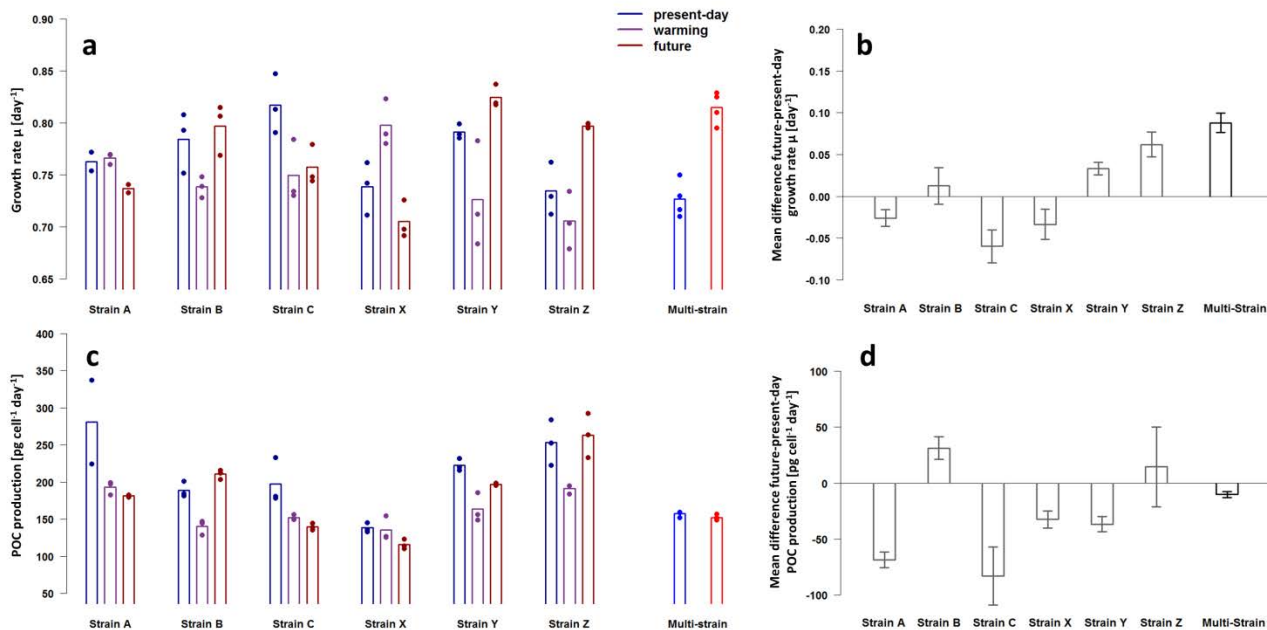


***Figure 1:*** *Specific growth rates (a, b) and POC production (c, d) of the monocultures and the multi-strain culture in the 3 scenarios (present: blue, warming: purple, future: red). Dots signify the value of the biological replicates, bars their respective mean. b) and d) show the mean difference and standard error of specific growth rate and POC production in the future treatment compared to the respective strain response under the present scenario.*

Elevated temperature and pCO$_2$ (future vs. present-day scenario) impacted the growth rate of strain Y and Z positively (by +4 and +8%), but negatively for strain A, C and X (by -3 to -7%), and had no effect on growth rate of strain B (Figure 1a, b). POC production under these conditions was elevated in strain B (+11%), not affected in strain Z, but lower in all others (-11 to -29%). Elevated temperature alone (warming vs. present-day scenario, i.e. at ambient pCO$_2$) increased growth rate only in one strain (strain X by 8%), while it slowed growth in three strains (strains B, C, Y by -6 to -8% Figure 1a) or had no effect in two others (strain A and Z). POC production was either not affected (strain X) or lower (-2 to -26%) and showed a significant negative trend in a linear model (F=11.51; p<0.01, slope: -46; Table S3b).

Responses of the multi-strain cultures to the applied treatments showed a smaller variability across biological replicates than the monocultures in most parameters (Figure 1, Table S1). While growth rate increased significantly in the future treatment (Figure 1a; One-way-ANOVA: F=62.71; p<0.001, Table S3d), POC quota decreased significantly under the same conditions (Table S1, One-way-ANOVA: F=84.01; p<0.001, Table S3d), causing POC production to stay constant in the two scenarios (Figure1c+d, One-way- ANOVA: F=4.0; p=0.09). Notably, POC production of all multi-strain cultures resembled those rates of the least productive monocultures (Figure 1c). Differences in photophysiological parameters (alpha, ETR$_{max}$, and in-situ electron transport rates (ETR)) between multi-strain cultures in the two scenarios were not significant (Table S3d).

**Table 1:** *Properties of 6 microsatellite loci and their respective primers. Measures of heterozygosity and Linkage Disequilibrium are based on the analysis of n=364 single genotype samples.*

| | repeat pattern | size range [bp] | Primer Sequence fwd | Primer Sequence rev | color tag | Multiplex | # of alleles | H$_O$ | H$_E$ | p H0/HE | Linkage diseqilibrium Locus | 1 | 2 | 3 | 4 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ThKF1 | CTG | 248-257 | TCGTATGGCTGCCATGAGAAG | GTAACTGCTGGGACGACCAC | HEX | no | 4 | 0.65 | 0.67 | 0.261 | 1 | * | + | + | - | - | - |
| ThKF2 | CA | 247-259 | AATTTGGAAGCCGCCGTAGA | GGGTCGGAGAGTTTGTTGCA | AT | no | 7 | 0.58 | 0.65 | 0.001 | 2 | + | * | - | - | - | - |
| ThKF3 | TCA | 187-264 | TCGCTGTCCTCGGTTTCAC | CAATGATGAGGTCCGGCGAT | FAM | no | 24 | 0.84 | 0.85 | 0.051 | 3 | + | - | * | - | - | + |
| ThKF4 | TTG | 246-258 | GGAGGAAAAACAACCGTTTGCT | TACAGGCCTTCCTTGCATGC | HEX | Multi#1 | 5 | 0.48 | 0.48 | 0.882 | 4 | - | - | - | * | - | + |
| ThKF6 | AAGTGA | 229-247 | AAATCCGCAGCCGAGAACAT | GAGAAGAGTCGCGCAGGATT | FAM | Multi#1 | 5 | 0.57 | 0.65 | 0.001 | 6 | - | - | - | - | * | + |
| ThKF7 | ACCAGC | 215-290 | ATTCCCATAGTCTCCCGACAGA | GGGGAGATCGTGATGCCTTC | FAM | Multi#2 | 14 | 0.80 | 0.84 | 0.485 | 7 | - | - | + | + | + | * |

**Microsatellite locus characteristics and genotypic composition of multi-strain cultures**

The six applied loci were found to be differently polymorphic, resolving 4-24 alleles across all samples (Table 1). Excluding stutter peaks, loci reliably yielded 1 or 2 peaks for each genotype, implying successful isolation and establishment of monocultures of our diploid organism. From repeated amplification of identical genotype DNA, we could establish a technical error rate of allele identification of 2.1%. Several DNA templates of closely related species of the same origin (*T. gravida, T. nordenskoeldii*) did not yield any PCR products, indicating that cross-amplification between species is unlikely to occur. Very low numbers of null-alleles can be assumed, since all 364 strain samples were amplifying with one or two alleles and expected as well as observed heterozygosity showed high similarity in most loci (except in loci ThKF2 and ThKF6). While some loci were tested positively for significant

Present-day scenario

Future scenario
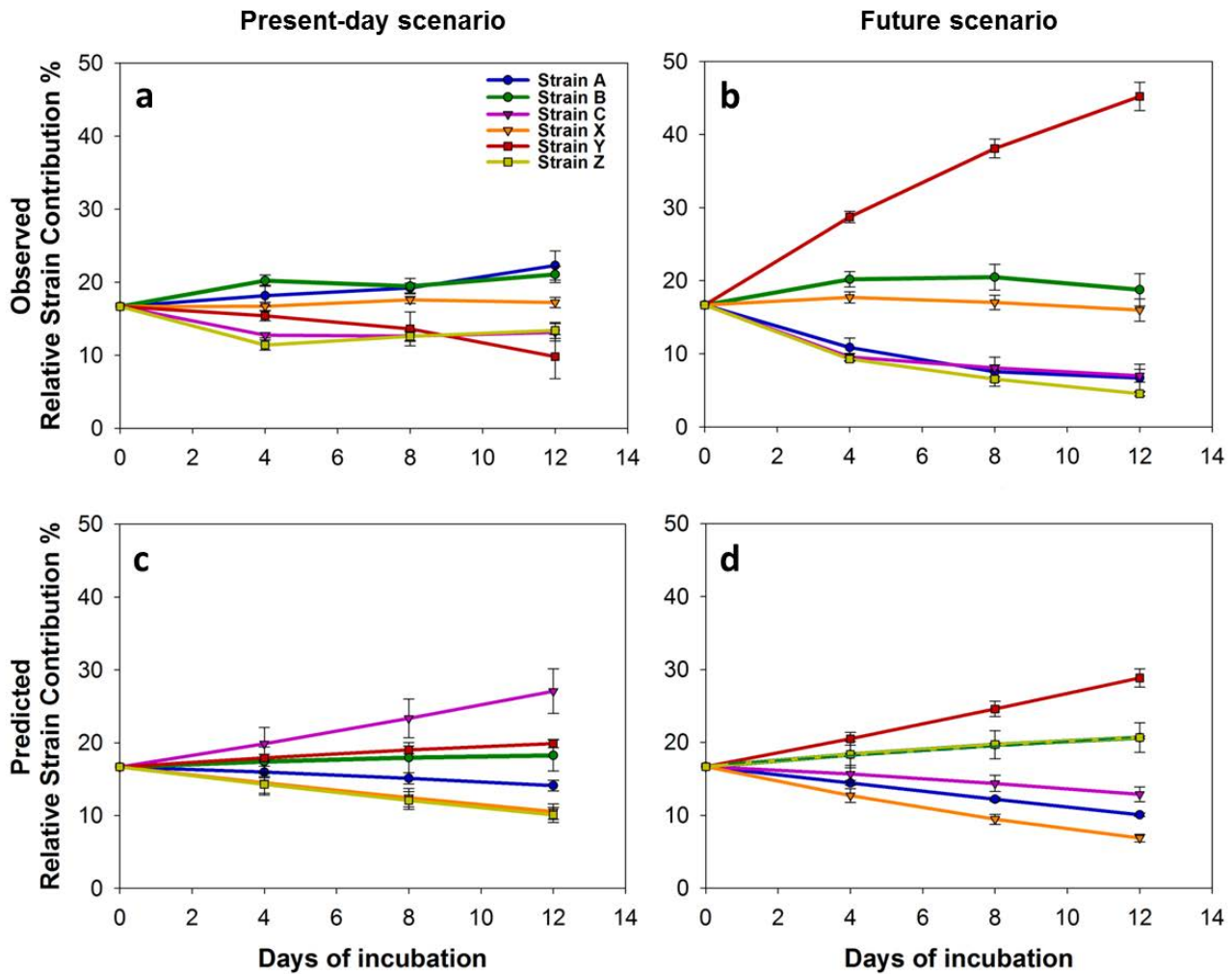
linkage disequilibrium (LD), the reciprocal combinations of them were not (e.g. LD was found in loci ThKF1 and 2 as well as ThKF1 and 3, but not in ThKF2 and ThKF3).

*Figure 2: Genotypic composition in the multi-strain culture expressed as their relative contribution to the population (%) as measured via asqPCR (a, b) and predicted from monoculture growth rates (c, d) in the present-day and the future scenario over the course of the experiment (13-14 generations). Error bars in the observed measurements (b+d) denote standard deviations of the four biological replicates. Error bars in the predicted composition show propagated uncertainties derived from standard deviations of specific growth rates in monoculture.*

By successful application of allele-specific qPCR (asqPCR) with our established microsatellites, we could follow the development of relative strain abundances in the multi-strain populations through filter samples taken at three time-points. While so far, this method has been used only for quantification of pairs of genotypes (John et al., 2015, Minter 2015, Sildever 2016), we succeeded to apply it to a range of six different genotypes within the same sample. The observed strain frequencies were highly reproducible across all replicate

incubations as reflected in the small standard deviations (Figure 2a, b), which complements the good reproducibility of physiological bulk responses of the multi-strain cultures (Table S1). In the present-day scenario, strain frequencies showed only small temporal changes throughout the experiment (~13 generations), except for a slight decrease in frequency of strain Y. In the future scenario, on the other hand, relative strain abundances diverged strongly and resulted in a clear dominance of strain Y (43-47%) within the same timeframe. Accordingly, the observed evenness in the two scenarios differed significantly (One-way ANOVA F=100, p<0.01; Table 2, Table S3d). No extinctions were observed in the timeframe of the experiment.

**Prediction of multi-strain cultures from monoculture responses**

The 'predicted' strain composition in the multi-strain culture was based on the growth rate constants measured in the monoculture responses, and therefore each strain's relative abundance changed linearly of over time (Figure 2c, d). This resulted in differing relative strain frequencies but similar diversity in both scenarios (evenness present-day: 0.97 and future: 0.94; Figure 2, Table 2). In comparison, actually observed strain composition in the present-day scenario changed slightly less than predicted (Figure 2a vs. c) and strains remained close to their original inoculation frequencies (16.6%) throughout the experiment. In the future scenario, the strain that had been growing fastest in monoculture under those conditions (strain Y) indeed dominated the final community, however to a larger degree than predicted (observed contribution final time point: 45% vs. predicted 28%, Figure 2b vs. d and Table S1). The predicted and observed evenness differed strongly in the future, but not the present-day treatment (Table 2). Linear regressions between predicted and observed strain frequencies showed that in the present-day scenario, the monoculture responses were a poor predictor of the actual development in mixed culture (R= -0.33, Figure S3a). In the future scenario, this correlation was slightly better (R= 0.67, Figure S3b), even though this was mainly driven by the correct prediction of strain Y becoming the dominant genotype in the mixture.

The predicted bulk responses of the multi-strain cultures (calculated based on strain composition and monoculture quota) will in the following be referred to as 'predicted values'. A comparison of these predicted and observed values can be found in Table 2 and Figure 3: for the majority of parameters, the predicted values were significantly different from the observed ones (p-values in Table S3e). Similarly, the mean of the monoculture response as well as the response of the fastest growing strain deviated considerably from the observed values. Predicted bulk growth rates were slightly but not significantly higher than the measured values in the present-day, but significantly lower than measured in the future scenario. Calculated for each strain individually, in both scenarios most observed growth rates differed strongly in mono- compared to multi-strain cultures (Figure 4). Observed POC and Chl *a* quota in all multi-strain cultures were much lower than predicted, causing production rates to be strongly overestimated, despite increased growth rates in the future scenario (Figure 3).

**Table 2:** *Predicted and observed responses in multi-strain incubation ± standard deviation. *signifies a significant difference between the predicted and observed value (One-way-ANOVA, α=0.05, see Table SI3e), except evenness which was not tested. Predicted numbers were calculated from the measured strain composition, assuming their respective values in monoculture. For reference, the mean of all monocultures as well as the properties of the fastest growing strain in monoculture are also depicted for both treatments.*

| | μ [day⁻¹] | POC production [pg cell⁻¹ day⁻¹] | k [day⁻¹] | POC [pg cell⁻¹] | Chl*a* [pg cell⁻¹] | C:N [mol:mol] | POC:Chl*a* [g:g] | Evenness |
|---|---|---|---|---|---|---|---|---|
| present-predicted | 0.75 ± 0.02 | 252 ± 7 | 1.08 ± 0.02 | 272 ± 12 | 8.6 ± 0.3 | 4.9 ± 0.1 | 29.31 ± 0.35 | 0.97 |
| present-observed | 0.73 ± 0.02 | 216 ± 4 }* | 1.05 ± 0.02 | 227 ± 5 }* | 7.1 ± 0.1 }* | 5.0 ± 0.1 | 30.57 ± 0.53 }* | 0.96 ± 0.02 |
| future-predicted | 0.77 ± 0.00 | 228 ± 1 | 1.12 ± 0.01 | 255 ± 2 | 7.9 ± 0.1 | 4.8 ± 0.0 | 28.87 ± 0.28 | 0.94 |
| future-observed | 0.81 ± 0.01 }* | 187 ± 4 }* | 1.18 ± 0.02 }* | 219 ± 5 }* | 6.8 ± 0.2 }* | 4.9 ± 0.1 }* | 27.46 ± 1.24 | 0.82 ± 0.02 |
| mean present | 0.77 ± 0.03 | 296 ± 55 | 1.11 ± 0.05 | 266 ± 58 | 9.0 ± 2.0 | 5.0 ± 0.4 | 30.0 ± 4.6 | NA |
| mean future | 0.77 ± 0.04 | 266 ± 59 | 1.11 ± 0.06 | 238 ± 76 | 8.2 ± 1.9 | 4.9 ± 0.6 | 29.4 ± 4.9 | NA |
| fastest grower present (Strain C) | 0.82 ± 0.03 | 285 ± 30 | 1.18 ± 0.04 | 241 ± 45 | 6.3 ± 0.8 | 4.7 ± 0.2 | 38.0 ± 0.2 | NA |
| fastest grower future (Strain Y) | 0.82 ± 0.01 | 284 ± 2 | 1.19 ± 0.02 | 239 ± 2 | 8.8 ± 0.4 | 4.8 ± 0.1 | 27.3 ± 1.3 | NA |
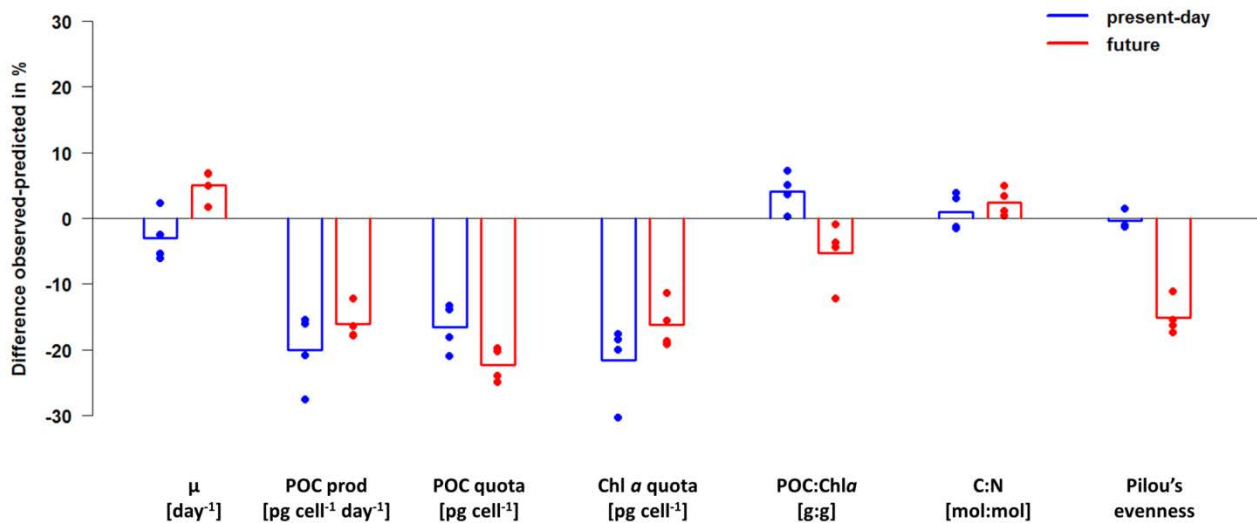


**Figure 3**: *Deviation of observed bulk physiological responses of the multi-strain culture compared to the predicted value as calculated from monoculture responses considering the observed final strain composition in the two tested scenarios (present-day: blue, future: red). Dots signify the value of the biological replicates, bars their respective mean.*
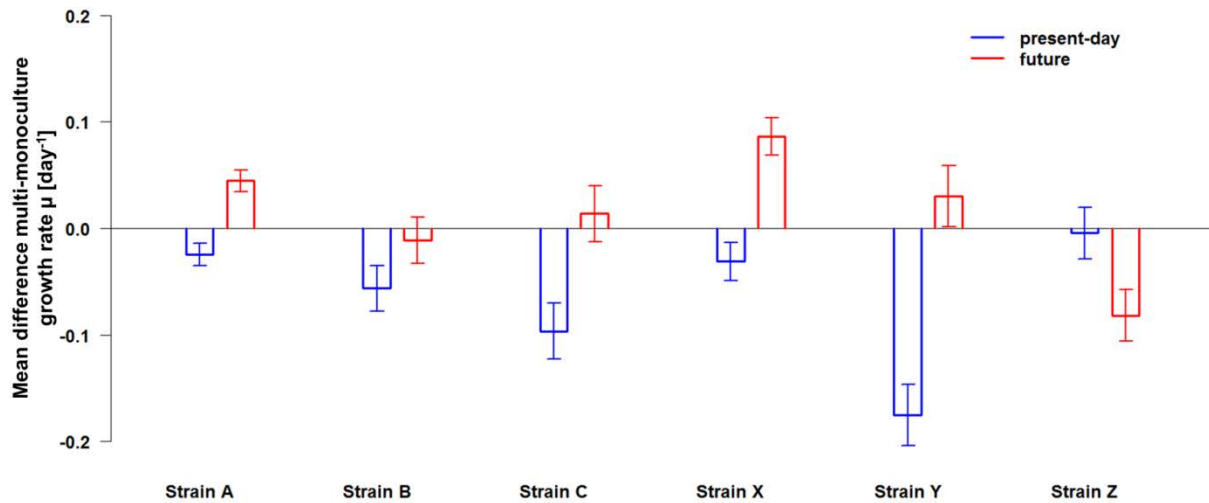
**Figure 4:** *Mean difference and standard error of each strain's growth rate in the multi-strain cultures (calculated from measured allele contributions) compared to the ones measured in monoculture. Since the diversity level was the only component changed, this represents the effect of genotype interactions.*

## DISCUSSION

### Wide and diverse temperature and $CO_2$ niches within the same population

All six strains tested grew well in the three applied treatments, i.e. the ambient conditions ('present-day scenario'), warming alone ('warming scenario') and warming in combination with elevated $CO_2$ ('future scenario'). The phenotypic treatment responses of strains were in the same range as the intraspecific variability among them under present-day conditions (Figure 1a, c). Provided that plastic responses can be maintained, we can assume that all strains have a sufficiently wide fundamental ecological niche to sustain growth and productivity under conditions anticipated for the coming decades. This is in line with reaction norms of *T. hyalina* strains isolated 2 years earlier from the same location (Wolf et al., 2018). Both datasets also show that underlying reaction norms appear to diverge between strains, which may be due to different physiological intracellular fine-tuning. The differences in growth rate constants caused by treatments as well as those between individuals varied in the range of 0.05 to 0.1 day$^{-1}$ (Figure 1a, b). While this may not seem much in absolute terms, this range is comparable with differences observed between species (e.g. Pardew et al., 2018; Schlie & Karsten, 2016) and certainly ecologically relevant as is readily visible in the predicted population composition (Figure 2c, d).

Although most strains did exhibit reproducible trait changes in response to the applied treatments, the pooled mean responses of all strains were hardly affected (Table S1). The treatment effects differed between strains in both magnitude and direction. The growth responses among strains towards high temperature and $CO_2$ (future scenario) were especially diverse, with rate changes between -7% and +8% compared to the present-day scenario (Figure 1b). Elevated temperature alone (warming scenario) often had a different effect than in combination with high $CO_2$: unlike usually expected for cold-adapted species (Eppley, 1972; Kremer et al., 2017; Thomas et al., 2012), three out of six strains grew slower and only one faster at 7°C compared to 2°C under present pCO$_2$. While POC production did not show a uniform development across strains either, the majority of strains decreased its rate in the future scenario, and even more so under warming alone. The relevance of these intraspecific differences is supported by the fact that the applied linear models were judged to be the best when including both, strain identity and treatment, including their interaction (summary in Table S3a). Furthermore, comparing models using only treatment or strain identity as explanatory variable, the ones using strain identity always explained the data better than those using treatment, showing that phenotypic intraspecific differences can surpass the influence of the applied future ocean scenario.

It has been assumed that treatments in experiments with natural assemblages carried out over tens of generations select for individuals with different response optima from the standing diversity (Collins et al., 2014; Scheinin et al., 2015; Wolf et al., 2018). This is partly because even if novel mutations do provide beneficial alleles, they would not have sufficient time to reach high frequencies unless they fall far outside the range of the present standing variation. In this study, two out of three strains from each of the two isolation backgrounds in the preceding natural community incubation grew faster in the treatment most resembling

their origin (i.e. strain A, B, C from the present-day vs. strain X, Y, Z from future conditions; Figure 1b). A similar pattern emerges when taking all measured parameters into account (e.g. in a Principal Component Analysis, Figure S2). This could be taken as a hint towards strain sorting within the former selection environment. Since six strains are a small sample size and the responses are not uniform, however, this cannot clearly support or falsify the idea of intraspecific sorting in the community incubation as hypothesized in Hoppe et al. (2018b).

Comparing all six strains in different scenarios, neither of the drivers had a generally positive or negative effect (Figure 1a-d). Due to such complexity of physiological responses, we cannot expect to find a representative trend in reactions to warming and high $CO_2$ using a small number of strains, even if they originate from the same population. This renders commonly applied parametrizations of climate change effects based on upscaling of physiological responses very difficult. Nevertheless, the demonstrated differences in growth rate of some strains hold a strong potential for rapid intraspecific sorting and thus for a rapid selection within a population. By applying allele-specific quantitative PCR, to our knowledge for the first time in such a setup, we were able to follow such sorting processes directly over short timescales and thus to resolve how this potential was realized in a simplified community.

**Rapid strain sorting in the future but not in the present-day scenario**

As described in the introduction, several ways of predicting the genotypic composition and yield of multi-strain cultures from its components in monoculture have been suggested. None of them fully explains our results as shown in Table 2. In line with selection effects, only under future conditions, the multi-strain growth rate resembled that of the fastest growing strain in monoculture. Here, all multi-strain cultures were subject to strong selection with the same strain dominating after 14 generations with 43-47% (strain Y). Compared to predictions based on growth rates in monocultures, sorting was even more pronounced in this treatment than anticipated (Figure 2b vs. d). The strong selection observed within the future scenario populations verifies the notion that strain sorting is a realistic mechanism that can strongly influence population composition and performance even on short timescales relevant for bloom dynamics (Godhe & Rynearson, 2017; Scheinin et al., 2015).

However, such directed rapid sorting does not always occur, as was revealed in the present-day scenario incubations (Figure 2a). Under those conditions, the fastest strain in monoculture failed to dominate the multi-strain cultures and bulk population growth rather resembled the lowest rate measured in the respective monoculture (Figure 1a, Table 2). Here, the strain composition provided little evidence for selection in the way that monoculture growth rates would suggest, since strain abundances diverged slightly less and with different strain proportions than predicted (Figure 2a vs. c). This suggests that strains must have changed their growth rate in multi-strain compared to monoculture. Hence, some other component of fitness must have been under selection in the colder, ambient $pCO_2$ environment, and the different strains seem to be roughly of equal fitness. Only strain Y, which dominated the future scenario, slightly decreased in frequency. This possibly hints towards a tradeoff causing divergent competitive abilities under the two treatments. Thus, in

both scenarios, the sorting processes in the multi-strain cultures show different dynamics than monoculture responses would suggest (Figure 2, Figure S3 and Table S1).

Furthermore, bulk growth rates of the whole population in the future scenario were found to be significantly higher than predicted from monoculture responses at the observed genotypic composition (Figure 3, Table 2). In the present-day scenario, however, the population growth rate was similar to the one predicted. In both scenarios, POC production was far lower than any prediction based on monoculture traits (Figure 3, Table 2). The reduced POC productivity in multi-strain cultures (Figure 1c and 3) is opposing the concept that diverse communities are more or at least equally productive compared to monocultures (Hector, 1998). In phytoplankton, however, this concept has been supported mainly in terms of growth rate instead of productivity (e.g. Bell, 1991; Hattich et al., 2017) and even for growth rate, negative diversity effects have been described (Roger et al., 2012). In an experimental evolution study, Collins (2010) found indeed that multi-strain cultures arrived repeatedly at lower yields than their respective monocultures after adapting to elevated $CO_2$, suggesting that genotypic diversity may act as a separate and potentially costly driver of adaptation. It has also been proposed that cell division rates lower than the unevolved original plastic response may be adaptive under long-term $CO_2$ enrichment (Collins, 2016; Schaum & Collins, 2014).

In spite of altered growth rates in the two scenarios in monoculture and considerable reshuffling in their strain composition, POC production changed remarkably little across the applied scenarios in all multi-strain cultures (Figure 1 c, d). Interestingly, this corresponds with theories on insurance effects (Yachi 1999) as well as with the primary production estimates of the community incubation the strains were originally isolated from, which did not change in different treatments either (Hoppe et al., 2018b; data KFb). It thus appears feasible that the mechanisms stabilizing POC production in our simplified populations may have contributed to the compensation of $CO_2$ effects in the natural assemblages, even though we cannot say to what extent. The stability of POC production in the multi-strain cultures is an effect of the opposing trends of growth rate and POC quota in both treatments. Hence, populations did not become more or less productive, which is also in line with the stable photophysiology (Table S1 and S3d), but merely reallocated their energy budget towards faster division rates in the future and increased carbon storage in the present-day treatment (c.f. Behrenfeld et al., 2008).

Considering the consistent divergence of the predicted and observed multi-strain bulk responses of POC and Chl *a* (Figure 3, Table 2), we can conclude that strains must also have changed their cellular quota growing alone compared to growing together with others. This means that, although highly reproducible, the genotypic composition as well as the cumulative traits even of a simplified population is not predictable from the strains' responses to the same treatments in monoculture. Since we controlled for all other confounding influences (e.g. all cultures were previously acclimated and remained in exponential growth under stable irradiances and nutrient-replete conditions), the single difference between the mono- and multi-strain cultures was their genotypic diversity. We therefore hypothesize that individuals alter their phenotype in response not only to their physico-chemical surroundings, but also to

their intraspecific context: the presence of other conspecific genotypes (i.e. diversity) may be a cryptic driver for trait responses that has often been neglected so far.

**Diversity as an additional response driver**

If the proximity of other conspecific genotypes acts as an additional driver, we should be able to quantify it by comparing the observed properties of the multi-strain incubations with the predicted ones (Figure 4). Indeed, for most parameters, this difference was reproducible and significant (Table 2). The scale and variability of this effect on growth rate within and between strains is similar to that of altered temperature and $pCO_2$ (cf. Figure 4 and 1b). Moreover, the resulting genotypic composition of populations was highly reproducible in all our incubations, a pattern that we also see in previous intraspecific competition experiments under a multitude of treatments (Bell, 1991; Collins, 2010; Lohbeck et al., 2012; Roger et al., 2012; Sjöqvist & Kremp, 2016). This suggests that the divergence of mono- and multi-strain culture responses is not a random artefact, but a definable eco-evolutionary driver that we simply do not understand yet.

Biomass buildup and strain composition, being the final consequences of all drivers combined in a multi-strain culture, may be understood as the result of an interplay of several selection pressures. Since the strongest drivers shape the community response the most, they are usually considered its best predictor (Boyd et al., 2015; Brennan et al., 2017). Therefore, the most successful individual in a selection environment is not necessarily adapted to be the fastest grower in a laboratory monoculture (Bach et al., 2018; Schaum & Collins, 2014), but is determined by the strongest drivers in the fitness landscape of interest. Under the future scenario, sorting in the multi-strain culture was much better predicted by the monoculture responses than under the present-day scenario (Figure S3: correlation coefficient R: present-day= -0.33, future= 0.67). This suggests that the effect of diversity was larger under present-day than under future conditions for most strains (Figure 4). We can assume that elevated temperature and $pCO_2$ exposed strains to stronger selection pressures than the present-day scenario, where experimental conditions resembled the environmental history of the strains. Therefore, in the future scenario, these abiotic treatment effects (Figure 1b, d) may have been more influential than the effect of intraspecific diversity (Figure 4). This could have caused our monoculture-based predictions to be more accurate for the future scenario, while in the present-day scenario biological interactions may have had a larger impact, causing the selective outcome to be less predictable from monoculture responses.

As quantified in this study, organisms seem to modulate their phenotype in response to the presence of other conspecific genotypes. A similar effect has been observed in incubations of a coccolithophore (Bach et al., 2018). There are numerous ideas for the underlying explanations of such diversity effects, and it is possible that they are caused by several interacting mechanisms at once, whose effects may add up or oppose each other. Explanations include direct and indirect competitive interactions (Collins, 2010), e.g. by chemical cues, mutual facilitation between genotypes (John et al., 2015), nutrient partitioning (Vanelslander et al., 2009), or interactions with the prokaryotic microbiome (Amin et al., 2015; Camarena-Gomez et al., 2018). However, direct evidences for such mechanisms in phytoplankton that

surpass assumptions are still rare and mainly descriptive (Brodie et al., 2017; Lima-Mendez et al., 2015). In the future, we need to gain a mechanistic understanding as to whether such effects can be explained by chemical cues or rather by more indirect competitive advantages.

**Ecological implications**

Our study suggests that intraspecific selection may have a larger impact when environmental conditions increasingly diverge from the environmental history of populations. Thus, intraspecific selection could buffer (or amplify) measurable effects on other levels, like species composition, productivity and elemental stoichiometry (Hoppe et al., 2018b). If strain sorting in response to warming and acidification entails extinctions in the long run, intraspecific diversity will decrease, and so would the species' adaptive capability towards other appearing pressures (e.g. nutrient limitation as the bloom enters a stationary phase). However, all existing evidence suggests that diatom populations are highly diverse (Godhe & Rynearson, 2017) and unlikely to be destabilized by moderate environmental shifts, especially in fluctuating environments (Gsell et al., 2012). Even in our comparably small artificial community of six strains and despite considerable sorting in the future scenario, measures of diversity like Pielou's evenness index remained high until the end of the experiment (0.85, Table 2). However, to fully answer the ecologically important question to which extent and into which direction intraspecific selection may eventually alter the diversity and productivity of future phytoplankton populations, we need to move towards experimental setups with increasingly realistic diversity and environmental variability levels (Kroeker et al., 2017; Sjöqvist & Kremp, 2016). This is particularly important as it is still challenging to resolve these processes in natural populations with commonly used methods since intraspecific diversity is often too high to identify such patterns (e.g. Godhe et al., 2016; Ruggiero et al., 2017; Rynearson & Armbrust, 2005).

Several conclusions can be drawn from this study: We add evidence to the increasingly recognized view that individuals of the same population do not apply one uniform strategy in response to elevated temperature and $pCO_2$. At the same time, within our experimental climate change scenario, even a low strain diversity buffered changes in the bulk productivity of the population. To which extent such stability can be generalized needs to be investigated, also in the context of other stressors (e.g. light or nutrient limitation). The high resolution of the strain composition of our multi-strain experiment allowed us to reveal two novel aspects: Firstly, different components of fitness seem to be under selection in different environments causing diverging selection dynamics and outcomes. Secondly, in the presence of conspecific genotypes, individuals alter their phenotype and do so to a similar extent as in response to our abiotic treatments. This provides further evidence that simple upscaling of single strain responses to populations is not reasonable without a better understanding of the mechanisms shaping intraspecific selection. Evaluating genotypic diversity as an additional, potentially quantifiable driver may be a step towards making natural community responses more predictable from laboratory experiments.

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**Supplement Material Wolf et al. "Company Matters"**

## Microsatellite development

For the development of microsatellite primers, DNA was extracted from three exponentially growing axenic monocultures of *T. hyalina* (one isolated in 2014, two in 2016). DNA was sequenced (150 bp paired-end) on an Illumina NextSeq500 sequencer (Illumina, San Diego, USA). Illumina BCL files were converted to fastq files and de-multiplexed using bcl2fastq (v2.17, Illumina) with default settings. Sequence reads were trimmed and assembled with CLC Genomics Workbench v9 (CLC bio, Qiagen, Germany). Microsatellites were identified within those three genome assemblies and characterised in Phobos v3.3.12 (Mayer et al., 2010) using a maximum unit length of 10, maximal mismatch score of -4, maximal gap score of -4 and maximum score reduction of 3. For qualified tandem repeats, 300 bp of flanking region were exported together with the microsatellite. A second output file was generated in Phobos by masking all microsatellites in the genome assemblies. Subsequently, the two output files per genome assembly were compared using in-house scripts and manual inspections to obtain only those loci with at least 50 bp of flanking regions on each site, at least 50 bp between two microsatellites and a minimum normalized repeat length of 4. Shared loci among the three individuals were identified by mapping one genome assembly against the others using the mem module of bwa (Li & Durbin 2009). The output format was transformed with samtools (Li et al. 2009). Mapped microsatellites were filtered to include only those without alternative hits, without soft masking (meaning low alignment quality) and with mapQ values above 25. Microsatellites with indels or substitutions in the 50 bp flanking region were excluded. Nine microsatellites with differing tandem repeat length for all three individuals were thus identified.

The according microsatellite primers (Table 1) were tested on eight *T. hyalina* strains with and without fluorescent markers (FAM, HEX, AT), in single and multiplex conditions. The following optimized PCR conditions were applied for all primers with the Type-it Microsatellite PCR kit (Qiagen, according to providers instructions) in a thermal cycler (Mastercycler Nexus gradient, Germany): 5min at 94°C prior to 30 cycles of 30s at 94°C, 90s at 57°C, 40s at 72°C and a final elongation step at 72°C for 10 min.

For microsatellite application and fragment size analysis PCR products were diluted with nucleotide-free water at 1:45 (1:35 for Mutiplex-PCR). Subsequently, 1 µL was added to 15 µL of Hi-Di formamide (Applied Biosystems, Germany) and 0.3 µL of the size marker genescan-500 [ROX] (Applied Biosystems). Size analysis was performed by capillary electrophoresis on a 3130xl Genetic Analyzer (Applied Biosystems). Microsatellite alleles were scored using Genemapper (version 4, Applied Biosystems). The software Arlequin (version 3.5.2.2; Excoffier & Lischer, 2010) was used to test pairs of loci for linkage disequilibrium. The acquired loci were characterized in DNA of 364 samples of single strains from a *T. hyalina* population collected 2016 in Svalbard (Wolf et al., in prep.). Six differently polymorphic (4-24 alleles) loci were found to yield reliable results in *T. hyalina* (Table 1) and were named 'ThKF' for *T. hyalina* from Kongsfjord. In this study, two of them (primer ThKF3 and 7) were applied for allele-specific-quantitative PCR (John et al., 2015; Meyer et al., 2006).

*Figure S1*: *Regressions of linearity test for asqPCR (2 examples) evaluating the allele frequencies measured in an artificial mixture of known DNA concentration from each strain: a) Regression of allele 208 with primer ThKF3 (unique for strain Y) b) Regression of allele 233 with primer ThKF7 (unique for strain B). Measured concentrations have a maximum of 50% because the strains contain the respective allele heterozygously. All alleles yielded a linear relationship with an R²-value > 0.99.*
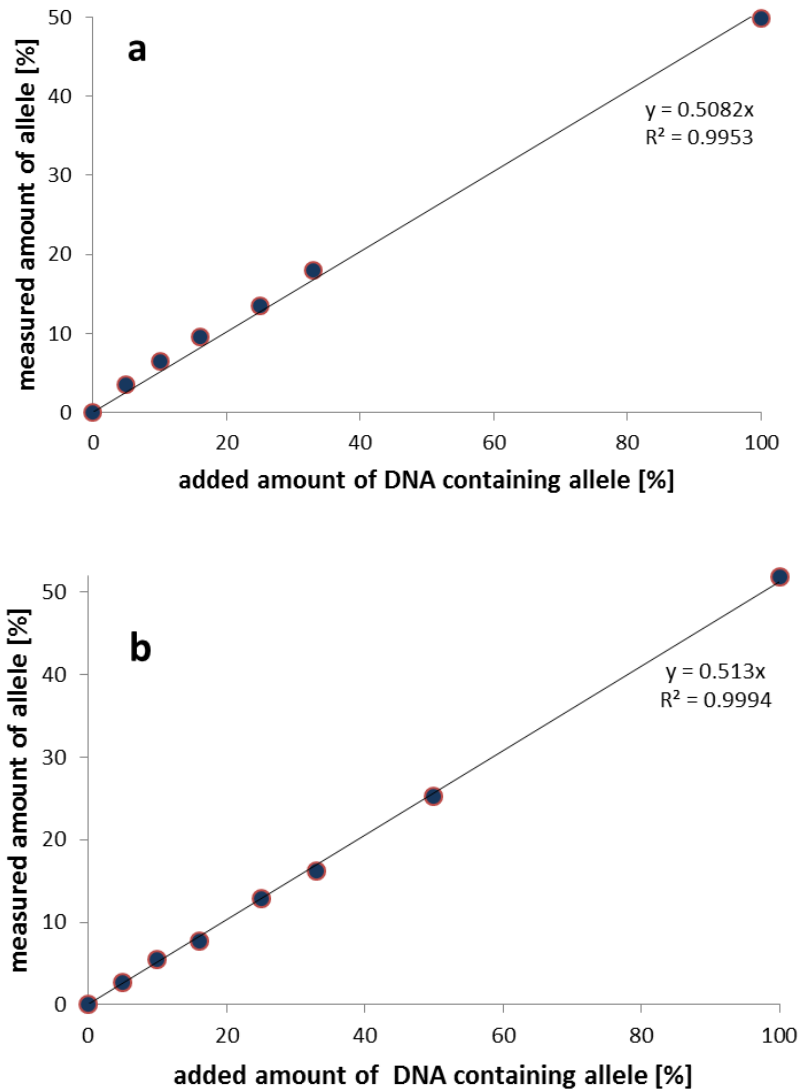
*Figure S2:* *Principal Component Analysis (PCA) of the physiological responses of strains in monoculture and the multi-strain culture (Mix) in the present (blue) and the future (red) scenario. Analysis based on the parameters: μ, Chl a quota, POC quota, POC production, C:N ratio and Chl a:POC ratio. Two loose clusters appear in the present-day condition (containing strains B, C, X and A, Y, Z), while strains are more spread out in future treatment. While the multi-strain culture (Mix) of the present-day treatment is close to the center (i.e. the mean of strains), it deviates more from the center under future conditions. The two components depicted here explain 78% of the variance.*
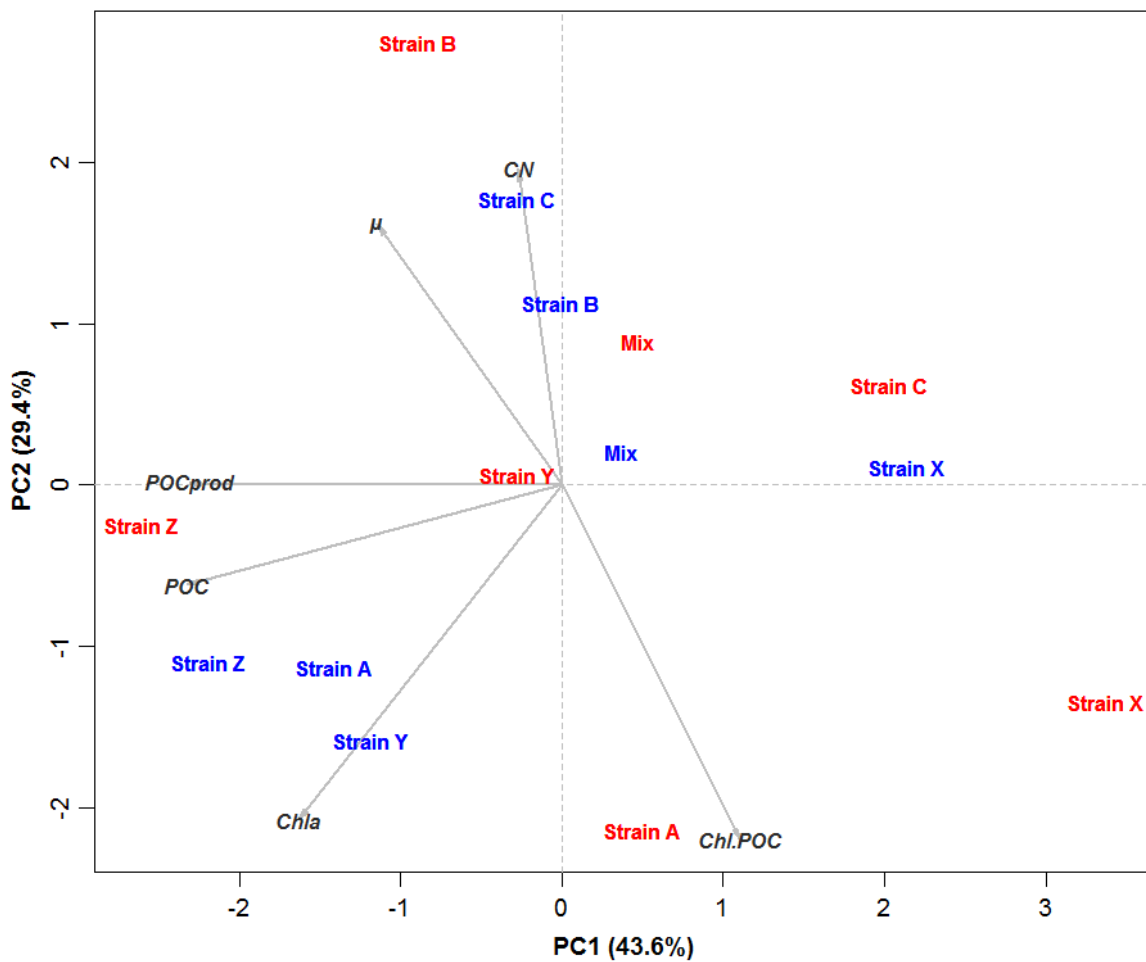
*Figure S3:* *Correlation of predicted and observed contributions of each strain in the multi-strain incubations using Pearson's R under the a) present-day (R= -0.33) and b) future scenario (R= 0.67).*
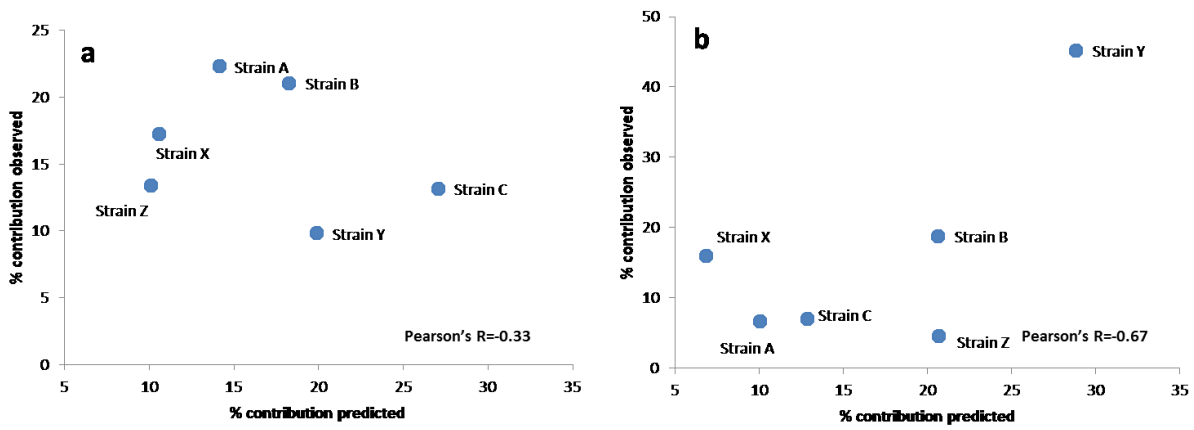
*Figure S4: Stacked relative strain contribution in the multi-strain culture in % as observed via asqPCR and predicted from monoculture growth rates in the present-day and the future scenario.*
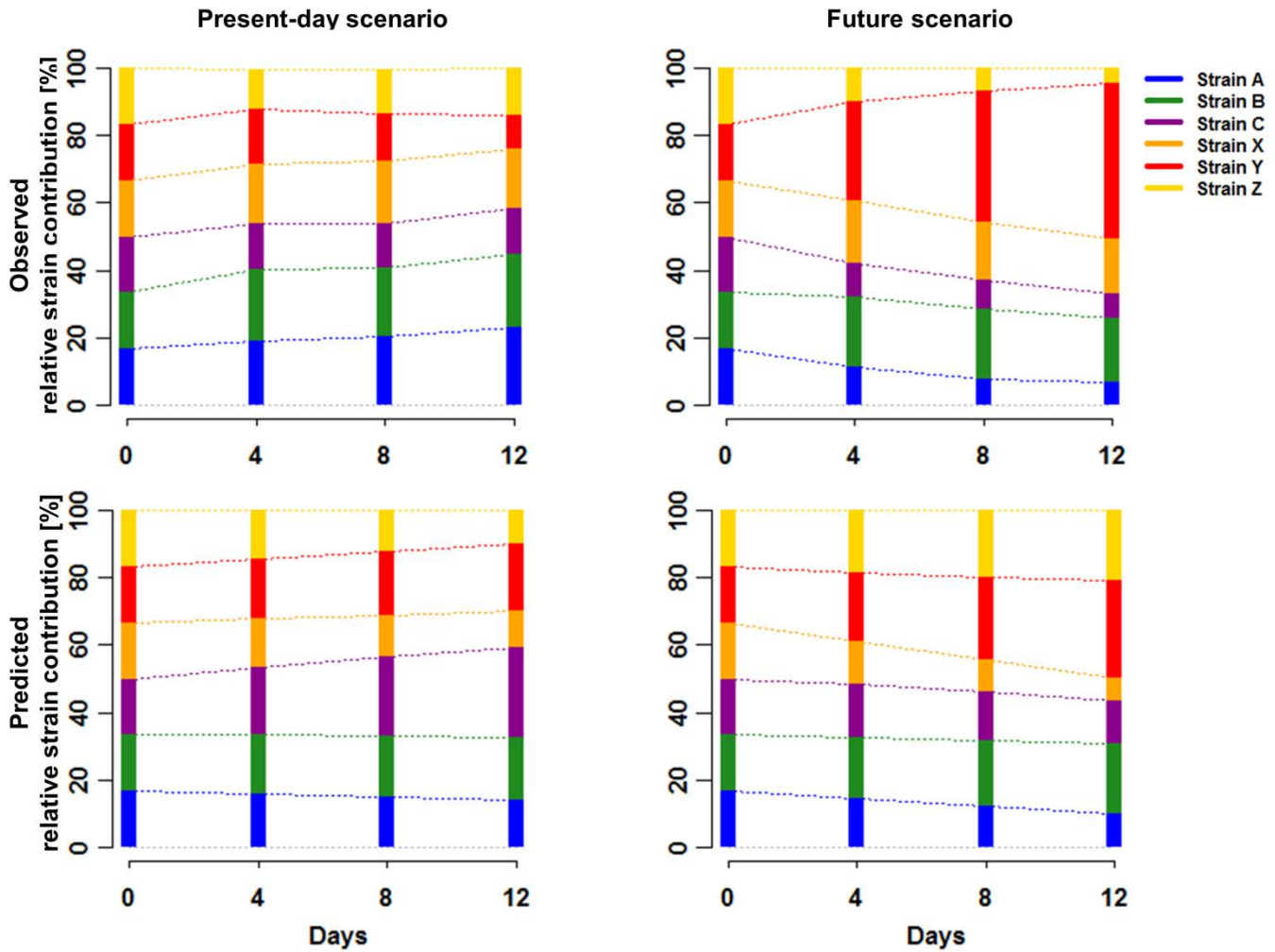
*Table S1: Physiological strain responses in monoculture and bulk responses of multi-strain cultures. Relative contribution shows the observed relative frequency of each strain in the multi-strain incubation at the final time point of the experiment (13-14 generations) in percent. α refers to maximum light-use efficiency (initial slope α, mole·m² (mol RCII)⁻¹ (mol photons)⁻¹), $ETR_{max}$ describes the maximum absolute electron transport rate through photosystem II (mol e⁻(mol RCII)⁻¹ s⁻¹), in-situ ETR refers to the irradiance of the growth conditions. NA stands for not acquired or not applicable.*

| culture | treatment | μ [day⁻¹] | contribution [%] | k [day⁻¹] | POC [pg cell⁻¹] | POC production [pg cell⁻¹ day⁻¹] | Chla [pg cell⁻¹] | C:N [mol:mol] | POC:Chla [g:g] | alpha | $ETR_{max}$ | In-situ ETR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Strain A | present | 0.76 ± 0.01 | 22 ± 2 | 1.10 ± 0.02 | 300 ± 14 | 330 ± 9 | 10.7 ± 0.2 | 4.9 ± 0.04 | 28.0 ± 1.7 | 1.37 ± 0.04 | 61.5 ± 6.8 | 45.0 ± 4.3 |
| | future | 0.74 ± 0.01 | 7 ± 2 | 1.06 ± 0.01 | 246 ± 5 | 262 ± 3 | 10.6 ± 1.2 | 4.8 ± 0.01 | 23.4 ± 2.2 | 1.28 ± 0.07 | 72.1 ± 3.4 | 56.6 ± 5.4 |
| | warming | 0.77 ± 0.01 | NA | 1.11 ± 0.01 | 252 ± 13 | 279 ± 13 | 9.2 ± 0.1 | 4.8 ± 0.2 | 27.5 ± 1.5 | NA | NA | NA |
| Strain B | present | 0.78 ± 0.03 | 21 ± 1 | 1.13 ± 0.04 | 241 ± 8 | 273 ± 15 | 7.9 ± 0.3 | 5.5 ± 0.7 | 30.6 ± 2.0 | 1.41 ± 0.04 | 60.9 ± 3.5 | 43.3 ± 3.4 |
| | future | 0.8 ± 0.02 | 19 ± 2 | 1.15 ± 0.04 | 264 ± 1 | 304 ± 9 | 7.0 ± 0.1 | 6.0 ± 0.7 | 37.9 ± 0.6 | 1.32 ± 0.06 | 44.5 ± 1.4 | 33.7 ± 2.5 |
| | warming | 0.74 ± 0.01 | NA | 1.07 ± 0.01 | 190 ± 14 | 202 ± 14 | 5.3 ± 0.5 | 4.5 ± 0.9 | 35.7 ± 3.2 | NA | NA | NA |
| Strain C | present | 0.82 ± 0.03 | 13 ± 1 | 1.18 ± 0.04 | 241 ± 30 | 285 ± 45 | 6.3 ± 0.8 | 4.7 ± 0.2 | 38.0 ± 0.2 | 1.26 ± 0.00 | 57.9 ± 3.0 | 45.9 ± 2.3 |
| | future | 0.76 ± 0.02 | 7 ± 1 | 1.09 ± 0.03 | 185 ± 8 | 202 ± 6 | 6.2 ± 0.3 | 4.8 ± 0.1 | 29.9 ± 0.4 | 1.26 ± 0.03 | 49.0± 0.1 | 38.9 ± 0.8 |
| | warming | 0.75 ± 0.03 | NA | 1.08 ± 0.04 | 203 ± 4 | 220 ± 5 | 7.4 ± 0.2 | 4.6 ± 0.4 | 27.5 ± 0.3 | NA | NA | NA |
| Strain X | present | 0.74 ± 0.03 | 17 ± 1 | 1.07 ± 0.04 | 187 ± 11 | 200 ± 9 | 7.5 ± 0.2 | 5.5 ± 0.3 | 25.0 ± 1.3 | 1.37 ± 0.10 | 38.8 ± 1.1 | 28.4 ± 2.7 |
| | future | 0.71 ± 0.02 | 16 ± 2 | 1.02 ± 0.03 | 164 ± 5 | 167 ± 9 | 6.5 ± 0.4 | 4.2 ± 1.0 | 25.5 ± 2.5 | 1.31 ± 0.03 | 52.1 ± 2.0 | 39.9 ± 2.4 |
| | warming | 0.8 ± 0.02 | NA | 1.15 ± 0.03 | 170 ± 16 | 196 ± 23 | 7.0 ± 1.0 | 4.4 ± 1.4 | 24.7 ± 4.0 | NA | NA | NA |
| Strain Y | present | 0.79 ± 0.01 | 10 ± 3 | 1.14 ± 0.01 | 281 ± 8 | 321 ± 12 | 11.2 ± 0.5 | 4.7 ± 0.1 | 25.1 ± 0.8 | 1.25 ± 0.02 | 46.8 ± 6.7 | 37.3 ± 4.7 |
| | future | 0.82 ± 0.01 | 45 ± 2 | 1.19 ± 0.02 | 239 ± 2 | 284 ± 2 | 8.8 ± 0.4 | 4.8 ± 0.1 | 27.3 ± 1.3 | 1.04 ± 0.03 | 55.4 ± 6.4 | 53.5 ± 6.7 |
| | warming | 0.73 ± 0.05 | NA | 1.05 ± 0.07 | 225 ± 11 | 236 ± 28 | 9.3 ± 0.6 | 4.8 ± 0.1 | 24.1 ± 0.4 | NA | NA | NA |
| Strain Z | present | 0.73 ± 0.03 | 13 ± 1 | 1.06 ± 0.04 | 344 ± 35 | 365 ± 44 | 10.4 ± 0.2 | 4.7 ± 0.1 | 33.2 ± 3.5 | 1.24 ± 0.03 | 66.0 ± 1.9 | 53.2 ± 2.9 |
| | future | 0.8 ± 0.002 | 5 ± 0 | 1.15 ± 0.00 | 330 ± 37 | 380 ± 43 | 10.1 ± 0.1 | 4.8 ± 0.3 | 32.6 ± 3.2 | 1.28 ± 0.01 | 56.4 ± 1.4 | 44.1 ± 1.1 |
| | warming | 0.71 ± 0.03 | NA | 1.02 ± 0.04 | 271 ± 6 | 276 ± 9 | 9.5 ± 0.5 | 5.2 ± 0.9 | 28.6 ± 0.9 | NA | NA | NA |
| Mean Monocultures | present | 0.77 ± 0.03 | NA | 1.11 ± 0.05 | 266 ± 55 | 296 ± 58 | 9.0 ± 2.0 | 5.0 ± 0.4 | 30 ± 5.1 | 1.32 ± 0.07 | 55.3 ± 10.3 | 42.2 ± 8.5 |
| | future | 0.75 ± 0.03 | NA | 1.08 ± 0.05 | 219 ± 38 | 235 ± 36 | 7.9 ± 1.7 | 4.7 ± 0.3 | 29.4 ± 1.1 | 1.25 ± 0.11 | 54.9 ± 9.5 | 44.5 ± 8.9 |
| | warming | 0.77 ± 0.01 | NA | 1.11 ± 0.01 | 238 ± 14 | 266 ± 15 | 8.2 ± 0.4 | 4.9 ± 0.4 | 28.0 ± 4.2 | NA | NA | NA |
| Multi-Strain | present | 0.73 ± 0.02 | NA | 1.06 ± 0.02 | 217 ± 5 | 230 ± 1 | 7.1 ± 0.2 | 4.9 ± 0.1 | 30.6 ± 0.7 | 1.29 ± 0.04 | 61.1 ± 3.9 | 47.6 ± 3.0 |
| | future | 0.81 ± 0.02 | NA | 1.18 ± 0.02 | 187 ± 4 | 219 ± 5 | 6.8 ± 0.3 | 4.9 ± 0.1 | 27.5 ± 1.4 | 1.31 ± 0.03 | 60.9 ± 3.7 | 46.6 ± 2.4 |

***Table S2:*** *Carbonate chemistry of each culture as mean and standard deviation of biological replicates: $pCO_2$ was calculated from measured pH and total alkalinity (TA) at the final time-point of incubation using $CO_2SYS$ (Pierrot et al., 2006) with the respective temperature, salinity of 32 and concentrations of 6.5 $\mu mol\ kg^{-1}$ for phosphate and 100 $\mu mol\ kg^{-1}$ for silicate.*

| Culture | Treatment | calculated $pCO_2$ [µatm] | pH $_{NBS}$ | TA [µmol kg$^{-1}$] |
|---|---|---|---|---|
| **Strain A** | present | 378 ± 11 | 8.14 ± 0.01 | 2212 ± 18 |
| **Strain A** | high temp | 389 ± 12 | 8.13 ± 0.01 | 2123 ± 9 |
| **Strain A** | future | 1191 ± 25 | 7.70 ± 0.01 | 2232 ± 16 |
| **Strain B** | present | 377 ± 10 | 8.15 ± 0.01 | 2226 ± 7 |
| **Strain B** | high temp | 407 ± 9 | 8.11 ± 0.01 | 2114 ± 6 |
| **Strain B** | future | 1249 ± 18 | 7.68 ± 0.01 | 2205 ± 2 |
| **Strain C** | present | 339 ± 9 | 8.19 ± 0.01 | 2242 ± 10 |
| **Strain C** | high temp | 433 ± 5 | 8.09 ± 0.00 | 2123 ± 7 |
| **Strain C** | future | 1229 ± 30 | 7.69 ± 0.01 | 2237 ± 4 |
| **Strain X** | present | 414 ± 4 | 8.08 ± 0.00 | 2102 ± 2 |
| **Strain X** | high temp | 427 ± 4 | 8.09 ± 0.00 | 2114 ± 8 |
| **Strain X** | future | 1180 ± 33 | 7.70 ± 0.01 | 2237 ± 10 |
| **Strain Y** | present | 408 ± 6 | 8.09 ± 0.01 | 2121 ± 10 |
| **Strain Y** | high temp | 416 ± 11 | 8.1 ± 0.01 | 2116 ± 1 |
| **Strain Y** | future | 1152 ± 22 | 7.72 ± 0.01 | 2254 ± 13 |
| **Strain Z** | present | 396 ± 9 | 8.13 ± 0.01 | 2238 ± 2 |
| **Strain Z** | high temp | 412 ± 6 | 8.11 ± 0.01 | 2121 ± 9 |
| **Strain Z** | future | 1191 ± 11 | 7.70 ± 0.00 | 2222 ± 4 |
| **Multi-Strain** | present | 350 ± 7 | 8.18 ± 0.01 | 2263 ± 40 |
| **Multi-Strain** | future | 1095 ± 65 | 7.74 ± 0.03 | 2238 ± 16 |

***Table S3a:*** *Linear models of all parameters measured in monoculture in the three treatments (present-day, high-temperature, future) with strain or treatment as the only explanatory variables or with both including their interaction (strain\*treatment). The p-value applies to the entire model and \* indicates slopes that are overall significantly different from 0. A lower AIC (Akaike Information Criterion) value indicates a relatively better quality of the model for the dataset. POC and Chl a are cell quota; alpha and ETR$_{max}$ describe maximum light-use efficiency and maximum as absolute electron transport rate through photosystem II, respectively.*

| Test | dependent variable | explanatory variable | p | significance (α=0.05) | AIC |
|---|---|---|---|---|---|
| linear model | μ | strain | 0.302 | | -328 |
| linear model | μ | treatment | 0.1216 | | -332 |
| linear model | μ | strain*treatment | 7.56E-07 | * | -376 |
| linear model | log(POC prod) | strain | 5.37E-08 | * | -179 |
| linear model | log(POC prod) | treatment | 0.01814 | * | -147 |
| linear model | log(POC prod) | strain*treatment | 2.84E-13 | * | -236 |
| linear model | Chl *a* | strain | 8.11E-14 | * | -5 |
| linear model | Chl *a* | treatment | 0.2337 | | 63 |
| linear model | Chl *a* | strain*treatment | 2.20E-16 | * | -60 |
| linear model | log(POC) | strain | 1.90E-11 | * | -214 |
| linear model | log(POC) | treatment | 0.04697 | * | -159 |
| linear model | log(POC) | strain*treatment | 2.20E-16 | * | -247 |
| linear model | log(Chl *a*:POC) | strain | 1.95E-08 | * | -226 |
| linear model | log(Chl *a*:POC) | treatment | 0.4075 | | -186 |
| linear model | log(Chl *a*:POC) | strain*treatment | 2.88E-10 | * | -259 |
| linear model | log(alpha) | strain | 0.0002527 | * | -154 |
| linear model | log(alpha) | treatment | 3.12E-02 | * | -139 |
| linear model | log(alpha) | strain*treatment | 8.88E-07 | * | -183 |
| linear model | log(ETR$_{max}$) | strain | 0.000179 | * | -160 |
| linear model | log(ETR$_{max}$) | treatment | 0.6458 | | -140 |
| linear model | log(ETR$_{max}$) | strain*treatment | 1.46E-07 | * | -195 |

**Table S3b:** *Linear models comparing monocultures in present-day and warming treatment (i.e. temperature effect). The p-value applies to the entire model and values lower than 0.05 indicate that slopes are overall significantly different from 0. A lower AIC (Akaike Information Criterion) value indicates a relatively better quality of the model for the dataset.*

| Test | dependent variable | explanatory variable | p | sign (α=0.05) | AIC |
|------|--------------------|--------------------|-----|---------------|-----|
| linear model | μ | Temperature | 0.06172 | | -227 |
| linear model | log(POC prod) | Temperature | 0.001813 | * | -109 |

**Table S3c:** *Linear models comparing monocultures in present and future treatment. The p-value applies to the entire model and values lower than 0.05 indicate that slopes are overall significantly different from 0. A lower AIC (Akaike Information Criterion) value indicates a relatively better quality of the model for the dataset.*

| Test | dependent variable | explanatory variable | P | sign (α=0.05) | AIC |
|------|--------------------|--------------------|-----|---------------|-----|
| linear model | μ | treatment*strain | 4.08E-06 | * | -265 |
| linear model | μ | treatment | 0.979 | | -215 |
| linear model | log(POC prod) | treatment*strain | 4.07E-07 | * | -149 |
| linear model | log(POC prod) | treatment | 0.177 | | -87 |

**Table S3d:** *One-way-ANOVAs of Multi-strain cultures comparing bulk responses measured in present and future scenario. The p-value applies to the entire model and values lower than 0.05 indicate that slopes are overall significantly different from 0.*

| Test | dependent variable | Group | F | P | sign (α=0.05) |
|------|--------------------|-------|-----|-----|---------------|
| One-way-ANOVA | μ | treatment | 62.71 | 0.000215 | * |
| One-way-ANOVA | POC prod | treatment | 3.999 | 0.0924 | |
| One-way-ANOVA | POC | treatment | 84.01 | 9.50E-05 | * |
| One-way-ANOVA | alpha | treatment | 0.797 | 0.406 | |
| One-way-ANOVA | In-situ ETR | treatment | 0.539 | 0.491 | |
| One-way-ANOVA | $ETR_{max}$ | treatment | 0.002 | 0.966 | |
| One-way-ANOVA | evenness | treatment | 100.9 | 0.000167 | * |

*Table S3e:* *One-way-ANOVAs of Multi-strain cultures comparing measured bulk responses with predictions based on strain composition and monoculture measurements as depicted in Table 2. The p-value applies to the entire model and values lower than 0.05 indicate that slopes are overall significantly different from 0.*

| Test | dependent variable | F | p | sign (α=0.05) |
|---|---|---|---|---|
| One-way-ANOVA | Present-day µ | 2.766 | 0.147 | |
| One-way-ANOVA | Present-day Chl *a* | 83.76 | 9.58E-05 | * |
| One-way-ANOVA | Present-day POC | 57.38 | 2.75E-04 | * |
| One-way-ANOVA | Present-day C:N (mol) | 0.279 | 0.616 | |
| One-way-ANOVA | Present-day Chl *a*:POC | 9.067 | 0.0237 | * |
| One-way-ANOVA | Present-day POC prod | 39.2 | 7.70E-04 | * |
| One-way-ANOVA | Future µ | 30.34 | 0.0015 | * |
| One-way-ANOVA | Future Chl *a* | 52.11 | 3.58E-04 | * |
| One-way-ANOVA | Future POC | 332.4 | 1.75E-06 | * |
| One-way-ANOVA | Future C:N (mol) | 6.01 | 0.0497 | * |
| One-way-ANOVA | Future Chl *a*:POC | 3.267 | 0.121 | |
| One-way-ANOVA | Future POC prod | 139.3 | 2.24E-05 | * |

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# Chapter 4   Publication III

**Revealing population dynamics of the Arctic diatom *Thalassiosira hyalina* using a novel microsatellite poolSeq barcoding (MPB) approach**

# Revealing population dynamics of the Arctic diatom *Thalassiosira hyalina* using a novel microsatellite poolSeq barcoding (MPB) approach

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# Chapter 4 Publication III

**ABSTRACT**

Whether an ecosystem remains stable under environmental change is not only decided at the level of species interactions, but also at the intraspecific level. In rapidly changing systems like the Arctic, the adaptive capacity of marine primary producers at the base of the foodweb plays a central role. Tracing intraspecific dynamics over space and time is an important approach for understanding population demography and adaptive processes. For methodological reasons, distinguishing populations to investigate their persistence and dynamics in natural contexts is still one of the major challenges in ecology and evolution, especially for planktonic microorganisms. Here we propose a new method, microsatellite poolSeq barcoding (MPB) that allows tracing allele frequency change in populations over time. We successfully applied the method to natural populations of the Arctic diatom *Thalassiosira hyalina* in community incubations as well as field samples. Comparisons with allele frequencies assessed by two established genotyping methods in different diversity contexts confirmed the validity of the new method. Using MPB, we could show allelic stability as well as shifts within a diatom population during an incubation experiment with natural phytoplankton communities and in field samples of two consecutive years. Our experimental studies revealed that a high $CO_2$ scenario combined with low temperature ('acidification' only) caused shifts at the intra- and interspecific level, while under other conditions (combined with warming or ambient) population composition remained surprisingly stable. This indicates that phenotypic plasticity may function as an effective response buffer under realistic future conditions, stabilizing ecosystem composition and functioning. In environmental samples, we found that the investigated natural diatom populations were homogenous and stable throughout a bloom season, but clearly differed between years. Our results add insights into the dynamics and potential role of selection and plasticity in natural diatom populations under stable experimental conditions as well as in a naturally variable environment across two bloom seasons. Furthermore, the novel MPB approach holds enormous potential to accelerate future data acquisition and facilitate the resolution of eco-evolutionary dynamics of natural populations in their environmental contexts.

**INTRODUCTION**

The way phytoplankton will respond to ongoing and future environmental changes is going to significantly impact earth system processes at many levels. Microscopic primary producers are the photosynthetic base of marine foodwebs worldwide and responsible for half of the global oxygen production (Field et al., 1998). In the Arctic environment, climate change has been progressing much faster than on global average (Larsen et al., 2014), which makes this region an important and ideal site to investigate organismic responses. Protists like phytoplankton are generally thought to be comparably resistant to rapid environmental changes because of their usually large census population sizes and short generation times of hours or days (Collins et al., 2014; Finlay, 2002). Whether effective population sizes are equally large, however, remains subject of debate (Gaebler-Schwarz et al., 2015; Watts et al., 2013). Furthermore, even minor changes in competitive abilities at the population level and species shifts within communities have the potential to strongly impact higher trophic levels and biogeochemical cycling and consequently the ecosystem functioning (Boyd et al., 2018; Hillebrand & Matthiessen, 2009; Rost et al., 2008).

Adjustments to changing environmental conditions can be driven by several mechanisms at different ecological levels. Every organism has the capacity to modify its phenotype within certain intrinsic limits by a plastic response to surrounding conditions (West-Eberhard, 2003). The optimal, or at least tolerable range of conditions within which an organism can dwell, define its plasticity. Since such plastic ranges can substantially differ within species (Alpermann et al., 2010; Brandenburg et al., 2018; Kremp et al., 2012; Pančić et al., 2015; Wolf et al., 2018), a population can furthermore adjust its genotypic composition, with the best adapted individuals being favored by selection, which is referred to as strain or lineage sorting (Becks et al., 2010; Collins et al., 2014; Scheinin et al., 2015). Whenever a change in the genetic composition of a population or species increases its fitness, this is called adaptive evolution and is typically steered by directional (adaptive) selection (Reznick & Ghalambor, 2001). Individuals that are better adapted to altered conditions than others can either emerge from new mutations, migrate from other locations or be already present within the standing genetic stock of a population. Consequently, populations with a wider range of diverse genotypes are more likely to contain some that are phenotypically fit for a new condition and can potentially adjust rapidly to occurring change. This is why large and highly diverse populations, such as found in phytoplankton, are considered to be more resistant to environmental variability (Bernhardt & Leslie, 2013; Yachi & Loreau, 1999), and why selection from standing genetic variation is likely to be essential for population resilience.

Data on spatio-temporal population dynamics can provide important insights into mechanisms and timeframes of such selective processes, and therefore into the potential of populations to adapt (Rynearson & Armbrust, 2004). However, detecting and tracing intraspecific characteristics of populations in space and time is particularly challenging for unicellular phytoplankton species. The often vast intraspecific diversity of phytoplankton is one of the reasons why such dynamic and selective processes within populations are methodologically so difficult to resolve. Only few studies have found indications for lineage

sorting in marine environments (Ruggiero et al., 2017; Scheinin et al., 2015). The main challenge in population genetic studies is the fact that individuals can only be distinguished via highly polymorphic loci. Typical approaches, such as amplified length polymorphisms (AFLP), single nucleotide polymorphsims (SNP analyses) or microsatellite analyses are applied for microalgae (Gaebler-Schwarz et al., 2015; Medlin & Töbe, 2011; Rengefors et al., 2017; Tahvanainen et al., 2012). Such neutral markers can detect selection only if the investigated loci are linked to genes under selection ('hitchhiking'; Weigand & Leese, 2018) but can allow inferences on genotypic diversity and population characteristics. Furthermore, since such methods usually cannot be applied directly in diverse community samples, they have to be performed separately for every strain. Consequently, for a single population sample, an adequate number of single cells has to be isolated and subsequently cultivated in monoculture in order to retrieve sufficient amounts of DNA for analysis. Since this is a demanding and highly time-consuming task (Medlin et al., 2000), such studies are forced to rely on the statistical power of subsamples, containing optimally a few dozens to hundreds of individuals while populations consist of billions of cells (Baverstock & Moritz, 1990). Due to the intricateness of this process, researchers are caught in a practical tradeoff between a higher resolution per population (i.e. number of isolates) and a higher frequency of temporal or spatial sampling at the cost of genotypic resolution. Pool Sequencing (PoolSeq; Futschik & Schlötterer, 2010) is a method that has been established in recent years and allows the analysis of single nucleotide polymorphisms for whole populations through a shotgun or targeted approach by mixing strains, tissue or DNA of conspecifics and sequence them all together. Although this allows the analysis of many individuals at once, it requires samples that contain only organisms of the targeted species, which is impossible to guarantee in environmental samples and may be the reason why it has not been applied to field samples of microplankton so far.

Diatoms are globally the most important marine primary producers (Armbrust, 2009; Hinder et al., 2012) and typically dominate photosynthetic biomass during the highly productive spring season in high latitude regions (Poulin et al., 2011). Therefore, on some model organisms extensive population genetic research for this taxon has been performed (e.g. Chen & Rynearson, 2016; Evans et al., 2004; Godhe & Härnström, 2010; Rynearson & Armbrust, 2000). With only few exceptions, marine populations have been found to be so diverse that within feasible sample sizes, genotypes are rarely found more than once, thus implying genotypic diversity of close to 100% (Godhe & Rynearson, 2017; John et al., 2004; Rengefors et al., 2017). Despite the usual absence of visible clonal dominance (but see Ruggiero et al., 2017), analysis of the pooled allele frequency patterns suggest that local populations often appear to be distinct, even at adjacent and frequently intermixing sites (Medlin, 2007; Rynearson & Armbrust, 2004). While some studies suggest that subpopulations in the same place can coexist and replace each other throughout a season (Erdner et al., 2011; Rynearson et al., 2006; Saravanan & Godhe, 2010), or that population structure develops over time (Tammilehto et al., 2017), other studies have shown that populations can remain relatively stable across timescales of decades or centuries (Härnström et al., 2011). From the available data, it appears increasingly likely that population structure

of phytoplankton such as diatoms is much more influenced by selection according to environmental conditions (i.e. local adaptation) than by genetic drift across space or time (Godhe et al., 2016; Sjöqvist et al., 2015). Our current knowledge on population structure and dynamics, however, is mainly based on observations in natural populations, whose selection environments are highly variable and the tedious sampling procedures allow only snapshots of their dynamic state without elucidating underlying processes.

The overarching aim of this study was to gain a better understanding of how global change may impact Arctic phytoplankton communities at the population level. The resistance towards environmental changes within a species can be caused by individual plasticity or genotypic shifts, but experimentally these two processes are difficult to disentangle (Gienapp et al., 2008; Merilä & Hendry, 2014). While individual plasticity can be tested in monoculture incubations, lineage sorting is often overlooked and still hardly measurable. We therefore conducted an incubation experiment with natural phytoplankton communities from an Arctic fjord (Kongsfjord, Svalbard), in which we aimed to induce directional selection that we could then resolve at the population level. Surprisingly, we observed only under one of the applied climate change scenarios (low temperature, high $pCO_2$) a shift in species composition together with a strong decrease in net primary productivity (Hoppe et al., 2018b). The resilience in species composition and productivity under all other settings posed the hypothesis that lineage sorting within species may have buffered responses in species composition and productivity. Applying previously established microsatellite primers of the diatom *Thalassiosira hyalina* (Wolf et al., under revision) to several hundreds of isolated strains from these incubations, we investigated how much the population of this species had diverged between treatments throughout the laboratory experiment.

Furthermore, we aimed to test a new 'microsatellite poolSeq barcoding' (MPB) methodology within this setup, which directly assesses microsatellite allele frequencies of an entire phytoplankton population from bulk community samples without previous strain isolation. The efficiency of the MPB analysis allowed us not only to trace the population dynamics over course of the experiment but also to monitor the natural spring bloom dynamics of the same and the following year at established study sites in the Kongsfjord.

## MATERIALS AND METHODS

### Community incubations and strain-isolation

In order to create selection environments that would accelerate lineage sorting of population, we conducted a community incubation experiment. Judging from similar studies in other regions (Hoppe et al., 2013; Tortell et al., 2008; Trimborn et al., 2017) we considered it plausible that exponential mitotic growth under stable treatment conditions can cause differing selective pressures within those incubations. Moreover, three strong dilutions (1:25) between the initial and the final time-point served as additional evolutionary bottlenecks, thus intensifying potential selection processes within each bottle. Details on the methods and results of the incubation experiment of Arctic phytoplankton assemblages can be found in Hoppe et al. (2018b), where it is referred to as KFb1 and KFb2.

In brief, the experiment was initiated in mid-April 2016 with a natural Arctic phytoplankton spring community from the Kongsfjord, Svalbard (mid-fjord station KB3, 78°55'N, 11°56'E) by gently pumping seawater from a depth of 24 m into 4 L polycarbonate bottles. The bottles were incubated in temperature-controlled chambers and aerated via continuous bubbling of air with the target $pCO_2$ of each treatment. The applied treatments each consisted of 3 replicates of *present-day* (at 1.8 ±0.1°C and 324±12 µatm $pCO_2$), *warming* (6.8 ±0.4°C and 347 ± 32 µatm $pCO_2$), *acidification* (at 1.8 ±0.1°C and 966±50 µatm $pCO_2$), and *future* (6.8 ±0.4°C and 1078±16 µatm $pCO_2$) and were exposed to continuous light at 50±2 µmol photons $m^{-2}$ $s^{-1}$. Macro nutrients were slightly increased in Redfield proportions yielding initial $NO_3$ concentrations of 20-22 µM. To avoid nutrient limitation as well as pH drift and to increase the effect of lineage sorting, three dilutions were performed at a ratio of 1:25, using nutrient-amended sterile-filtered seawater from the initial sampling time point. Depending on the growth rate of the respective community, the final time-point was reached after 16-22 days, which is equivalent to 27-31 generations according to the bulk community growth rates of 1.3 to 1.7 divisions per day (k, estimated by nutrient drawdown).

At the final time-point of the experiment, ~65 single cells of the diatom *Thalassiosira hyalina* (Grunow; Gran, 1897) were isolated from each of three replicate bottles of the *present-day* and the *future* treatment (yielding a total of 365). Cells were picked manually under a light microscope and washed three times in sterile seawater. Single-cell isolation of each strain was repeated after 10-14 days of growth in 48-well-plates at 6.8°C and 50 µmol photons $m^{-2}$ $s^{-1}$ in 1–3 mL sterile nutrient-enriched seawater. Each of the resulting strains was checked microscopically for contamination with other algal species, before grown as 250 ml monocultures at 3°C and 5-10 µmol photons $m^{-2}$ $s^{-1}$.

**Origin of filter samples for microsatellite poolSeq barcoding**

For the establishment of microsatellite poolSeq barcoding (MPB), three kinds of filter samples were collected: Firstly, samples of known strain composition were used for quantitative calibration of the allele frequencies observed by MPB. These 'multi-strain' samples contained known frequencies of certain alleles of only 6 genotypes of *T. hyalina*, and had been quantified by allele-specific qPCR before (Wolf et al., under revision). Secondly, filter samples from the final time-point of the community experiment (*tfin*) were analyzed for a semi-quantitative comparison of allele frequencies derived from MPB and from the single-strain genotyping. These contained a species- and genotype diversity that should be more comparable to the natural situation. For further pooled analysis, filter samples were also collected during each dilution (*t1-3)* of the community experiment of the *present-day* and *future* treatment, as well as of the last dilution and the final time-point of the *acidification treatment (t3+tfin*). Lastly, the performance of this new method was tested on field samples of the natural population from natural spring blooms 2016 and 2017. Field samples were collected regularly (every 2-6 days) at the midfjord station KB3 in Kongsfjorden, as well as occasionally at stations KB2 (closer to the fjord opening; 78° 58,74´ N, 11° 43,50´ E) and KB5 (closer to the glacier inside the fjord; 78° 53,79´N, 11° 26,45´ E). Sampling took place at between April 29th and May 16th 2016 as well as between April 18th and May 26th 2017.

Filtration volume of the community incubation was 300-500 ml from each bottle, volume of the field samples consisted of 1-2 L of seawater. All samples for MPB were filtered after thorough mixing of the sampling bottle on 10 µm PC filters (Whatman Nucleopore), which were stored at -80°C until further analysis.

**Microsatellite genotyping of monocultures**
The detailed methods for genotyping of monocultures can be found in the Supplement material of Wolf et al. (under revision). In brief, DNA was extracted with the NucleoSpin Plant II kit (Macharey-Nagel GmbH, Germany) according to manufacturer's instructions with an additional cell disruption step in a cell homogenizer (Fast Prep FP120, Thermo Fisher, USA). The six microsatellite primers (Table SI1) were applied to DNA samples in equimolar concentrations with fluorescent markers (FAM, HEX, AT), in single or multiplex runs with the following PCR conditions using the Type-it Microsatellite PCR kit (Qiagen) according to provider's instructions: 5 min at 94°C prior to 30 cycles of 30s at 94°C, 90s at 57°C, 40s at 72°C and a final elongation step at 72°C for 10 min. Fragment analysis was performed by capillary electrophoresis on a 3130xl Genetic Analyzer (Applied Biosystems) and size assigned relative to a GeneScan ROX standard (Thermo Fisher). Microsatellite alleles were scored using Genemapper (version 4, Applied Biosystems). From samples measured repeatedly throughout all runs, an error rate in allele length assignment of 2% was calculated. The software Arlequin (version 3.5.2.2; Excoffier & Lischer, 2010) was used to calculate expected and observed heterozygosity ($H_e$ and $H_o$), assess deviations from Hardy-Weinberg equilibrium within each experimental bottle and determine population differentiation using $F_{ST}$ among pairs of bottles. Population structure was also tested using the software STRUCTURE (version 2.3.4; Pritchard et al., 2000) without population prior. Linkage disequilibrium (LD) was calculated in the software LIAN (version 3.7; Haubold & Hudson, 2000) and Bonferroni correction of significance level was applied to account for multiple testing.

Out of the six microsatellite loci used for single-strain genotyping, primers ThKF3 and ThKF7 were selected for MPB analysis. The choice was based on their relatively large allelic richness and relatively small fragment size observed within the genotyping of our 365 monocultures (ThKF3: 180- 270 bp, 24 alleles; ThKF7: 200-300, 14 alleles, Wolf et al. (under revision)). PCR triplicates were run per locus using 10 ng of sample DNA with the following PCR conditions using the Type-it Microsatellite PCR kit (Qiagen, according to providers instructions) in a thermal cycler (Mastercycler Nexus gradient, Germany): 5 min at 94°C prior to 30 cycles of 30 s at 94°C, 90 s at 57°C, 40 s at 72°C and a final elongation step at 72°C for 10 min. PCR products were visualized on an 1.5 % agarose gel. Bands within the approximate size range of microsatellite sequences (150-300 and 150-350 bpfor ThKF3 and ThKF7, respectively) were excised, purified using the NucleoSpin gel and PCR clean-up kit (Macherey–Nagel, Düren, Germany) and additionally purified using AMPure XP Beads (Beckman Coulter; Brea, USA–CA). PCR triplicates were pooled and dual indices as well as Illumina sequence adapters were attached by means of an Index PCR using the Nextera XT Index Kit (Illumina). Final PCR products were again purified using AMPure XP Beads. The library was validated using an

Agilent 2100 Bioanalyzer Software and a DNA 1000 Chip (Agilent Technologies; Santa Clara, USA–CA) to verify the size of the resulting fragments. The final DNA libraries were pooled at equimolar concentrations and run in a MiSeq System (Illumina) after combining the denatured PhiX control library (25%) and the denatured amplicon library.

**Microsatellite poolSeq barcoding (MPB) analysis**

Amplicon sequencing on an Illumina MiSeq sequencer was used to produce 2x300 bp paired-end sequences. De-multiplexing and FASTQ sequence generation was performed by the 'Generate FASTQ' workflow of the MiSeq Reporter software. This resulted in about 11 million raw amplicons for primer set ThKF3 and about 12 million raw amplicons for primer set ThKF7. Amplicon contingency tables were constructed for each primer set using an in-house developed but modified metabarcoding pipeline (Sprong et al., in prep.). Trimmomatic (version 0.38; Bolger et al., 2014) was used to crop reads to a length of 275 bp and additionally to truncate the pre-trimmed reads at that base position at which an averaged Q-score in a sliding-window of length 3 dropped below 8, scanned from the 5'-end to the 3'-end. The paired-end reads were merged with VSEARCH (version 2.3.0; Rognes et al., 2016), allowing a maximum of 5 mismatching bases while requiring a minimum overlap length of 50 bp. Sequences which could not be merged were discarded. As most amplicons were expected to be shorter than the remaining read length, VSEARCH was adjusted to allow the merging of staggered reads. Resulting non-overlapping segments outside the targeted sequence were truncated on-the-fly. Target-flanking sequence segments with 100% forward and reverse primer match were truncated from the amplicons by the tool cutadapt (version 1.9; Martin, 2011) and amplicons were only kept in the sequence pool if both the segment of the forward and of the reverse primer were found during truncation. The remaining sequences were further selected by applying a feature filter (VSEARCH; version 2.3.0): Sequences were discarded, if i) they were longer than 320 bp or shorter than 120 bp, ii) if they carry any base ambiguity or iii) if the expected number of miscalled bases per sequence (sum of all base error probabilities) was above 0.1. Each sample was independently checked for chimeric sequences by VSEARCH (version 2.3.0) utilizing the UCHIME (Edgar et al., 2011) algorithm in de-novo mode and predicted chimeras were removed from the sample files. All sequences were pooled and amplicon contingency tables were created for both primer sets using an adapted script from https://github.com/torognes/swarm/wiki/Working-with-several-samples (retrieved: December 2018). Alleles represented by only one amplicon (singletons) were assumed likely to be errors and removed from the tables. For primer set ThKF3 about 6 million reads, and for primer set ThKF7 about 7.5 million reads passed all filtering procedures. The average length of the ThKF3 amplicons was about 180 bp, and about 205 bp for the ThKF7 amplicons. The strict quality filtering and the shortage of the amplicons guarantee a high soundness of the sequence information. Only very few samples did not yield sufficient PCR products or failed to pass the quality filters. For primer ThKF3, this concerned only one sample of the field bloom 2016 (KB3_t12). More samples had to be excluded for primer ThKF7, including all incubation experiment samples of the *acidification* treatment as well as eight of the field bloom samples (one of 2016 and seven of 2017).

The generated amplicon tables for both primer sets were further processed using the software R. Sample libraries were standardized to the median of total read numbers in all samples. Allele frequencies expressed as percentage of the total read number per sample and different alleles with the same lengths received unique names (e.g. 214.1, 214.2, etc.). In order to exclude sequencing errors in the form of rare amplicons, all allele frequencies contributing less than 1% to the whole sample were set to 0. This resulted in the loss of 10% (ThKF3) and 8% (ThKF7) of all reads in the calibration samples, but highly reduced the error probability (see result section). The sequences of all remaining amplicons were checked to contain the expected microsatellite repeat structure. These results were compared to those of the fragment analysis of isolates from the same experimental incubations.

## Calibration of allele frequencies

The MPB results were calibrated with two different sample types. First, allele frequencies of multi-strain samples previously analyzed by asqPCR (see Wolf et al., under revision) and therefore containing a known genotypic composition, were compared with those of the same multi-strain samples analyzed by MPB. Since fragment analyses yield only information on allele length, all alleles of the MPB analysis that had the same number of basepairs were summed up (even if containing point mutations) for this comparison. Allele frequencies (in percent) of all samples from the fragment analysis and the MPB analysis were compared by linear regression yielding Pearson's r and its significance level, and by calculating the mean deviation between them for each allele length. Second, the same comparison based on allele lengths was performed for samples of the genotyped *present-day* and *future* community incubations. In this case, however, the allele frequencies of the fragment analysis contained only a limited subsample of genotypes from a highly diverse population which introduces stochastic effects. The final results of the MPB were evaluated using principle component analyses (PCA).

## RESULTS

### Microsatellite genotyping/ fragment analysis

A total number of 365 strains were isolated from the respective three replicate bottles of the final time-point of the *present-day* and *future* experiment (Present-day_A, B, C and Future_A, B, C). Re-isolation, transport and genotypic analysis of single strains was successfully accomplished in 83% of originally isolated strains. Among all analyzed single strains (using 7 microsatellite loci), only 7 multi-locus genotypes (MLGs) were found twice, yielding an overall genotypic diversity of 96%. Within each bottle, this number varied between 90 and 100%. Although a strong clonal dominance within the bottles can thus be excluded, all but one pairs of identical genotypes originated from the same replicate bottle. Expected and observed heterozygosity were very similar in all replicate bottles and thus no significant deviations from Hardy-Weinberg equilibrium were detected ($H_0 \approx H_E$). Similarly, none of the bottles showed significant linkage disequilibrium after Bonferroni Correction. All $F_{ST}$ values between pairs of bottles were very low, and differences were not significant except for two bottles

from different treatments (Table1). This generally suggests no substantial differentiation between the populations within incubation bottles of either treatment at the final time-point of experiment. This result was supported by the Bayesian clustering analysis using the software STRUCTURE (Pritchard et al., 2000), where no population subdivision among genotypes was detected.

**Table 1:** *Statistics summary for microsatellite genotyping with six microsatellite markers for six incubation bottles of the community incubation experiment.*

| | Run time [days] | # approx. generations | # genotyped | # unique MLG (% diversity) | $H_O$ | $H_E$ | $F_{ST}$ and LD | Present-day-A | Present-day-B | Present-day-C | Future-A | Future-B | Future-C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Present-day-A** | 20 | 27 | 64 | 64 (100%) | 0.642 | 0.667 | **Present-day-A** | NA | - | - | - | - | * |
| **Present-day-B** | 20 | 27 | 65 | 63 (96.9%) | 0.623 | 0.669 | **Present-day-B** | 0.00193 | NA | - | - | - | - |
| **Present-day-C** | 20 | 27 | 63 | 57 (90.5%) | 0.633 | 0.653 | **Present-day-C** | -0.00188 | 0.00131 | NA | - | - | - |
| **Future-A** | 15 | 31 | 55 | 53 (96.4%) | 0.645 | 0.682 | **Future-A** | 0.00373 | -0.00077 | 0.00259 | NA | - | - |
| **Future-B** | 15 | 31 | 58 | 56 (96.6%) | 0.601 | 0.633 | **Future-B** | 0.00221 | 0.00192 | -0.00003 | 0.00578 | NA | - |
| **Future-C** | 15 | 31 | 60 | 60 (100%) | 0.663 | 0.681 | **Future-C** | 0.00677* | 0.00255 | 0.00189 | 0.00205 | 0.00129 | NA |
| **Present-day-strains** | **20** | **27** | **192** | **184 (95.8%)** | **0.632** | **0.663** | | | | | | | |
| **Future-strains** | **15** | **31** | **173** | **169 (97.6%)** | **0.636** | **0.665** | | | | | | | |
| **Total** | | | **365** | **351 (96.2%)** | **0.634** | **0.664** | | | | | | | |

For each bottle are given: run time of the incubation experiment, approximate number of generations as calculated from assemblage growth rate, number of genotyped individuals, number of multilocus genotypes (MLG) and genotypic diversity in %, observed ($H_O$) and expected ($H_E$) heterozygosity and significance for Linkage disequilibrium within bottles (p for LD). Sum or average values are marked in bold. The right part of the table shows pairwise $F_{ST}$ values (lower diagonal) and significant linkage equilibrium (LD; upper diagonal) at a significance level (*p<0.05). Note: none of the differences in heterozygosity nor LD within bottles were significant.

## Calibration of microsatellite barcoding

Correlation between MPB allele frequency and allele-specific qPCR frequencies (John et al., 2015; Meyer et al., 2006; Wolf et al., under revision) was high and significant for both microsatellite loci for the controlled 'multi-strain' experiment (Calibration 1: Pearson's r>0.5; p<0.001; see Figure 1a and b). This indicates very accurate allele identification and quantification. The mean error of all alleles per sample was 1.0 ±0.8 % for ThKF3and 1.3 ±0.4 % for ThKF7 (see Table 2). A PCA of the 'multi-strain' samples (Figure SI2) revealed separate clusters indicative of their distinct genotypic composition and development over time.

Comparison of MPB with genotypic data of a natural populations within the community incubation experiment showed a substantially greater variation, yet a high degree of accordance (Calibration 2: Pearson's r=0.44; p<0.001 for both primers; Figure 1c and d). After applying the same 1% low-frequency filter as described above, a total of 74% (ThKF3) and 79% (ThKF7) of all amplicons were retained. Considering that this comparison includes the subsampling bias of genotyping a limited number of isolates in the fragment analysis, the mean error of all alleles per sample was still low, with 1.6 ±0.1% for ThKF3 and 1.3 ±0.2% for ThKF7.
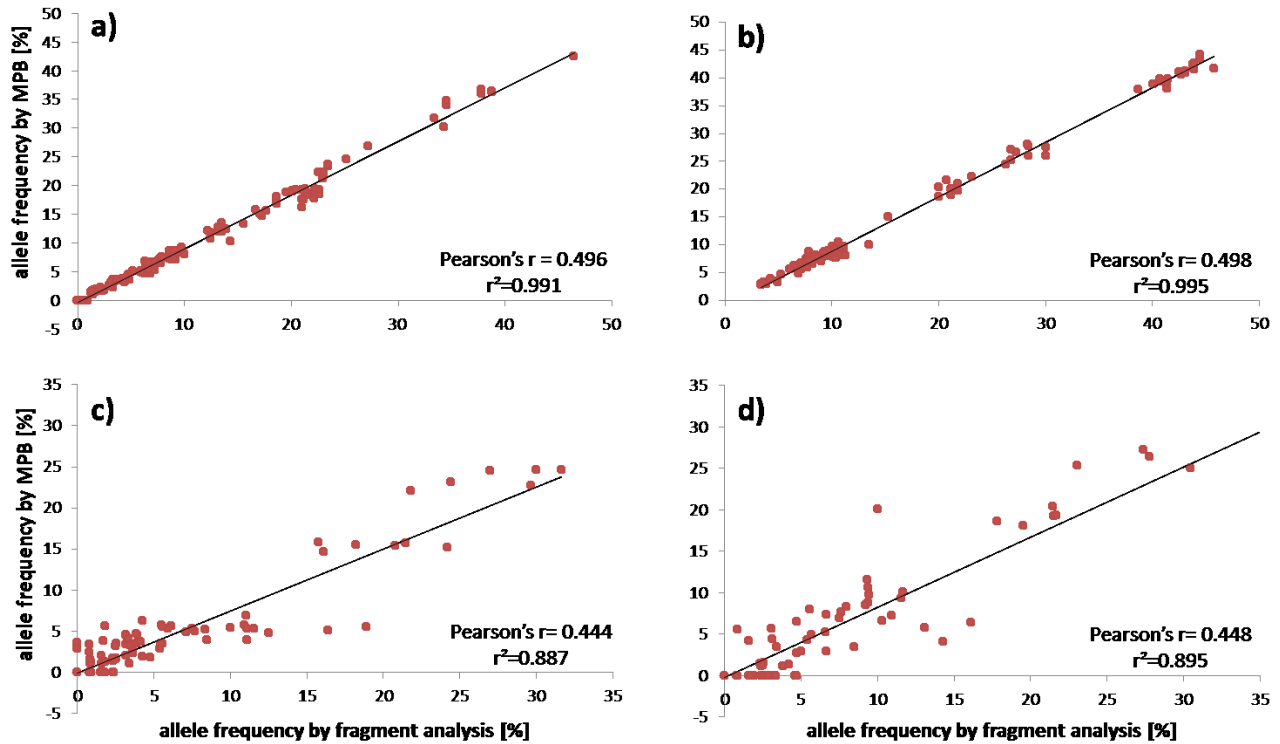
***Figure 1:*** *Calibration results of relative allele abundances per sample pooled across calibration samples as measured by fragment analysis (x-axis) and by MPB (y-axis) using both primers ThKF3 and ThKF7. Pearson's correlation coefficient (r) was used to evaluate the linear regression. Calibration 1 for Primer ThKF3 (a) and ThKF7 (b): correlation of allele frequencies in 18 multi-strain samples of artificial populations with limited diversity (six strains of T. hyalina) and known composition from asqPCR; Table 2 shows the detected error for each allele separately. Calibration 2 for Primer ThKF3 (c) and ThKF7 (d): correlation of allele frequencies in 6 community samples of phytoplankton assemblages including natural T. hyalina populations at final time-point of the incubation experiment. Here, fragment analyses were based on 55-65 isolated strains per sample.*

| ThKF3 | Mean error [%] | Mean allele abundance [%] |
|---|---|---|
| 205 | 0.29 | 1.09 |
| 208 | 2.65 | 12.91 |
| 211 | 0.53 | 4.00 |
| 214 | 1.37 | 30.59 |
| 217 | 0.12 | 1.69 |
| 220 | 1.26 | 8.98 |
| 223 | 0.89 | 12.89 |
| 226 | 1.79 | 17.40 |
| 229 | 0.32 | 5.32 |
| 232 | 0.35 | 0.91 |
| 235 | 0.91 | 4.21 |
| | | |
| **ThKF7** | | |
| 233 | 1.89 | 10.14 |
| 239 | 1.71 | 42.54 |
| 245 | 0.91 | 9.33 |
| 251 | 1.32 | 24.28 |
| 257 | 0.90 | 8.11 |
| 269 | 1.03 | 5.60 |

***Table 2***: *Mean error in the relative frequency and mean relative frequency of each allele per sample at locus ThKF3 and ThKF7 as estimated via calibration with multi-strain samples of known composition.*

**Analysis of natural populations by microsatellite poolSeq barcoding (MPB)**

Using primer ThKF3 for the experimental incubations of the naturally diverse populations, a PCA revealed no differentiation or cluster formation between the allelic composition of *T. hyalina* populations of the *present-day* and *future* treatments; neither between the final time-points of different incubations, nor over time (*t1-tfin*) (Figure 2). All samples of the *acidification* treatment, however, formed a distinct cluster separate from the other community samples. The arrows in Figure 2 show that the majority of the depicted pattern is explained by differences in the three most abundant alleles rather than rare ones (three versions of 214 (24%), as well as 226 (15%) and 236 (6%)). Primer ThKF7 yielded similar results for the *present-day* and the *future* treatment (Figure SI3).

A PCA of relative allele distributions within field samples taken throughout the Kongsfjord spring blooms in 2016 and 2017 are depicted for primer ThKF3 in Figure 3. Throughout each year, allele composition developed without a clear direction over time, but populations of both years revealed a pronounced differentiation and formed two distinct clusters. Only one sample from an intermediate time-point of 2017 as well as the earliest and the latest samples of 2016 did not conform to this pattern. Products of primer ThKF7 did not resolve a differentiation between the majority of these field samples and revealed no clusters. The resulting PCA can be found in the Supplements (Figure SI4).
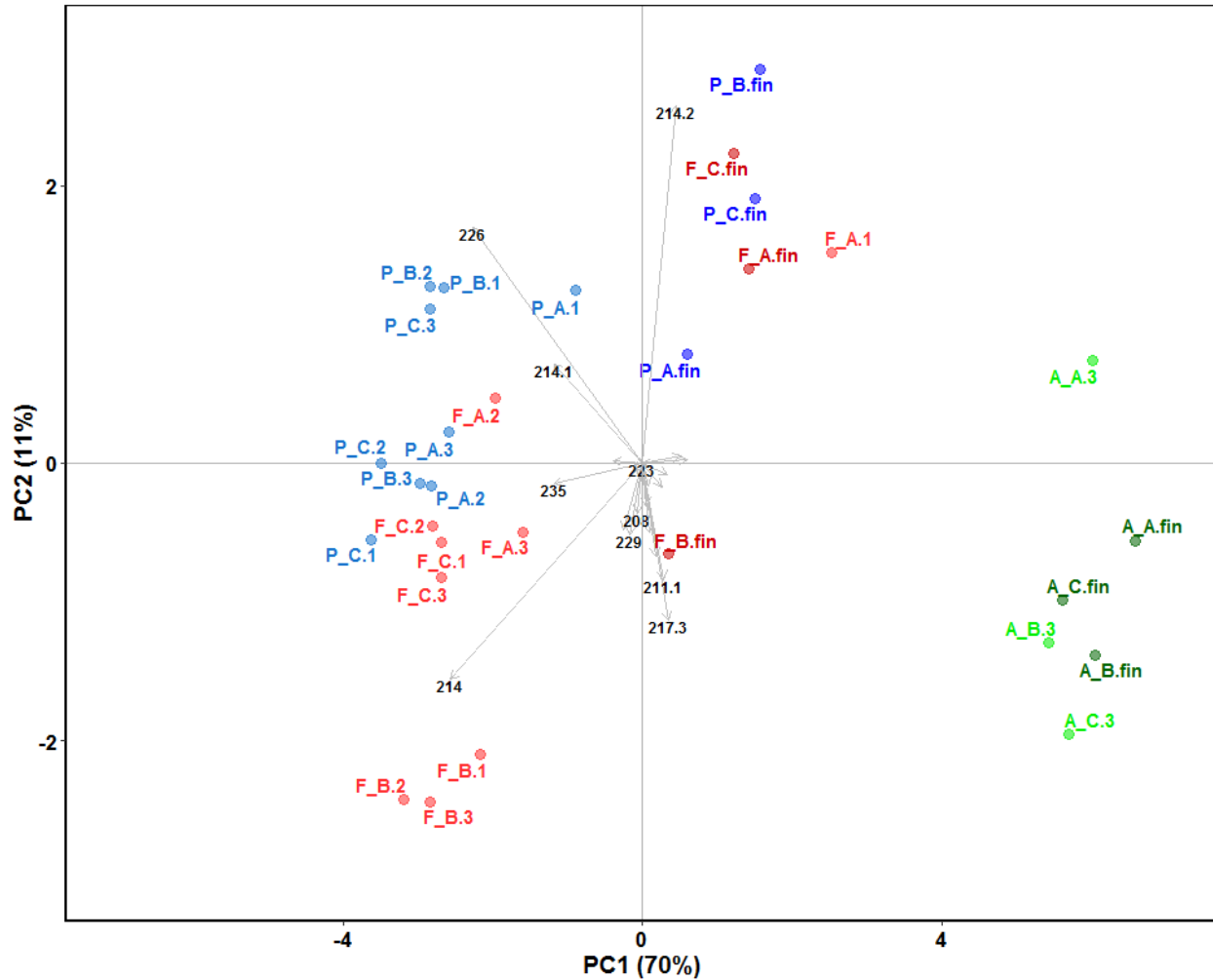
*Figure 2: Principal component analysis of relative allele frequency data of T. hyalina obtained by MPB using primer ThKF3 on community samples of the incubation experiment with a natural phytoplankton assemblage. Three replicate incubations exposed respectively to the present-day (P: 2°C and 400µatm pCO$_2$; blue) and the future treatment (F: 7°C and 1000µatm pCO$_2$; red) were sampled at four time-points (t1, t2, t3 (light color); tfin (dark color)). The three replicate incubations of the acidification treatment (A: 2°C and 1000µatm pCO$_2$, green) were analyzed only at time-points t3 and tfin. Black numbers refer to the allele lengths, which were used as underlying variables for PCA. Only the 10 most influential ones are identified here. Different allele versions of the same lengths (homoplasy) have unique names (e.g. 214.1, 214.2).*
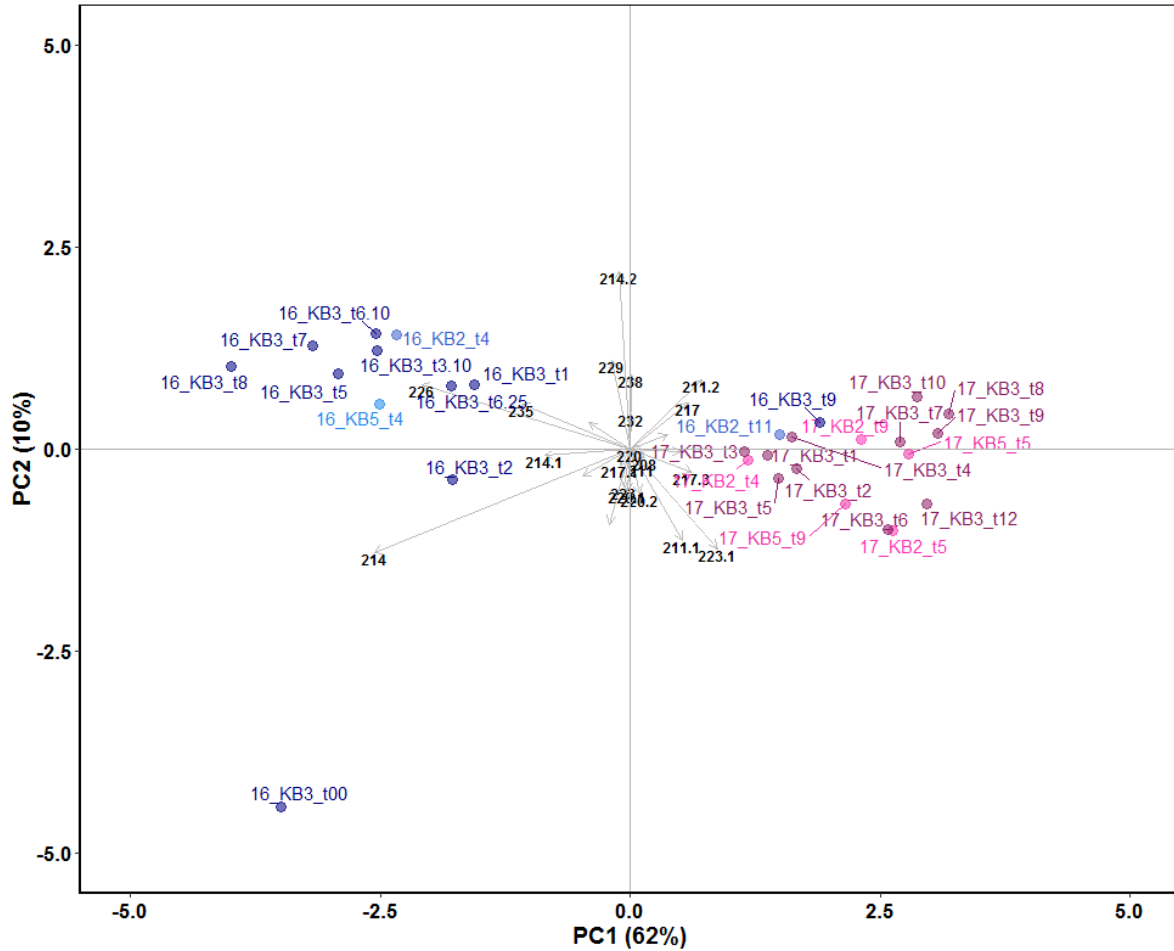
***Figure 3****: Principal component analysis of relative allele frequency data of T. hyalina obtained by MPB using primer ThKF3 on field samples of the natural phytoplankton spring bloom from the Kongsfjord in 2016 (blue) and 2017 (purple). Black numbers refer to the allele lengths, which were used as underlying variables for the PCA. Only the 20 most abundant ones are identified here. Different allele versions of the same lengths (homoplasy) have unique names (e.g. 214.1, 214.2)Samples of 2016 were taken at 10m or 25m between April 29th and May 16th 2016 (blue), samples of 2017 at 25m between April 18th and May 26th 2017 (purple). The sampling stations throughout the Kongsfjord are makes by color shades (KB3 (dark blue/purple); KB2 and KB5 (light blue/pink)). The exact date of each field sample can be found in the Table SI2.*

**DISCUSSION**

**Genotyping of natural assemblage incubations reveals no differences in population structure**

Despite strongly divergent selective environments in the *present-day* and *future* treatments of the community incubations, the genotyping results of hundreds of strains of *T. hyalina* did not indicate any considerable differences between the experimental populations (Table 1). It yielded no obvious signs of pronounced intraspecific selection. Although the few multi-locus-genotypes (MLGs) that were identified twice among the isolates originated mostly from the same experimental incubation bottle, from an overall genotypic diversity of 96% we cannot make inferences about genotypic shifts within the *T. hyalina* population. Furthermore, neither a Bayesian clustering analysis nor pairwise comparisons between incubation bottles based on $F_{ST}$ values revealed signs of population differentiation between them (Table 1). This may hint at a lack of changes in genotypic composition, but might also suggest a soft genetic sweep, where advantageous alleles are widely distributed among the standing genetic variation and thus show no linkage to any of the neutral microsatellites (Weigand & Leese, 2018). Isolates from all tested incubation bottles (*present-day* and *future*) showed only small deviations from *Hardy-Weinberg-equilibrium* ($H_O \approx H_E$) and no significant linkage disequilibrium (Table 1). This suggests that genetic drift played no major role despite recurrent dilutions. Still, soft sweeps (selection on standing genetic variation that was not linked to single genetic backgrounds) may have occurred but would not have been picked up with the current markers. These results do not support the initially posed hypothesis that a pronounced intraspecific dominance shift among lineages within a species may have been the cause for the large degree of compensation observed in the incubated community.

**Microsatellite poolSeq barcoding (MPB) depicts differences in diverse populations**

Since it is a new approach to use targeted markers in a pooled analysis of several individuals, especially in environmental field samples, we tested the reliability of MPB in two different kinds of setups containing differing degrees of diversity. The first calibration step using samples of a simplified artificial population of six strains of *T. hyalina* of known composition ('multi-strain') yielded accurate results (Figure 1, Table 2). The deviation from the previously measured fragment analysis was very small and the amount of error did not appear to depend on allele length. Furthermore, a PCA (Figure SI2) reflected the expected temporal development and difference in strain composition between treatments of these previously analyzed multi-strain samples very well. We therefore conclude that microsatellite poolSeq barcoding (MPB) is a valid approach for the semi-quantitative and qualitative evaluation of allele frequencies in a low-diversity setup, comparable to allele-specific qPCR (John et al., 2015; Meyer et al., 2006; Minter et al., 2015; Wolf et al., under revision). A marked advantage to fragment analysis based methods, however, is the resolution of otherwise cryptic point mutations (homoplasy). A few allele lengths were found to exist several times in different versions (e.g. two alleles with 214 bp, see arrows Figure 3+4), likely revealing allele versions

of the same length. Since alleles of the same lengths were summed up, this did not affect the method comparison.

The second calibration step, comparing the community incubation MPB samples with allele frequencies of the isolated genotypes was potentially subject to shortcomings in both methods: the technical errors of MPB in form of PCR and sequencing mistakes or bias by subsampling of the genotyping, where only ~65 isolates out of several hundred thousand cells per bottle were analyzed. Still, the comparison of MPB and fragment analysis yielded only small discrepancies (Figure 1, Table 2). The mean error was only slightly larger than in the multi-strain samples, and there was a strong linear correlation between the two allele frequency measures (Figure 1). The strong similarity of results from both methods and the accurate technical calibration with multi-strain samples suggests that MPB is a versatile method. It appears capable of assessing allele frequencies, and thus population characteristics and dynamics, based on the entire range of genotypes present in a filter sample from natural diversity instead of tediously established subsamples.

A first practical application of MPB on the different experimental treatments within the community incubation revealed that there was no apparent difference between those populations grown under *present-day* and those under *future* conditions (Figure 2), which is in line with the results from microsatellite genotyping of single isolates (Table 1). No directional temporal pattern (between time-points *t1* and *tfin*) was found among community samples from those two treatments in either method. While no strains had been isolated from the *acidification* treatment, MPB allowed us to also analyze filter community samples of the bottles exposed to this treatment. Here, the populations showed a pronounced shift in allele frequencies compared to the *present-day* and *future* samples and must therefore have contained a population of different genotypic composition (Figure 2).

Interestingly, the *acidification* treatment was also the only one causing a significant decrease in primary productivity of the assemblage and a shift in the species composition (Hoppe et al., 2018b). Especially within the context of the broader experimental dataset of this publication, this hints towards a tipping point of community stability in terms of productivity as well as community structure (Botero et al., 2014)(see also Figure SI1). Notably, this apparently also had a visible signature on the intraspecific level. The identification and comprehension of such tipping points caused by interacting drivers are important for our understanding of ecosystem functioning and represent challenges to the adaptive capability of species and populations. However, it should be considered that the total biomass-buildup and abundance of *T. hyalina* within the community in the *acidification* treatment dropped substantially (Hoppe et al., 2018b). Minor shifts in the genotypic composition within a small population may seem more pronounced than within a larger and more diverse population, which could have biased the analysis here. One should note, however, that this bias is comparable to that of different sizes of subsampling during strain isolation of traditional genotype analyses. Nonetheless, the detected change in allele frequencies proves the sensitivity of our primer to allelic shifts.

Since the analysis of experimental community incubations yielded strong indications for MPB to be sufficiently sensitive to detect differences in the population composition of

*T. hyalina*, we also tested how this new method would perform on natural field samples. The analysis of 15 samples from the spring bloom 2016 and 16 samples from spring bloom 2017 with primer ThKF3 revealed a clear cluster for each of the two years with only few outliers (Figure 3). It appears that the *T. hyalina* populations from the two consecutive years each contained a distinct intraspecific allele composition at this locus. This demonstrates that even in a fully diverse natural community, MPB with only one locus can detect differences in population composition on the annual scale. Since no clear temporal dynamics are visible within each year and ordination plots depict differences only in relative terms, we cannot know with certainty whether the population composition throughout the spring seasons was fairly stable or whether allele dynamics within one bloom were too subtle for us to detect.

**The potential of microsatellite poolSeq barcoding (MPB) for population genetic research**

Our results show that a pooled analysis of relative frequencies of microsatellite alleles is possible in laboratory setups as well as in environmental field samples with natural diversity. While it cannot entirely replace the information gained by genotyping single individuals, in the age of high-throughput sequencing it holds the potential to take sampling capacities to a new level. In many ways, the advantages and disadvantages of this method are parallel to those discussed for pool-sequencing approaches (Futschik & Schlötterer, 2010; Schlötterer et al., 2014).

The four most prominent benefits of MPB are certainly i) its resource efficiency, ii) easy applicability to bulk field samples, which iii) avoids subsampling bias and iv) the potential to resolve homoplasy (i.e. different alleles of same length). The analysis of population dynamics and selection processes within natural populations of non-model species represents a major challenge in ecology and evolution (Weigand & Leese, 2018). Especially in unicellular planktonic organisms, MPB can facilitate population genetic research in full communities and field studies, since it allows a new extent of temporal and spatial resolution of natural or experimental intraspecific patterns. Furthermore, microsatellite poolSeq barcoding removes the potentially large bias of subsampling only a small fraction of individuals, which may not be statistically representative of the entire population. This is especially beneficial in organisms with immense population sizes and large genotypic diversity, such as phytoplankton (e.g. Alpermann et al., 2009; Tahvanainen et al., 2012). Finally, using sequencing instead of fragment analyses has the further advantage of analyzing alleles based on their real genetic code instead of their length. This encompasses the ability to differentiate alleles of the same length and also to identify possible cross-amplifications with other species.

In spite of these considerable advantages, it needs to be taken into account that MPB cannot provide any information on haplotypes or assign alleles to certain genotypes, since it relies purely on the pooled allelic composition of the sample, as in other poolSeq approaches (Schlötterer et al., 2014). This implies that many measures commonly used in population genetics cannot be retrieved, which precludes inferences for example about reproductive strategies. Potential technical problems include sequencing errors or DNA strands to be

skipped or sequenced twice (c.f. Futschik & Schlötterer, 2010). Strict quality filters (e.g. low expected number of errors per read), full length overlap of paired reads and exclusion of low frequency amplicons (i.e. singletons) can control these problems (Callahan et al., 2017; Edgar & Flyvbjerg, 2015). Microsatellite loci should be chosen to be as short and as polymorphic as possible, since a short amplicon length potentially decreases sequencing errors and PCR bias. It also allows better quality control during reverse complementation in the data processing, since an overlap of the entire amplicon is possible (Kozich et al., 2013). Nevertheless, we can only make inferences about how informative a neutral marker gene is for our population, e.g. its proximity to genes under selection or how evenly its alleles are distributed throughout the population. This is, however, the case for all microsatellite-based studies and not specific to this novel approach.

**Eco-evolutionary implications for intraspecific population dynamics**
The application of MPB enabled us to resolve intraspecific patterns in *T. hyalina* populations to an extent that would otherwise not have been feasible. By removing the bias of subsampling from a large and highly diverse population and by adding temporal resolution to the measured allele frequencies, we have gained valuable information on the investigated Arctic diatom populations in the laboratory and in the field under natural conditions.

*Experimental community incubations*
Phenotypic investigations of single strains from community incubations as well as a simplified competition experiment revealed the potential for strong selection among lineages of *T. hyalina* (Wolf et al., under revision). The absence of detectable genotypic as well as of allelic shifts between the *present-day* and *future* incubations, however, suggests that intraspecific selection did not play a major role in the observed compensation of phenotypic effects on the community (Hoppe et al., 2018b). Instead, it implies that the phenotypic plasticity of the majority of lineages may have sufficed to buffer the imposed environmental conditions of the treatments. Within a well-controlled and non-variable environment in the laboratory, selection could be expected to work in fairly directional way (Morrissey & Hadfield, 2012). Therefore our results suggest that those treatment conditions were within the phenotypic plasticity range of most genotypes and thus not imposing sufficient selection pressure. This is in line with the high plasticity observed in this and another important species of the community incubation (Hoppe et al., 2018a; Wolf et al., 2018). In a previous competition experiment with *T. hyalina* under a scenario resembling the ambient conditions, we also found that strains can sustain an equilibrium of coexistence (Wolf et al., under revision). Moreover, response diversity, which is often linked to genotypic diversity, broadens a population's tolerance range and at the same time enhances the adaptive capacity (Elmqvist et al., 2003; Reusch et al., 2005). A highly diverse population like the investigated one is therefore likely to be more resistant or resilient than a low diversity population.

An alternative explanation for the absence of a shift in allele frequencies could be that either the characteristics under selection or the microsatellite alleles are widely and evenly distributed throughout the population. In contrasted to a 'hard sweep', where the polymorphic marker gene is physically linked with those genetic loci under selection, this

would imply a 'soft sweep', where the selected genetic variants are not associated with a single genomic background (Weigand & Leese, 2018). Accordingly, even if intraspecific selection did take place, it would have remained undetectable to our analysis on the basis of a neutral marker. Such a homogenous allele distribution would imply that the majority of genotypes originates from a persistent, frequently recombining population with limited influence from intermixing other *T. hyalina* populations. This is surprising in view of the study site, as explained below.

Regardless which of these two options actually explains the stability in the allelic pattern of the *present-day* and *future* incubations, the allelic shift observed in the *acidification* treatment revealed that there is a limit to the stability of this allele pattern under certain conditions. At a $pCO_2$ of 1000µatm and a low temperature of 1.8°C, the plasticity of the *T. hyalina* populations apparently failed to sustain their competitive abilities. This development is congruent with the observation of changed species composition and reduced NPP in the entire assemblage (Figure SI1 and Hoppe et al., 2018b). It is also in line with the frequently observed pattern that under low temperatures, negative effects of ocean acidification manifest already at lower $pCO_2$ levels than in warmer conditions (Hoppe et al., 2018a; Sett et al., 2014; Wolf et al., 2018). Thus, *T. hyalina* was likely not the only species affected by those conditions. Here, selective bottlenecks seemed tight enough to decrease *T. hyalina* population size and cause a change in genotype composition that resulted in a detectable allelic shift. Although a decrease in population size adds a stochastic bias to the measurement, it is still likely that the remaining genotypes at the final time-point were a selection of the fittest ones under the applied conditions. The fact that community samples from replicate bottles all cluster together (Figure 2) suggests that directional selection and not only genetic drift played a role in this clear shift.

*Natural populations in in-situ spring blooms*
The change in allele frequencies between the spring blooms of 2016 and 2017 and the lack of allelic pattern within either year (Figure 3) suggests that populations differed between years, but reveals no clear directional development within them. Furthermore, the occasional samples from other adjacent locations (KB2 and KB5, Figure 3 and Figure SI5) do not reveal any spatial structure and imply a fairly homogenous panmictic population throughout the entire Kongsfjord. Although the lack of differentiation within one season could be a matter of lacking resolution of our marker, genetic shifts throughout a bloom are likely to be rather chaotic than directional. This applies especially to a variable environment like our study site, where small-scale selection pressures constantly change (Bell, 2010; Fransson et al., 2016; Svendsen et al., 2002).

Among the field samples of 2016 it is notable that those samples, which do have a larger ordination distance to the main cluster of 2016 originated from the early or late season (Figure 4). While early samples of 2016 deviate from the clusters of both years (*t0-t4)*, late samples approximate the cluster of 2017. This is of interest since during these later time-points in the season (*t9; t11,* i.e. mid-May) nutrient concentrations strongly declined and eventually terminated the diatom dominated phase of the bloom (Smoła et al., in prep.).

Therefore, this was likely the period when selection pressures changed radically and a different set of genotypes became dominant. This later version of the population may have made the major contribution to the resting stages in the sediment, which can strongly influence the population composition of the next season (Godhe & Härnström, 2010; Sundqvist et al., 2018) or may even have remained in the water column (Kvernvik et al., 2018).

The population differentiation between years may also be caused by a number of possible influences: the evolutionary bottleneck of drastically decreased population sizes during winter; the germination season with its particular habitat filters during the germination of a subset of cysts; as well as seasonally differing wind-and current-driven advection of waters outside the fjord. These oceanographic influences could add new genotypes, just as it frequently supplies Atlantic species (Hop et al., 2002). It is a known phenomenon that mainly the Atlantic West-Spitsbergen current but also the Arctic-type coastal water of the East Spitsbergen current can at times strongly influence the Kongsfjorden system (Svendsen et al., 2002). While these mixing events can also occur throughout the bloom, their genotypic impact may be much larger over a longer time period as well as when the locally adapted population is smaller and not exhibiting exponential growth.

**Conclusions and future directions**

The results of this study show that our novel MPB approach accurately reproduces the results from traditional genotyping and asqPCR in phytoplankton population samples of differing diversity and environmental context. As a new method, MPB has the potential to facilitate the identification and tracing of differentiation processes in natural protist populations as well as to enable better temporal and spatial resolution in population genetic studies. Applying both, traditional genotyping and microsatellite barcoding, we found surprisingly little evidence for lineage sorting within natural populations of our model organism *T. hyalina*, in an incubation experiment as well as throughout spring blooms. This suggests that the phenotypic plasticity of individuals comprising the investigated natural populations may cover a wide range of conditions, thus preventing changes in lineage abundances. Furthermore, selection dynamics in highly diverse populations may be more subtle than clonal dominance of a few successful lineages. Nevertheless, pronounced differences in populations detected in one experimental treatment as well as between populations of two consecutive years in natural field samples show that, under given conditions, resolvable population shifts do occur. The mechanisms that drive phytoplankton population dynamics or stability in highly productive systems (such as those of *T. hyalina* in the coastal Arctic) are still not well understood. Gaining such knowledge will require more research in natural and experimental settings, especially including increased temporal and spatial resolution. The newly developed microsatellite poolSeq barcoding method may help to achieve this ambitious goal.

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**Supplement Material**
**Wolf et al.: Revealing population dynamics of the Arctic diatom *Thalassiosira hyalina***
**using a novel microsatellite poolSeq barcoding (MPB) approach**

*Figure SI1: Species composition of the community experiment at final time-point of incubation (see Hoppe et al. (2018) for details). Species abundances are depicted as percentage of total cell number as counted by light microscopy.*
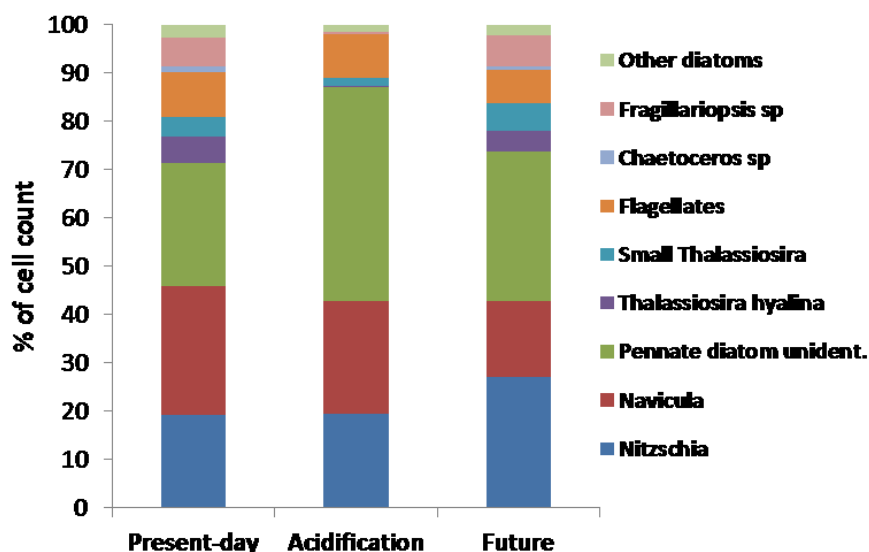


*Table SI1: Properties of 6 microsatellite loci of Thalassiosira hyalina and their respective primers. Repeat pattern of the locus, size range of fragments (including primer sequence) and number of alleles found within all genotype samples (365 isolates), primer sequences, observed and expected heterozygosity and the significance of difference (p $H_0/H_E$). Measures of heterozygosity and linkage disequilibrium are based on the analysis of all n=365 single genotype samples. + denotes the detection of significant linkage. Modified after Wolf et al. (under review).*

| | repeat pattern | size range [bp] | Primer Sequence fwd | Primer Sequence rev | # of alleles | $H_O$ | $H_E$ | p H0/HE | Linkage disequilibrium Locus | 1 | 2 | 3 | 4 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ThKF1 | CTG | 248-257 | TCGTATGGCTGCCATGAGAAG | GTAACTGCTGGGACGACCAC | 4 | 0.65 | 0.67 | 0.261 | 1 | * | + | + | - | - | - |
| ThKF2 | CA | 247-259 | AATTTGGAAGCCGCCGTAGA | GGGTCGGAGAGTTTGTTGCA | 7 | 0.58 | 0.65 | 0.001 | 2 | + | * | - | - | - | - |
| ThKF3 | TCA | 187-264 | TCGCTGTCCTCGGTTTCAC | CAATGATGAGGTCCGGCGAT | 24 | 0.84 | 0.85 | 0.051 | 3 | + | - | * | - | - | + |
| ThKF4 | TTG | 246-258 | GGAGGAAAAACAACCGTTTGCT | TACAGGCCTTCCTTGCATGC | 5 | 0.48 | 0.48 | 0.882 | 4 | - | - | - | * | - | + |
| ThKF6 | AAGTGA | 229-247 | AAATCCGCAGCCGAGAACAT | GAGAAGAGTCGCGCAGGATT | 5 | 0.57 | 0.65 | 0.001 | 6 | - | - | - | - | * | + |
| ThKF7 | ACCAGC | 215-290 | ATTCCCATAGTCTCCCGACAGA | GGGGAGATCGTGATGCCTTC | 14 | 0.80 | 0.84 | 0.485 | 7 | - | - | + | + | + | * |

*Figure SI2: Principal component analyses (PCA) of allele frequencies of multi-strain samples used for calibration of microsatellite locus ThKF3 (a) and ThKF7 (b). Samples of the present-day treatment ('P') in blue and of the future treatment ('F') in red. for each replicate bottle ('A,B,C')and time-point for bottle A (t1-tfin) The comparison with the genotypic composition as measured in these samples using asqPCR by Wolf et al. (under review) illustrates that both loci reflect the genotypic composition and development found there: In the present-day treatment, strains remained close to their initial composition, while under future conditions, substantial sorting took place, which can be recognized in the increasing distance to 0 over time in samples F.A.t1, F.A.t2 and F.A.tfin.*
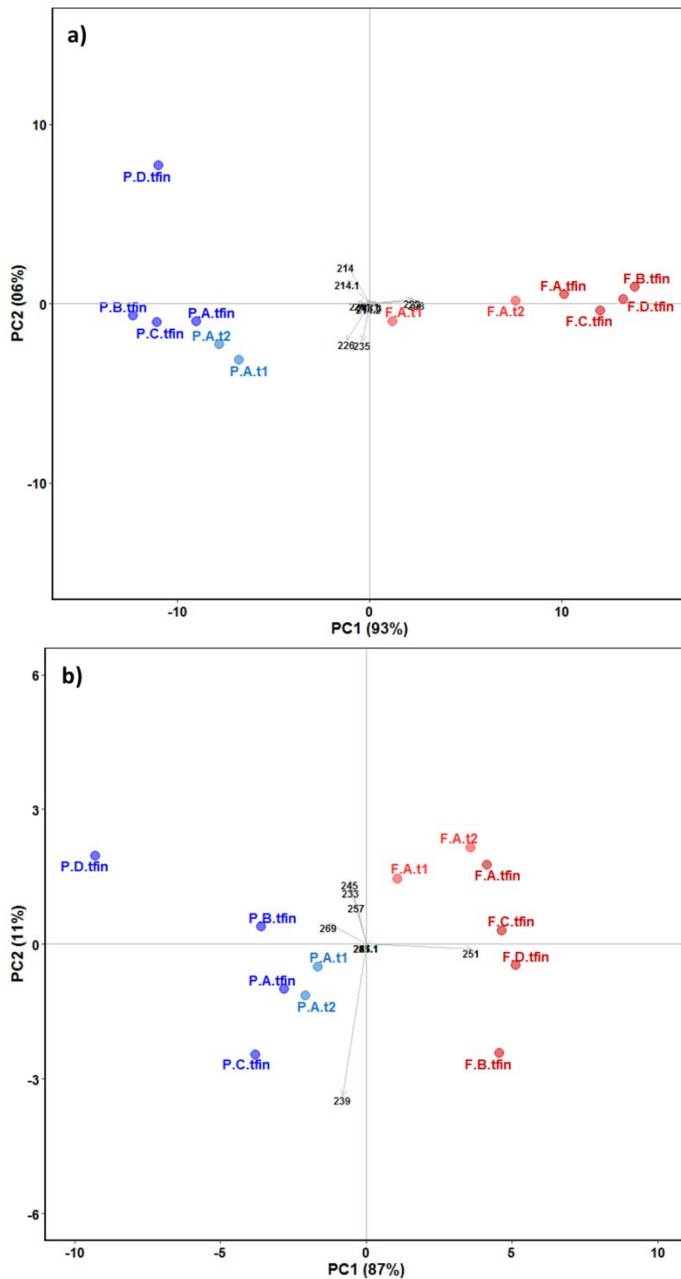
*Figure SI3: Principal component analysis of relative allele frequency data of T. hyalina obtained by MPB using primer ThKF7 on community samples of the incubation experiment with a natural phytoplankton assemblage. Three replicate incubations exposed respectively to the present-day ('P': 2°C and 400μatm pCO₂; blue) and the future treatment ('F': 7°C and 1000μatm pCO₂; red) were sampled at four time-points (t1, t2, t3: light colors; tfin: dark colors). Black numbers refer to the allele lengths, which were used as underlying variables for PCA. Only the 10 most influential ones are identified here. Different allele versions of the same lengths (homoplasy) have unique names (e.g. 214.1, 214.2).*
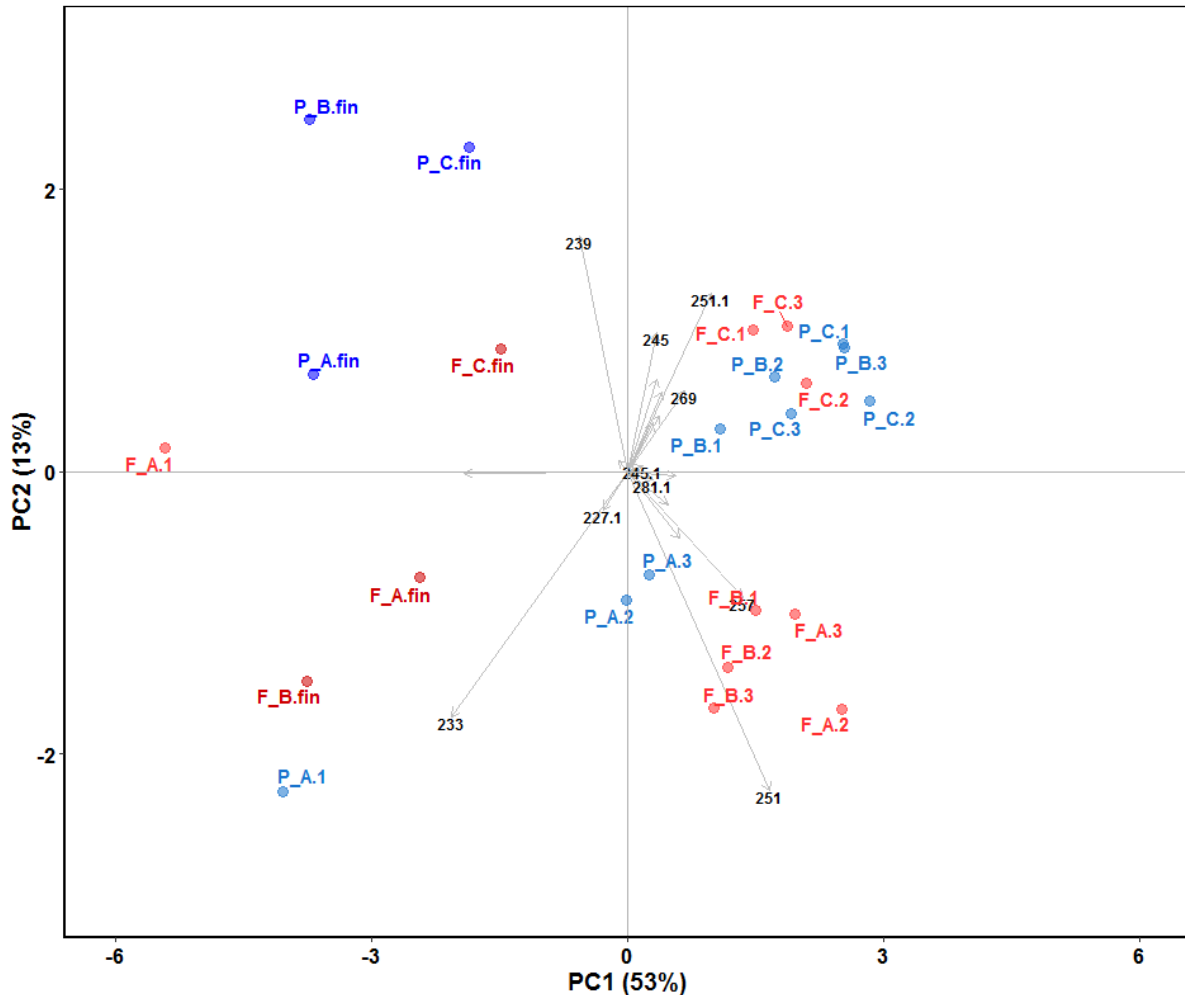
*Figure SI4*: Principal component analysis of relative allele frequency data of T. hyalina obtained by MPB using primer ThKF7 on field samples of the natural phytoplankton spring bloom from the Kongsfjord in 2016 (blue) and 2017 (purple). Black numbers refer to the allele lengths, which were used as underlying variables for the PCA. Samples of 2016 were taken at 10m or 25m between April 29th and May 16th 2016 (blue), samples of 2017 at 25m between April 18th and May 26th 2017 (purple). The exact date of each field sample can be found in the Table SI2. Black numbers refer to the allele lengths, which were used as underlying variables for PCA. Only the 20 most abundant ones are identified here. Please note that not all samples yielded results with primer ThKF7 that passed the quality control within the data processing and are therefore missing here.
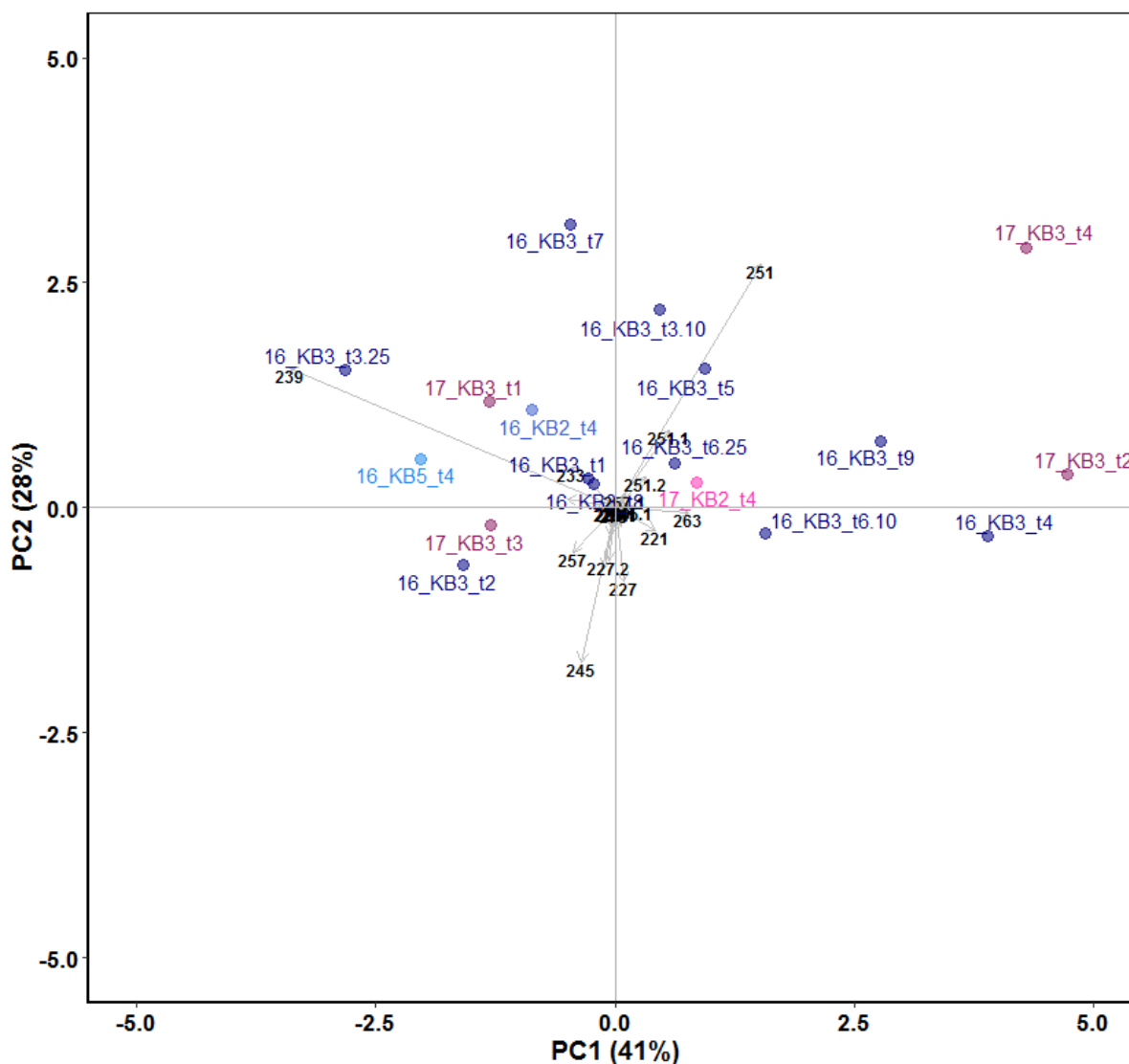
***Table SI2***: *Field Samples and their respective sampling date as depicted in Figure 4 within a PCA.*

| Sample | Date | Sample | Date |
|---|---|---|---|
| 16_KB3_t00 | 14.04.2016 | 17_KB3_t1 | 18.04.2017 |
| 16_KB3_t1 | 21.04.2016 | 17_KB3_t2 | 20.04.2017 |
| 16_KB3_t2 | 25.04.2016 | 17_KB3_t3 | 24.04.2017 |
| 16_KB3_t3.10 | 27.04.2016 | 17_KB3_t4 | 26.04.2017 |
| 16_KB3_t3.25 | 27.04.2016 | 17_KB3_t5 | 02.05.2017 |
| 16_KB3_t4 | 29.04.2016 | 17_KB3_t6 | 04.05.2017 |
| 16_KB3_t5 | 03.05.2016 | 17_KB3_t7 | 08.05.2017 |
| 16_KB3_t6.10 | 06.05.2016 | 17_KB3_t8 | 11.05.2017 |
| 16_KB3_t6.25 | 06.05.2016 | 17_KB3_t9 | 16.05.2017 |
| 16_KB3_t7 | 09.05.2016 | 17_KB3_t10 | 19.05.2017 |
| 16_KB3_t8 | 13.05.2016 | 17_KB3_t12 | 26.05.2017 |
| 16_KB3_t9 | 16.05.2016 | | |
| | | 17_KB2_t4 | 26.04.2017 |
| 16_KB2_t4 | 29.04.2016 | 17_KB2_t5 | 02.05.2017 |
| 16_KB2_t11 | 23.05.2016 | 17_KB2_t9 | 16.05.2017 |
| 16_KB5_t4 | 29.04.2016 | 17_KB5_t5 | 02.05.2017 |
| | | 17_KB5_t9 | 16.05.2017 |

***Figure SI5:*** *Sampling locations in the Kongsfjord. The majority of our field samples from 2016 and 2017 was taken at KB3, occasional additional sampling was performed at KB2 and KB5. Modified after Fransson et al. (2016)*
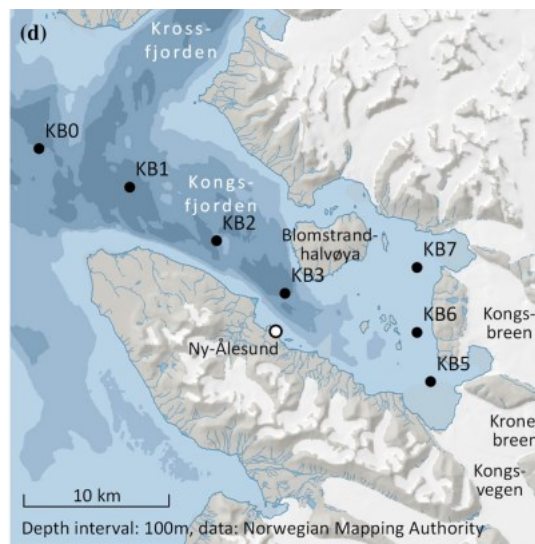
**References:**

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Wolf, K. K. E. and others under review. Company matters: The presence of other genotypes alters traits and intraspecific selection in an Arctic diatom under climate change. Global change biology.

# Chapter 5   Synthesis

## 5.1 Major findings of this Study

### 5.1.1 Compensating communities

Of the ten experiments using coastal Arctic phytoplankton communities described within *Publication I*, nine exhibited a strikingly high buffering capacity in Net Primary Productivity (NPP) towards elevated $pCO_2$, while species composition remained unchanged in seven of them. This result stands in stark contrast to similar studies in other regions, like the Antarctic (Hoppe et al., 2013; Tortell et al., 2008). Neither different light nor temperature treatments systematically affected this trait stability. Only the Davis Strait communities (DS) buffered alterations in productivity by a shift of functionally redundant species.

The only incubation with reduced productivity under high $pCO_2$ was the one conducted at the lowest temperature (1.8°, KFb_1), possibly hinting towards a tipping point of plasticity under high $pCO_2$ at low temperatures. In the most different treatments (*present-day* and *future*) of both experiment with Kongsfjorden communities KFa and KFb, warmer temperatures in combination with elevated $pCO_2$ did have a positive effect on primary productivity (*Appendix* Figure A1). This is to be expected in a nutrient-replete experiment considering only bottom-up controls (i.e. no grazers), but species shifts were minor even in those treatments.

The exceptional resistance of the majority of the incubations in NPP and species composition implies compensatory mechanisms on other ecological levels than among species (sensu *chapter 1.4*). For an adaptation at the observed speed (2-3 weeks), this could only be achieved by a sufficiently high physiological plasticity of individuals (i.e. acclimation within wide reaction norms) or by population plasticity through sorting of diverse strains (i.e. selection of standing stock). In order to assess the relevance of those two mechanisms, I chose one of the dominant diatoms in the KFa experiment, *Thalassiosira hyalina*, as model species and established monocultures from isolated cells for further investigations (see also Wolf et al., 2018)

### 5.1.2 Plastic and diverse individuals

In combination with a former study on isolates from KFa (Wolf et al., 2018), single strain experiments with isolates from the final community of experiment KFb (*Publication II*) revealed that both, individual and population plasticity may play an important role in the adaptation of *T. hyalina*. The majority of the six tested strains in *Publication II* responded with changes in a number of physiological parameters (among them growth rate and POC production) that were on an ecologically relevant scale, but none that would seem *per se* detrimental to their existence. Although many of the applied treatments surpassed the range of natural variability that cells usually experience under current conditions (see *Appendix* Figure A2.1), this was in line with my expectations for strains that originate from a relatively variable environment like the Kongsfjord (see *chapter 1.6*). Nevertheless, between the strains there was a surprisingly high degree of phenotypic differences, not only under the control conditions but also in the extent and even direction of response to the treatments. A principal

component analysis within the additional data (*Appendix* Figure A3) illustrates that each strain responded with a unique pattern or strategy of intracellular reorganization, as measurable in cellular quota and elemental ratios. This demonstrates that the plasticity of a population, which contains such diverse phenotypes as a standing stock, must be much larger than the plasticity of individuals. Concurrently, rapid selection among them could be a likely mechanism of adaptation.

The physiological investigations on isolates from the community experiments in *Publication I*, i.e. from KFa (Wolf et al., 2018) and KFb (Publication II), yielded differing implications regarding a dominance of the best adapted strains to the applied physico-chemical treatments. While the optimum ranges of the two isolated strains from KFa (Wolf et al. 2018) perfectly correlated with their former selection environment (i.e. the respective treatment of the community incubations of *Publication I*), the six strains isolated from KFb (*Publication II*) showed no clear response pattern according to their former selection environment. Here, only two of three strains performed better within treatments corresponding with their condition of isolation. Thus, the role of strain or lineage sorting as a mechanism underlying the observed resistance of natural assemblages as hypothesized in *Publication I* was not evident to act as straight forward as previously expected.

### 5.1.3    Selection at work: genotypes influence each other

Evolution and selection are well investigated processes since centuries. Still, in organisms like phytoplankton, where the identification and even the concept of individuals is elusive (*chapter 1.4*), intraspecific dynamics are difficult to resolve. Extending a fingerprinting method based on microsatellites (allele-specific qPCR), we were able to follow the selective processes within an artificial population of our six strains isolated from experiment KFb over the course of a competition experiment (*Publication II*). Based on the preceding characterization of the six strains in isolation, I predicted which of them should become dominant and therefore how the composition of the population would be expected to develop.

For both, the *present-day* condition and the one of elevated temperature and pCO$_2$ (*future*), the responses measured in monoculture suggested that the fastest growing strain in the respective treatment should largely dominate the artificial population. My observations revealed, however, that selection among genotypes does not merely function by one strain outgrowing the other at its fastest rate possible under the respective condition. Instead, strains alter their phenotype in response to the presence of others, and these changes are on a similar scale as in response to the applied abiotic treatments. This was true for growth rate, as visible in the unexpected dynamics of population composition, but also for other cell traits, which caused the bulk population quota and production rates to strongly diverge from our expectations (i.e. by more than 20%).

These results show that even within a highly simplified population and experimental setup, measurements in monoculture failed to predict the resulting genotypic composition. Moreover, even if we were able to forecast selection outcomes, we would still not be able to anticipate bulk values of the resulting culture. Furthermore, I found that different

environments appear to select for different components of fitness, which was revealed by strongly diverging and yet highly reproducible selection dynamics and outcomes in the two scenarios. Despite these reproducible differences, POC production was remarkably similar in the multi-strain cultures under both treatments, although considerably lower than predicted. This suggests that even a very low diversity can buffer changes in overall productivity. The reduced POC production compared to predictions also implies that interactions with other genotypes, however these may mechanistically function, could be energetically costly. The here observed stability was surprisingly similar to that in productivity in the community incubations in *Publication I*. Even more striking was the virtually perfect reproducibility not only of the selection outcomes but also of their temporal dynamics within the biological replicates. This provides a strong indication that we are far from grasping which factors drive lineage sorting, even in such a simplified setup. Nevertheless, selection appears not to be steered by coincidence but by reproducible forces. Hence, we may eventually have a chance to understand and maybe even to predict selection outcomes.

### 5.1.4    A step towards reality: Selection at natural diversity levels

The previous studies provided evidence that *T. hyalina* populations possess the potential for both, large population plasticity as well as selection between lineages. However, none of the phenotypical investigations on isolated strains could unambiguously resolve, whether lineage sorting had indeed taken place within the community incubations. In *Publication III*, we could finally approach this question through microsatellite genotyping and a novel method, microsatellite poolSeq barcoding (MPB) that allows a substantial acceleration of sampling and analysis of populations. At the end of the experiment KFb, all incubation bottles of the *present-day* and the *future* treatment still contained close to 100% diverse genotypes, which renders it highly unlikely that lineage sorting was strong enough to cause dominance of certain clonal genotypes (clonal dominance).

Analyses of the allele composition at our microsatellite loci throughout the community experiment supported this finding and argue against directional selection or genotypic shifts in either of the incubations of KFa or KFb within those treatments (see *Appendix* Figure A4 for Kfa). This suggests that individuals were sufficiently plastic to sustain their original population structure under these conditions. The treatment of low temperature at high $pCO_2$ (*acidification*), however, the same one that had presented the only exception to the resilient communities within P*ublication I*, showed a clear deviation in its allele composition from the others. The potential tipping point, already identified through species composition and productivity, was thus also visible on the intraspecific level. In this case the selective pressures were strong enough to cause a collapse in the *T. hyalina* population. The intraspecific shift implies either that here, bottlenecks and genotypic drift played a role or that the population was diminished in spite of intraspecific reorganization.

Temporal and spatial dynamics of natural protist populations are especially challenging to resolve with traditional approaches. Our new method also enabled us, however, to easily follow the allele composition of *T. hyalina* within the natural spring blooms 2016 and 2017.

Similarly to the majority of incubated populations, the allele patterns did not change strongly throughout the bloom seasons of each year, but did diverge clearly between the two consecutive years. In contrast to stable experimental conditions, the absence of genotypic shifts throughout weeks in a naturally variable environment, which is influenced by interregional ocean currents, seems unlikely. Two alternative reasons for the observed stability in allele composition are possible: Frequently changing selection pressures in the field could make selective processes chaotic enough to evade clear shifts into a certain direction. It is also possible that a population development was not detectable because selected gene variants were not associated with certain genotypes or our marker was not linked to any genes under selection (soft sweep). Fundamentally changed selection pressures or genetic drift throughout the winter months may in both cases still explain the differences observed between the years.

Irrespective of these uncertainties, the results imply that the investigated population is homogenous and likely recombines frequently within its boundaries. The outcomes also hint towards a more subtle understanding of lineage sorting, which appears not to be rigid enough in a fully diverse population to cause measureable clonal dominance, even in a stable experimental environment. Instead, plasticity may play a larger role than previously assumed and selective processes may act more on entire, potentially unrelated phenotypes than on single clones. This study illustrates that we are only beginning to understand the processes that steer resilience and selection within species, but also provides a new possibility to facilitate population genetic data acquisition and thus eventually our understanding of underlying mechanisms.

## 5.2    The elusive beauty of simplified experiments

Hypothesis-driven research aiming for process-understanding of natural ecosystems is often based on the idea of downscaling ecological complexity in order to identify casual relationships between its components. Based on this simplification, investigations typically take place in a controlled laboratory setting, where isolated mechanisms ideally reveal themselves reproducibly, while they may be masked in natural settings. Implicitly, this also means that those mechanisms are eventually meant to be scaled up again and projected onto a realistic ecological context. While working within such simplified and well-controlled laboratory systems (which are by no means simple in the true meaning of the word), it is easy to forget how much they diverge from the natural situation. The natural environment is typically highly variable and encompasses a suite of biological interactions, which take place most prominently in form of grazing and competition between species, but also among conspecifics. The here presented work illustrates the progressive realization of intraspecific complexity, which I found mirrored in the co-occurring literature in the research community of marine global change biology within the past years. While the majority of these insights are not new to biology, their existence or at least importance for phytoplankton has been ignored

or scattered across hardly connected research communities, which have started a dialogue just recently (e.g. Boyd et al., 2018; Collins et al., 2014).

The design of my PhD project was based on the assumption that physiological responses to an altered environment determine the most and least successful organisms in a system and that those winners then drive the community traits in a future environment (e.g. Boyd et al., 2015; Feng et al., 2009; Hare et al., 2007). The various physico-chemical drivers of this selection process and their interactive effects make the physiological response of a species even more non-linear than individual drivers and thus much more challenging to predict (e.g. Boyd et al., 2010). Based on my previous work on *T. hyalina* (Wolf et al., 2018) and in line with findings in other species, I expected that single strains of an Arctic diatom could not forecast a species' response (e.g. Kremp et al., 2012; Langer et al., 2009) and that selection of winners and losers could theoretically also take place *within* rather than among species (*Publication I and II)*. This implies also that both, individual plasticity as well as genotypic diversity in form of lineage sorting (i.e. adaptive evolution) have the potential to broaden the physiological scope of populations by providing winner genotypes for many occurring situations (Collins et al., 2014). With this knowledge, the observed stability of species composition and productivity in a range of incubations of diverse phytoplankton assemblages (*Publication I*) was less surprising. Still, the relative contribution to this resilience by phenotypic plasticity on the one and intraspecific selection on the other hand remained cryptic.

In recent years, a few studies have investigated in artificial populations, whether selective processes would in fact favor those genotypes that were identified in monoculture as the best adapted ones and therefore determine, i.e. forecast the traits of this population. Their results are often contradictive and could be based only on selection outcome instead of tracing the population dynamics (e.g. Hattich et al., 2017; Roger et al., 2012; Sjöqvist & Kremp, 2016). The temporal resolution of selective processes in *Publication II* could reveal that selective patterns differ depending on treatment conditions. While current knowledge lacks a mechanistic explanation of such ambiguity, this provides the inconvenient implication that our predictions on winners and losers are often not appropriate, even within a highly simplified experiment (i.e. stable environments with two environmental drivers, no species interactions and very small genotypic diversity). The relevance of such predictions of fitness from the laboratory to the natural environment has recently been questioned in a few studies (Bach et al., 2018; Ruggiero et al., 2017). Furthermore, not only the competitive ability of genotypes but also their cell composition and productivity differed in isolation or in the presence of other conspecifics, ruling out the validity of quantitative upscaling to the species level altogether (e.g. *Publication II,* Figure 3). Interactions between conspecifics have been described before in phytoplankton in the context of predation defense (John et al., 2015; Wohlrab et al., 2016), but were, to my knowledge, measured only once in cultures containing only one species (Schaum, 2014), where chemical signaling was suggested as a likely mechanism of interaction. In a variable environment, selection is likely to be more complex than competitive exclusion by limiting resources (sensu Paradox of the plankton, *chapter 1.4*). Even in the absence of environmental fluctuations, however, I observed other mechanisms,

such as intraspecific interactions, to play a role in slowing selection down (as in the *present-day* treatment of *Publication II*) or speeding it up (as in the respective *future* treatment).

Within my studies, the question of the importance of selective processes in natural populations could finally only be answered by population genetics (*Publication III*). By developing a new way of microsatellite analysis (microsatellite poolSeq barcoding, MPB), I could show that natural populations also exhibited unexpected intraspecific stability as well as strong composition shifts only under specific conditions (*Publication III*), thus confirming results from the artificial population experiment (*Publication II*) .The allelic composition in populations throughout the two most different experimental treatments (*present-day* and *future*) as well as throughout the natural bloom revealed little directional development. A clear shift of population composition was observed only in one experimental treatment (*acidification*) and between blooms of consecutive years. These observations illustrate how sudden diverse, seemingly resilient populations can collapse and that selective processes in natural, diverse systems may be much more subtle than often assumed for clonal organisms.

Over the course of my investigations, it became more and more obvious that only a simplified scenario can reveal underlying mechanisms of what we observe (e.g. by examining plasticity and selection separately), but also that we are still far from grasping even very basic concepts of intraspecific processes and therefore their relevance and interdependence. The insights from this work could only be gained by integrating findings from well-controlled laboratory experiments on monocultures with those of artificial and ultimately natural populations. While the relevance and interplay of intraspecific diversity, plasticity and selection as a buffer in natural systems may be a common concept in evolutionary biology (Fox et al., 2019), it is only beginning to be integrated into marine global change biology research (Kroeker et al., 2017).

## 5.3 Implications for the prediction of phytoplankton community dynamics and their stability in an Arctic environment

The here presented observations about simplified and natural population dynamics open up many questions, some of which I would like to further discuss in the following sections: If correct predictions from monoculture studies are so difficult, what do population responses and selection outcomes depend on and will knowledge about genotypic interactions help us to project them? Which intraspecific buffering mechanisms are important, how do they work and when do they fail? What have we learned about the investigated populations and what may this mean for their future? And finally, what does this mean for future experiments? Do investigations on single strains still make sense? How can we find results in the lab that are meaningful for the field? And on a broader scale, how can this baffling complexity be integrated better in order to eventually achieve a more realistic view on marine systems and their projection?

### 5.3.1 Intraspecific interactions and predictions from laboratory experiments

*How to assess the response of a diverse population?*

The notion that single genotypes cannot realistically represent the physiological properties of a species has been claimed for at least 40 years (Gallagher, 1980). Still, it seems to be continuously pushed aside by the temptation of working with this simplified concept for reasons of practical feasibility in intricate physiological experiments. Part of the problem may be that there are still no established alternatives for such investigations on single strains. Even when screening a range of different monocultures, it is debated how their responses can be integrated into a realistic population response: Neither the best performer, nor the worst, nor their mean appears to yield reliable estimations (c.f. Collins, 2010; Hattich et al., 2017; Roger et al., 2012; Sjöqvist & Kremp, 2016). In highly diverse organisms like phytoplankton, it is likely that the experimental identification of overarching patterns or representative averages for a population or species would require experiments with sample sizes larger by one or two orders of magnitude. Such large-scaled screening experiments would only be possible in considerably simplified setups that allow phenotypical screening with a much higher throughput (e.g. Brennan et al., 2017; Fontana et al., 2014; Gross et al., 2017).

*Monoculture responses do not predict competitive success due to intraspecific interactions*

Although it is often assumed that monoculture growth rates are representative of selective success in a competitive setup (e.g. Gsell et al., 2012; Pardew et al., 2018), opposing results have been described in several studies. Ruggiero et al. (2017) identified a genotype of the diatom *Pseudo-nitzschia multistrata* that dominated a laboratory competition experiment with four strains as well as phases of a natural bloom population. When tested in monoculture, however, it did not display the fastest growth rate. In an experimental evolution experiment, Schaum and Collins (2014) found that lineages which evolved to grow at slower rates were more stress-resistant and exhibited stronger competitive abilities than fast growers. This illustrates that the competitive ability of a given strain within a population is not simply determined by its physiological ability to 'outgrow' the others under the physical conditions at hand.

Resolving selective processes temporally in artificial populations under two climate change scenarios over the course of an experiment (*Publication II*) yielded some insight as to why there is no simple answer to this problem of prediction: intraspecific interactions influence both, the selective processes among conspecifics and their phenotypic traits. These two separate aspects are analogous to the two variables determining the population response in the concept of the Price equation (*chapter 1.3.2*): environmentally induced changes in traits of individuals or their offspring ('transmission') and selection among them. In biodiversity research, this is mainly applied to productivity or yield. In such contexts, trait changes are often referred to as the 'complementary' effect, which is only vaguely defined and could encorporate a range of processes (Fox, 2005). It can therefore account for deviations from the 'selection' effect, where the population adopts the winner's trait. Inconveniently, my results suggest that both, individuals' traits and selection, appear to be also influenced by intraspecific interactions that we cannot properly grasp yet (*PublicationII*). To my knowledge,

the alteration of cell traits by intraspecific diversity in phytoplankton has been quantified for the first time here and seems currently not reliably predictable. To dare quantitative upscaling from monoculture characteristics will require a much more profound understanding of populations and their dynamics.

*Predictability may depend on the environmental conditions*

In the simplified populations, selection appeared to be more predictable in an environment that deviates significantly from current conditions, like the *future* scenario (*Publication II*). This suggests that the accuracy of growth rate based predictions of selective processes depends on the conditions rates are measured in. One could therefore hypothesize that fitness and selection were more strongly driven by the responses to the more altered environmental conditions in the future scenario, which had also determined the responses in the monocultures that our predictions were based on. Thus, in the *future* scenario, the predicted winner indeed dominated the genotypic composition, although individual's traits still changed (*Publication II* Figure 2 and 3). In contrast, the strain composition in the *present-day* scenario of the same experiment remained close to its initial state and deviated strongly from the predictions based on monoculture results (*Publication II* Figure 2).

In a treatment resembling more ambient conditions, the phenotypic response range of genotypes may be more flexible and their traits may therefore also be more easily affected by interactions between conspecifics. In two laboratory experiments, Fontana and coworkers (2017; 2019) could show that under limiting conditions, coexisting individuals diversify their phenotypes and reduce the overlap of their requirements. A similar evasion of competition has been observed under nutrient limitation in the field between diatom species (Alexander et al., 2015). While in my experiment the cultures were not limited, these studies illustrate the kind of mechanisms that competitors may actively apply on the inter- and intraspecific level. Under *present-day* conditions, such trait diversification among strains may have been feasible, while under conditions diverging more from the ambient situation (such as high $pCO_2$), phenotypic response ranges may be less flexible.

Indirect or even direct inter- or intraspecific interactions potentially come at an energetic cost (e.g. via the synthesis of growth inhibitory or stimulating components or defense substances), as are many cellular processes that allow adjustments to a stressful environment (e.g. proton pumps at elevated pH or nutrient uptake systems). Therefore, one could hypothesize that in a more habitual environment, such interaction mechanisms could play a larger role because more resources (i.e. energy and educts) could be allocated to them. This could also explain why the productivity of the multi-strain cultures in *Publication II* was in both scenarios lower than expected from monocultures. While biodiversity research finds that high species diversity often increases the yield of an ecosystem (Hector et al., 2002), my results suggest that competitive intraspecific interactions may cause an additional cost (see also Collins, 2010). The costs imposed by environmental stressors on a population, however, likely also depend on the present diversity, since more efficient genotypes for a respective situation may be present and shape the population response.

*Predictability may depend on the tested organism*

In multi-strain experiments that include genotypes of different species, predictions from monocultures appear to work better than in experiments with conspecific genotypes (Low-Décarie et al., 2011; Pardew et al., 2018). Hence, predictability may generally improve when the response differences between the components of a mix are larger. This would be true when the reaction norms of individuals diverge more because they belong to different species or at least populations, but also when more extreme or stressful treatments are applied as discussed above, which push some organisms towards the limits of and some beyond their optimum range. It has also been observed that the responses of some species are more predictable than those of others (Bestion et al., 2018a). However, as long as such experiments rely on testing single strains of those species, their meaning for natural systems remains debatable. In order to judge whether inter- and intraspecific competition can be directly compared, we would require a better understanding of the mechanisms behind them. Chemical interactions between species (allelopathy) have been found to be an important part of phytoplankton competition, but not many compounds have been identified yet (Cembella, 2003; Legrand et al., 2003). The production of such chemicals can cause growth inhibition and cell death and may also be affected by factors like nutrient limitation, pH and temperature (Legrand et al. 2003, Tillmann & Hansen, 2009). Other chemically mediated responses induce defense traits (Lundholm et al., 2018; Wohlrab et al., 2017).

In diatoms, polyunsaturated aldehydes are candidates for signaling compounds and have been found to act in grazer defense and to induce programmed cell death (Ianora et al., 2004; Pohnert, 2000). A signaling pathway was identified that may reduce photosynthetic efficiency in neighboring cells (Vardi et al., 2008). Direct signaling between conspecific microalgae and diatoms is still not well studied, but increasingly found in all major algal groups (Venuleo et al., 2017). They may involve facilitation (John et al., 2015), sexual induction (Sato et al., 2011) and even growth inhibition and suicide commands that could regulate population growth (Brownlee, 2008; Casotti et al., 2005). Such findings show exciting parallels to better researched elaborate bacterial interactions such as quorum sensing (Hmelo, 2017). Furthermore, species- or even strain-specific interactions with or between the microbiome of other cells may also be possible mechanism, which is currently under increasing investigation (Amin et al., 2015; Bunse et al., 2016; Camarena-Gomez et al., 2018). It is therefore likely that biological interactions between and within phytoplankton species are much more intricate than the mere competition for nutrients and light.

*Some hope for predictions*

It appears that intraspecific diversity and interactions add even more complexity to the responses of phytoplankton assemblages, of which "we are currently unable to make even a rough calculation" (Collins & Gardner, 2009). At first, this seems hardly encouraging news. Nevertheless, it may reveal one of the reasons why we are continuously struggling to explain discrepancies between experimental results. The selection outcomes within most studies comparing mono- and multi-strain cultures, including *Publication II*, are highly reproducible (e.g. Roger et al., 2012; Collins et al., 2010). This offers hope that the observed deviations from

our predictions are not random, but are steered by mechanisms that we may be able to eventually grasp. Furthermore, and maybe more importantly, the apparently large functional diversity within species and their adaptive dynamics may point towards a larger capacity of assemblages to buffer and adapt to the anticipated changes than previously expected. In order to anticipate limits to this capacity, however, an understanding of its mechanisms is required.

### 5.3.2. Buffering mechanisms and tipping points in a changing environment

In the introduction, I introduced three ecological levels on which buffering against environmental changes can occur: the individual (plasticity and acclimation), the population (lineage sorting from standing diversity or mutation) and the community (shift between functionally redundant species). Throughout the herein presented studies, we found indications for all three mechanisms, as well as for experimental conditions that limited the buffer capacity of all of them. Disentangling these mechanisms in the laboratory and observing their role in a natural system is difficult (Gienapp et al., 2008; Merilä & Hendry, 2014), but can help us to understand how these processes may interact and what role they can play in population dynamics or stability.

*Resistance to applied treatments lies on the intraspecific level*
Although the majority of the isolated strains tested in monoculture showed decisively different responses to the applied treatments in their POC production (*Publication II*), I observed large resistance in productivity under the same settings when looking at different levels of diversity: The majority of natural community incubations showed no changes in NPP towards elevated $pCO_2$ (*Publication I*) and even a comparably minute diversity of six genotypes buffered the effects of the tested scenarios on POC production entirely (*Publication II*). The *in-situ* productivity estimations throughout the spring bloom in the Kongsfjord, where the assemblages originated from, were also in a similar range as the ones measured in the laboratory (38.2 ±12 µg C (µg Chl $a$)$^{-1}$ day$^{-1}$, Hoppe et al. (in prep.)). The concurrent lack of a pronounced species shift in most incubated phytoplankton assemblages from the Kongsfjord suggests that the observed resistance must reside to a large amount on the intraspecific level. At the same time, since the dominant species remained equally competitive, they must employ similarly effective buffering mechanisms, which sustain roughly equal plasticity in all of them within this framework. Although *T. hyalina* was not a key player in all community incubations and thus may not have been the main driver of the observed resistance in the natural communities, it suggests that other Arctic species could show similar characteristics. These findings stand in stark contrast with comparable investigations in the Southern Ocean, where similar treatments caused substantial shifts in productivity and species composition (Hoppe et al., 2013; Tortell et al., 2008; Trimborn et al., 2017). This illustrates that the efficiency of physiological or ecological buffers may differ between ecosystems and is likely influenced by organisms' environmental history. In order to know what such sensitivity or resilience depends on, it is necessary to gain a better understanding of the mechanisms behind them.

*Plasticity can sustain diversity and thus facilitate evolutionary adaptation*

Since individual plasticity and rapid strain selection could be disentangled in Wolf et al. (2018) and *Publication II*, we know that both have the potential to influence the results of the natural community incubations. The genetic analysis I performed suggests that within the range of the environmental drivers where no species shift was observed, phenotypic plasticity may have played a larger role than genotypic shifts (*Publication III*): Neither the traditional application of microsatellites, nor the new poolSeq barcoding technique detected any measurable signs of selection or differentiation between the populations studied here. This can either mean that no directional sorting took place, or that neither of the methods was able to resolve it. It also implies that the enormous diversity I found within *T. hyalina* populations was largely maintained throughout the incubation experiment (*Publication I and II*). This is notable, since considerable evolutionary bottle-necks and drivers for selection were induced by the setup, such as several strong dilutions of incubation bottles and a stable environment with conditions that diverge from the organisms' environmental history. In line with this, in a mathematical modelling approach, Menden-Deuer and Rowlett (2014) demonstrated that variability between individuals can sustain coexistence and diversity. Wide and variable individual reaction norms as measured in Wolf et al. (2018) for *T. hyalina* strains from the Kongsfjord, combined with trait diversification between conspecific genotypes (sensu Fontana, section 6.3.1), may thus maintain the large intraspecific diversity. If the effects of environmental conditions can be buffered by phenotypic plasticity, this may have beneficial effects for the populations. The maintenance of genotypic diversity could preserve the population's resilience by retaining the potential for buffering against conditions with more detrimental effects though lineage sorting. Furthermore, plasticity can also accelerate evolutionary processes (Lande, 2009) as long as it can provide partly adaptive phenotypes for a given situation (Baldwin effect, *chapter 1.5*).

Such interdependencies illustrate that individual plasticity and genotype selection are probably not disconnected but rather facilitate each other. It is therefore likely that both are important mechanisms for the resistance against change we observe in diverse communities. The relative importance of each may depend on the specific properties of the ecosystem and the evolutionary pressure imposed by the environmental conditions that occur at a certain point in time. For example, an unpredictable and variable environment may produce more plastic phenotypes and sustain a higher diversity (von Dassow et al., 2014), so that a genotypic shift would occur only at more extreme conditions. When such an 'extreme' condition or even a tipping point is reached may therefore depend strongly on the extent of plasticity as well as diversity within a population. While no genotypic shift could be detected in a natural population under the applied *future* treatment (*Publication III*), very similar conditions triggered the clear dominance of one strain when only six genotypes were present (*Publication II)*. At least in this stable environment, a low diversity could still buffer a response in population productivity through strain sorting and intraspecific interactions. This is in line with studies on the species level, where the degree of diversity necessary to buffer changes has been found to increase strongly in a more variable or stressful environment (García et al., 2018).

*Lineage sorting in diverse populations may not cause clonal dominance*

It is hard to imagine that a natural population should not experience selective processes, neither in experimental conditions nor throughout an entire bloom season. Therefore it seems likely that potential intraspecific shifts were simply not detectable for us until the population collapsed (as in the *acidification* treatment, *Publication III*). Although the new microsatellite poolSeq barcoding (MPB) method resolves the entire allelic range within the population, it was based only on a single locus in our case with some 30 polymorphic alleles. Still, this locus appears to be informative, since its allelic composition was extremely similar to that of traditional microsatellite genotyping (calibration 2 in *Publication III*) and yielded similar results as analyses based on six loci. I therefore hypothesize that lineage sorting in natural populations may rarely involve the dominance of a single or a few related genotypes in form of a clonal expansion (Ruggiero et al., 2017), at least not when individuals are plastic and phenotypically diverse. Instead, subtle frequency shifts across large parts of the population are more likely, which are consequently more difficult to resolve. If several genotypes can express a well-suited phenotype for an experimental scenario, a large group of lineages may become dominant. Each of them would become only slightly more abundant, and if all of them originated from a frequently recombining population, this group could contain a similar pattern of our microsatellite loci as the entire population. Such a shift would remain undetected. Furthermore, in a naturally variable environment, drivers of selection can change so quickly that intraspecific shifts occur not in a unidirectional way. Therefore, only a very large shift, such as between two years (*Publication III*), would become visible. As we can only describe relative differences between the populations, lineage sorting may have occurred on a cryptic level. Hence, the view of certain genotypes as winners of a situation may be a simplification, just as it likely is at the species level.

*Sudden tipping points and what they may depend on*

Within the here presented experiments, we did detect a limit to the stability of the tested populations and species assemblages suggesting that tipping points indeed occur unexpectedly (Scheffer & Carpenter, 2003). Elevated $pCO_2$ combined with the lowest temperature (1.8°C, experiment KFb, *Publication I*) caused a sharp decline in NPP along with a less pronounced species shift. Here, *T. hyalina* clearly decreased in abundance, and the concurrent shift in allele frequencies shows that the genotypic composition must have been strongly influenced as well (*Publication III)*. This is an impressive illustration of the importance of interactive effects of multiple drivers, also at high diversity. A larger impact of acidification under lower temperature would be physiologically allegeable: Since the cellular machinery (such as proton pumps that can regulate intracellular pH levels) is likely to work slower in colder conditions, they may not be able to fully compensate for the changed carbonate chemistry. However, no such effect was observed in the experiment KFa at 3°C under different irradiances (*Publication I* and its SI Figure 1). This may imply that the limits of stability can be breached by very subtle differences in the combined environmental drivers (here of 1.2°C), which is a known phenomenon in ecology (Botero et al., 2014; Wassmann & Lenton, 2012). If such a temperature threshold proves to be a general pattern, the underlying physiological mechanisms and tradeoffs would certainly be worth to investigate. The

observed difference may also be attributable, however, to a difference in the initial communities within our setup. If populations are locally adapted and show long-term persistence (c.f. Härnström et al., 2011; Sjöqvist et al., 2015), the location or region at which we sample will have a large effect on our results and concurrent interpretations (Schaum et al., 2012). And this may still be a simplification, since the phytoplankton communities in the two experiments KFa and KFb were sampled from the same location and season, but assemblages of the two different years (KFa in 2014 and KFb in 2016) appeared to vary in their population composition, responses and stability (*Publication I, III, Appendix* Figure A4). Both possibilities, sudden tipping points and large local or interannual variability of the community are likely, and thus render predictions on the future buffering capacity of coastal arctic phytoplankton very difficult.

*How do we define stability?*
The definition of a tipping point or a buffering capacity is a somewhat subjective one. In two of three incubations where a profound species shift occurred (e.g. Davis Strait, DS_1+2; *Publication I*), NPP remained largely unchanged. Therefore, in those incubations it seems like productivity was buffered on the level of functionally redundant species. While no species is entirely like another, a dominance shift between them will almost certainly cause differences in some community traits (e.g. elemental rations and quotas). Whether we consider these traits important will define our idea of stability and thus determine whether we consider such shifts a response or a buffer. In fact, the same considerations can be applied to intraspecific shifts, since even the traits of different strains of the same population can diverge drastically (*Publication II* and Wolf et al., 2018). The results of *Publication II* show that interactions between strains can strongly change bulk characteristics of a population from what we would expect. Therefore, dominance shifts and interactions on a genotypic level may be relevant even if those parameters in focus (like productivity) remain stable. This is especially the case when scaling characteristics up to larger contexts. The results of the phenotypic investigation of different genotypes in Wolf et al. (2018) are a good example of this: Independently of the treatment, one strain contained twice the amount of biogenic silica compared to the other. When we speak about stability, the definitions of tipping points and ecological buffering will much depend on the specific research questions and the traits we are measuring. This could also concern cryptic life-cycle traits, which are strongly related to fitness but rarely monitored (Hinners et al., 2017). It is therefore debatable, whether a change in species or genotypic composition can be considered a breached tipping point, if it entails only implications for higher trophic levels (such as grazer defense or food quality); or likewise, whether we can speak of stability if a change in genotypic composition could increase carbon export by doubling cell quotas.

Applying the measures typically used to assess ecosystem function, like productivity and species composition, the combined buffer capacity of plasticity, intraspecific sorting and species shifts appears to make coastal Arctic and subarctic phytoplankton assemblages more resistant to environmental change than expected based on experiences from other regions. It will be an interesting endeavor to investigate which environmental factors may cause such

more or less stable communities and if the resistance and adaptive abilities at hand will be sufficient to cope with further changes of the coming decades.

### 5.3.3 Implications for *Thalassiosira hyalina* populations in the Kongsfjord

*Phenotypic and genotypic diversity throughout the fjord*

The incubations of *T. hyalina* monocultures (*Publication II;* Wolf et al., 2018) illustrate that individuals of this coastal diatom have wide tolerance ranges towards $pCO_2$ under different temperatures that exceed the conditions they usually experience within their main growing season during the spring bloom (March-May, c.f. *Appendix* Figure A2.1). Nevertheless, the optima and response to interacting environmental drivers differed greatly between individuals and indicate phenotypically highly plastic and diverse populations (*Publication II)*. It is likely that this may be induced by the high environmental variability in the fjord. The physico-chemical conditions are influenced by very different water masses from inside (calving and runoff from glaciers) and outside the fjord (advection from Atlantic (West Spitzbergen Current) and Arctic (East Spitzbergen Current) currents; Svendsen et al., 2002a). Next to strong seasonal variations of inflow, irregular advective events are driven to a large degree by wind and eddies (Tverberg & Nøst, 2009), which makes temperature, salinity, alkalinity and nutrient regimes variable as well as often unpredictable. These environmental conditions create a perfect selection environment for plastic phenotypes (e.g. De Jong, 2005; Schaum et al., 2015). In addition, the prevailing shallow coastal shelfs provide the possibility for species with resting stages (such as *T. hyalina*) to store their genetic material in 'seed-banks' in the sediment (Lennon & Jones, 2011). Moreover, frequent recombination among genotypes is suggested by a large accordance to Hardy-Weinberg equilibrium (*Publication III*). In conditions like this, a population has the potential to be well enough adapted to sustain a relatively stable genetic composition (in spite of a supply of non-native genotypes).

*Drivers of population stability and differentiation in the Kongsfjord*

Throughout the development of a natural spring bloom, the allelic pattern hardly shifted. In my view, this is not surprising in highly variable natural conditions as observed in the Kongsfjord where drivers of selection may change so quickly that no large, unidirectional and thus measurable intraspecific shifts occur (Bell, 2010; Rynearson & Armbrust, 2005). Independently of their vicinity to the fjord opening, all three stations sampled showed the same allelic composition (*Publication III* Figure 3 +SI5), which could mean that the native population is homogenously distributed throughout the fjord. It also suggests that genotypes from outside the population do not seem to be influencing it substantially throughout a season or that the population expands beyond the boundaries of the fjord into the Fram Strait area. Between the spring blooms of 2016 and 2017, however, the genotypic composition changed enough for our barcoding method to detect it (*Publication III* Figure 3). Although it is likely that the populations of the two seasons are intricately connected through resting stages in the sediment, the inflow of new genotypes from outside the fjord is to be assumed in a system like the Kongsfjord where large amounts of water from Atlantic and Arctic regions are advected throughout the year. While this influx of genotypes may not be able to compete with

a locally adapted population during a bloom phase, such non-native cells may still leave spores in the sediment for the next season. A hint towards a bentho-pelagic coupling of the subsequent years through resting stages is the observation that samples from the late phases of the bloom 2016 increase their similarity to the population of 2017 (P*ublication III* Figure 4). Interestingly, this shift in allelic composition of the bloom population in 2016 coincides with a sharp decline in nitrate concentrations the in the fjord in mid-May (*Appendix* Figure A.2.1a and Smola et al. (in prep.)) as well as a temporary drop in *T. hyalina* abundance, which recovers afterwards (*Appendix* Figure A.2.2). I hypothesize that the onset of nitrate limitation and the concurrent selection pressures may have caused a genotypic shift, yielding a population composition more fit for nutrient-limited conditions. This second subpopulation may subsequently have provided the majority of seeding material for the next year. Throughout the winter season, only few active cells are present in the water column (Kvernvik et al., 2018), and resting stages in the sediment or deep water layers likely play a prominent role for the population composition of the next year (McQuoid & Godhe, 2004; Sefbom et al., 2018). Since during spore formation, overwintering and early germination, different qualities than during the spring bloom may be of competitive advantage, the winter may constitute a considerable evolutionary bottle-neck.

*Thalassiosira hyalina populations are resistant but far from invincible*
Although my investigations imply a high resistance of current *T. hyalina* populations towards environmental change, their sudden collapse in the experimental *acidification* treatment illustrates that little is required to make a seemingly stable community change – especially if strong competitors belonging to other species can quickly take over. Even more than this experimental result, the fact that the timing and composition of phytoplankton blooms in the fjord are known to vary strongly between years (Hegseth & Tverberg, 2013) shows that many different drivers must shape the conditions that finally determine bloom phenology and composition. Two of the most important aspects, for example, are the influence of nutrient availabilities and grazing pressure, which were entirely ignored within this conceptual work. Still, within a time-series we could resolve a striking stability of primary productivity throughout blooms although they were dominated by species of entirely different functional groups (Hoppe et al., in prep.). This illustrates that in spite of intraspecific processes playing a decisive role, species shifts do not lose their importance as a buffer in ecological functioning. Irrespective of reshuffling within an ecosystem on all buffering levels, total productivity may be determined by more overarching regimes (such as total nutrient supply and length of the growing season through irradiance), which are more profound but often slower to change. Such alterations of the system may give fast adapting organisms like the ones investigated here more time for sufficient adjustments.

## 5.4    Future Directions

The here presented work is a further piece of evidence that on every ecological level (individuals within populations, species within a community and finally phytoplankton within the entire ecosystem) not only interactions with the physico-chemical but also with the biological environment and even among conspecifics have a large impact on ecosystem characteristics. Furthermore, each of these ecological levels is continuously shaped by the surrounding selection pressures, which alter the characteristics of individuals (via plasticity), populations (via sorting) and communities (via species shifts). Therefore, understanding phytoplankton responses to its physico-chemical conditions at its present state can provide only limited insight on its ability to cope with future conditions since it describes merely its current state in an isolated scenario (Sauterey et al., 2015). The unequivocal relevance of diversity and evolutionary processes, even on short timescales and in simplified setups, urges us to find ways to reconcile physiology, ecology and evolution in the way we assume the world to function, which is expressed for example in models.
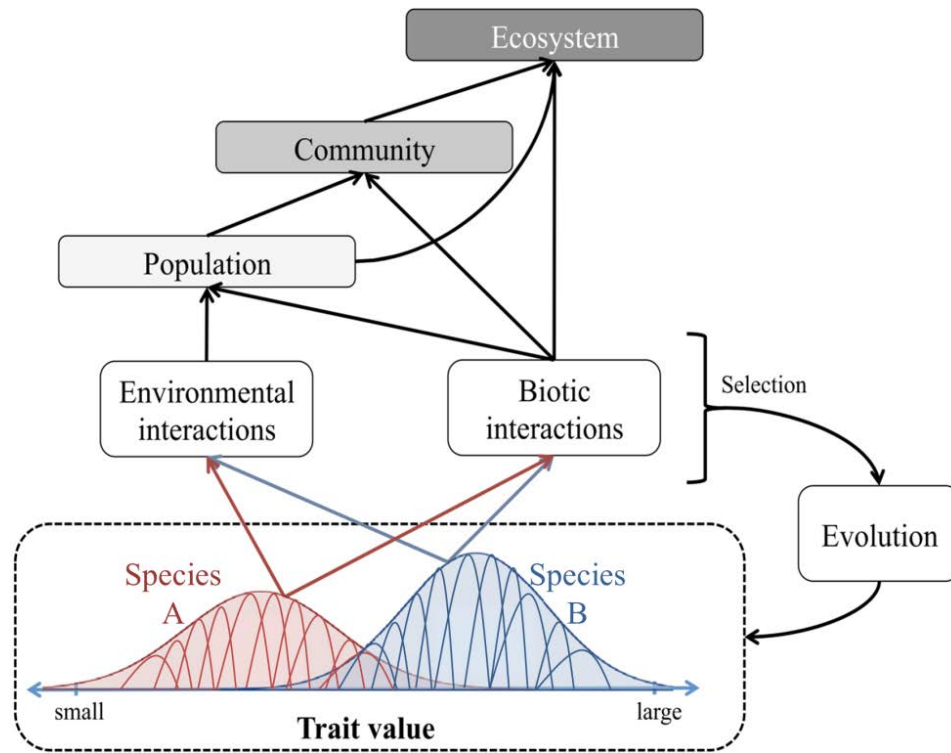


**Figure 6.1**: *Conceptual overview of different ecological levels and their interactions through ecology and evolution from a trait-based perspective. The physiological traits of species or populations are variable and composed of the traits of the diverse individuals within them. This trait variation structures species' interactions, determining the patterns that arise at population, community, and ecosystem levels. Finally, trait values can change through evolutionary processes as a species' or population's context determines the selective environment it faces. Modified after Kremer et al. (2017b).*

This idea is well summarized from the view of trait-based modelling in Figure 6.1. Large-scale Earth-System models may not be able to include small-scale dynamics of these complex processes within biology because we lack sufficient process-understanding and because models are limited by computational power (Asch et al., 2016). A way forward, however, could be the use of specialized models, which may be able to apply and conceptualize findings from experiments and field observations in a way that eventually allows incorporation into larger-scale models (Tréguer et al., 2017). Trait-based or smaller scaled models are continuously developed and improved in order to better represent and investigate the role or mechanisms of diversity and evolution (e.g. Follows & Dutkiewicz, 2011; Follows et al., 2007; Irwin et al., 2015; Le Quéré et al., 2005; Menden-Deuer & Rowlett, 2014; Sauterey et al., 2015). Most likely, there will not be one single best way to integrate the interplay of plasticity, ecology and evolution (Kremer et al., 2017b), but biologists within these disciplines can increase their communication and try to keep the relevance of the other factors in mind when designing their studies.

### 5.4.1    The quest for the appropriate experimental design

Our attempts to understand natural systems are bound to the eternal compromise between the two ends of investigative setups: controlled and understandable but unrealistically simplified laboratory conditions on the one hand and the natural but uncontrollable *in situ* environment on the other where the complexity of drivers often conceals causal relationships. Each approach has its advantages, and the type of question to be answered determines the appropriate method. If understanding and predicting phenotypic responses to environmental change is the ambitious goal, the integration of both ends of this complexity is necessary. Each of them needs the other to provide the theoretical and parametric base for models that can test and apply the gained knowledge.

*Experiments with single strains in monoculture*
Discrepancy between phenotypes measured in a laboratory and those expressed in the natural environment can be explained by the divergence of laboratory and field conditions in many apparent physico-chemical aspects. Furthermore, depending on the origin of organisms, local adaptation of populations may cause substantial differences in the mean reaction norm of their members. My results illustrate that intraspecific diversity within populations and interactions between conspecifics may be yet another component that causes variable responses. The inconsistency between responses in lab or in nature becomes potentially stronger on timeframes that allow for evolution (depicted in Figure 6.2), but similar phenomena are valid on short timescales if biotic interactions are taken into account.

All these arguments imply that it is risky to consider the response of a single strain in monoculture as a realistic representative for the species and use the measured values for upscaling or parametrization on an ecosystem level. Such experiments are and will remain indispensable, however, to understand causal relationships and physiological mechanisms that underlie a response pattern. While they may not be the best framework to investigate *how* an organismal group will respond to change, they are certainly the only way to find out

*why* certain responses are observed and can therefore elucidate their underpinnings and boundaries. Since such investigations usually require highly intricate and costly studies, it is important to confirm that the examined driver (or driver combination!) is indeed of paramount relevance for the overarching question and its significance has been repeatedly observed.

Because laboratory experiments involve by definition strong simplifications of the physico-chemical and biological environment, projections on 'winners' or 'losers' within the complex natural system are unlikely to be realistic (Bach et al., 2018; Dutkiewicz et al., 2013; Webster et al., 2017). Such assumptions can only be dared if they take the role and interactions of other abiotic and biotic drivers, but also the adaptive capacity of an organism into account (Fox et al., 2019). I suggest that phenomena observed in monoculture experiments should therefore only be generalized if they are highly replicable among lineages, consistent with observations in a natural context, or can be explained by underlying physiological mechanisms.
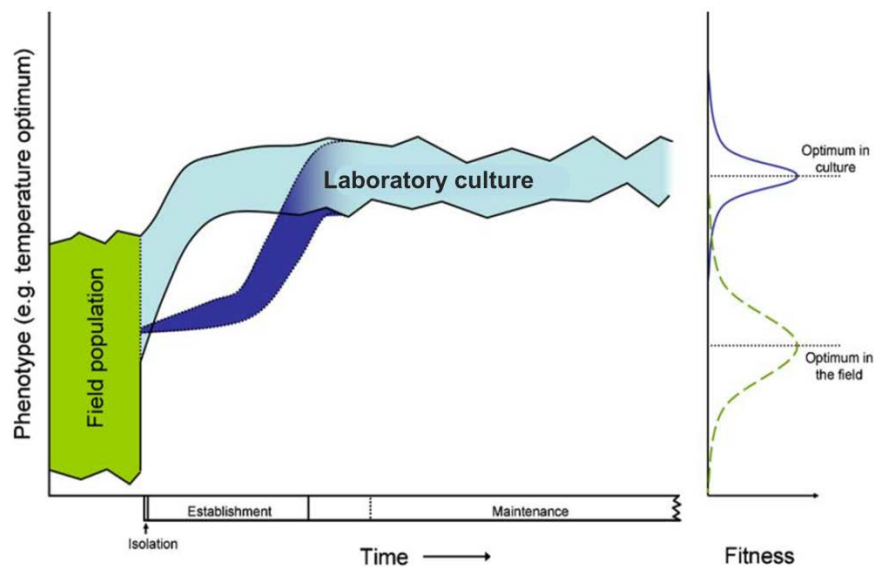


***Figure 6.2***: *Discrepancies between in situ and in vitro responses can be induced by subsampling effects of phenotypically diverse populations, by adaptation to the physico-chemical laboratory conditions, but also by biological interactions. The combination of these influences may cause a fundamentally different reaction norm or optimum range to be measured in a laboratory culture compared to the natural field population. The measured temperature optimum, as in this example, could be above, below or within the optimum range of the natural population. This effect can be amplified by evolutionary adaptations on longer timescales. Modified after Lakeman et al. (2009).*

*Experiments with diverse strains or populations*

While monoculture experiments can focus on mechanisms behind responses to the physico-chemical environment, understanding interactions with the biological inter- or intraspecific surroundings require working with artificial or natural populations and species assemblages. Since in planktonic microbes, cells in suspension cannot be easily separated, such setups reduce the available information on individual strain or species and mainly yield bulk parameters on the entire assemblage. To some degree, this disadvantage can be tackled by membrane-separated incubation chambers that allow the growth medium but not the cell lineages to intermix (e.g. Dunker et al., 2017). Molecular approaches like asqPCR (*Publication II*) may facilitate our mechanistic understanding of intraspecific interactions in controlled environments. Depending on the parameters to be measured, novel flow-cytometric techniques allow quantification of single-cell traits even in fully diverse assemblages (Fontana et al., 2016; Pomati et al., 2013).

The problem but also advantage of such mixed cultures is that they also include adaptive processes like sorting, which are an important component of the response to changed conditions. It remains difficult, however, to disentangle the plasticity of individuals (i.e. acclimation) and that of the entire population (i.e. selection and sorting). On the int*er*specific level, abundance shifts between phytoplankton species can be comparably easily morphologically or genetically identified. The new methodologies of extended asqPCR and microsatellite poolSeq barcoding (*Publication II+III*) may be able to diminish the problem of detecting selection also on the intraspecific level since shifts in the genotypic composition can be more easily measured. While reaction norms of single strains depict their individual phenotypic plasticity, reaction norms of mixed cultures may be more variable due to intraspecific shifts, but could also illustrate a more realistic population plasticity that integrates selective and interactive processes. If reproducible, such reaction norms may yield at least slightly more realistic parametrization for modelling approaches.

*Observations in the field*

The bias of simplification can only be avoided by observations in the field. The challenge here is to identify and monitor the relevant drivers that cause a response over short or long timescales. Mesocosm or on-deck incubations, which expose the natural community to certain drivers in partly controlled variable in-situ conditions, offer a useful intermediate step that can even include grazers (Hoppe et al., 2017b; Riebesell et al., 2013). Nevertheless, they can also be subject to several substantial biases, such as differences in initial communities and environmental fluctuations (Moreno de Castro et al., 2017). Studies in analogous natural systems that are already experiencing conditions expected elsewhere in the future may also offer insights into possible responses in the context of the entire ecosystem (Rastrick et al., 2018). If in the ecosystem of interest, monitoring of the most influential drivers could be achieved (e.g. by means of automated moorings, long-term observatories or regular manual sampling), along with close resolution of species composition, correlations of responsiveness can be drawn (see below). In such a context, microsatellite poolSeq barcoding opens the exciting new possibility to monitor the intraspecific dynamics within certain species as well.

With its help, it may become possible to differentiate changes in species composition, genotypic composition or otherwise to deduce phenotypically plastic adjustment on the physiological level. If we could determine on which ecological level a response towards drivers is based, it may allow for better inferences about its causes and further consequences for ecosystem properties. Still, to which extent the knowledge about one community or population can be transferred from one location to another, needs to be carefully considered. If we improve our understanding of the physiological and ecological mechanisms that drive the ecosystem, however, we also have better chances to realistically assess which systems can be compared.

### 5.4.2    Perspectives towards predicting complex natural ecosystems

It seems already an intimidating goal to understand and eventually predict a multi-dimensional system with numerous interacting abiotic and also biotic influential factors by performing one- or two-dimensional experiments. In addition to this, the drivers as well as the responding organisms are continuously and rapidly changing due to environmental alterations and evolution. This is especially valid for protists in a region as affected by climate change as the Arctic. However, the overarching goal of prediction is what ecosystem models ultimately aim for (Mouquet et al., 2015) and what experiments are expected to facilitate. A sign of relief is to be found in studies suggesting that within a large range of drivers, it is typically only a few dominant ones that shape an adaptive response (Boyd et al., 2015; Brennan et al., 2017). Thus, steps towards better prediction, e.g. of phytoplankton blooms, can indeed be taken if those dominant drivers can be identified along with the important (i.e. fitness related) traits of the investigated group of organisms (McGill et al., 2006). But how to identify these relevant traits and drivers in systems we often hardly understand?

Especially in remote regions, emerging technologies that allow field observations through data acquisition across large temporal and spatial scales may be of considerable aid in this endeavor. Automated sensor-based monitoring (e.g. Cottier et al., 2005; Pomati et al., 2011) can provide high resolution data on a range of environmental parameters that may potentially act as drivers. Concomitantly, the potential effects can be observed by measuring biological characteristics through flow-cytometry, optical or and –omics approaches, which can monitor various cell traits (Fontana et al., 2014), abundance of size classes (Bracher et al., 2017; Brewin et al., 2011) or taxonomic groups (Bowler et al., 2009; Guidi et al., 2016), population genetic characteristics (MPB) or even expression of target genes (Alexander et al., 2015). Correlations between these kinds of measurements can provide valuable indications for causal relationships (Hunter-Cevera et al., 2016). Since processing vast amounts of data becomes increasingly easy, more sophisticated machine-learning approaches can refine the identification of important drivers, which is currently being realized in simple systems (Thomas et al., 2018).

These approaches can uncover more cryptic correlations in the field and thus provide a promising tool to generate hypotheses on those mechanisms that shape dynamic ecosystems. It will still be the task of experimental research, however, to confirm and refine our

knowledge on the identified drivers and provide the mechanistic foundations of such correlations, which can eventually shape predictive models. Concurrently, the results presented within this thesis are pointing towards the necessity for experiments to validate their findings in setups that increasingly approach the natural situation. If the prediction of responses of future ecosystems is to be the broader objective, natural systems need to be considered in a way that treats biological interactions, diversity and adaptation as crucial components.
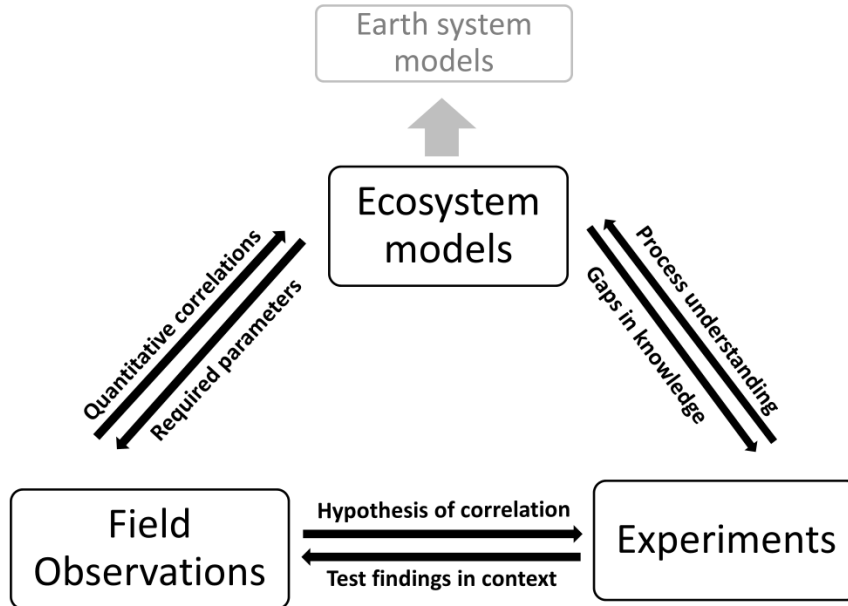


**Figure 7.7:** *Conceptual idea on how experiments, field observations and modelling approaches could profit from each other in order to eventually improve understanding and predictive power of the ecosystem. Detected inaccuracies in ecosystem models can define where observational parameters or process-understanding is required most. While field observations can provide them with quantitative parametrizations, they can also identify potential correlations. Experiments, which may take place in the laboratory or simplified in-situ conditions, can test these hypothetical dependencies and verify and refine them in an increasingly natural context. This gained process-understanding can then be delivered back to be incorporated conceptually into an ecosystem model and eventually on a broader scale into earth system models.*

## 5.5 Conclusions

More than 100 years ago, Haaken Gran began to investigate phytoplankton blooms and noted that "such a universal phenomenon [...] must have a universal acting cause" (Gran, 1902), but also that bloom development is "[...] much more irregular than it would be if merely such simple factors as warmth and light controlled production". Today, much knowledge has been gained on many other factors that influence primary productivity in the ocean, and still it appears that the universally acting causes, which Gran referred to and which are required for models and prediction, are only partly understood. Progress can only be made if all basic mechanisms driving a response, not only physico-chemical but also biological ones, are identified and included. A few of them, namely plasticity, diversity, adaptation and intraspecific interaction, have been addressed in this thesis and were shown to have substantial influence on the performance of populations and communities. In most cases observed here, this influence was stabilizing. The Arctic communities focused upon in this work were found to have a high potential to successfully adapt to future conditions, and much of this resilience seems to rest within the plasticity of populations and individuals. The wide reaction norms of individuals, their high diversity and the predominant maintenance of this diversity under future scenarios as well as over time in natural conditions indicate that populations are well buffered against environmental change on several ecological levels. Nevertheless, the fact that these results as well as the sudden and adverse response to cold and acidified conditions were unexpected, illustrates that our comprehension of phytoplankton physiology and population dynamics is still insufficient to anticipate their future. My work has shown that especially the consequences of diversity and selection within species are not as simple to forecast as often assumed. Some of the herein established methods may advance the still methodologically challenging research on these issues. The investigated populations originated from coastal ecosystems that are already well in the course of change. It may be highly elucidating to compare similarities or dissimilarities in other regions like the high Arctic or Antarctic, which are until now less impacted environments.

# Chapter 6   References

# 6 References

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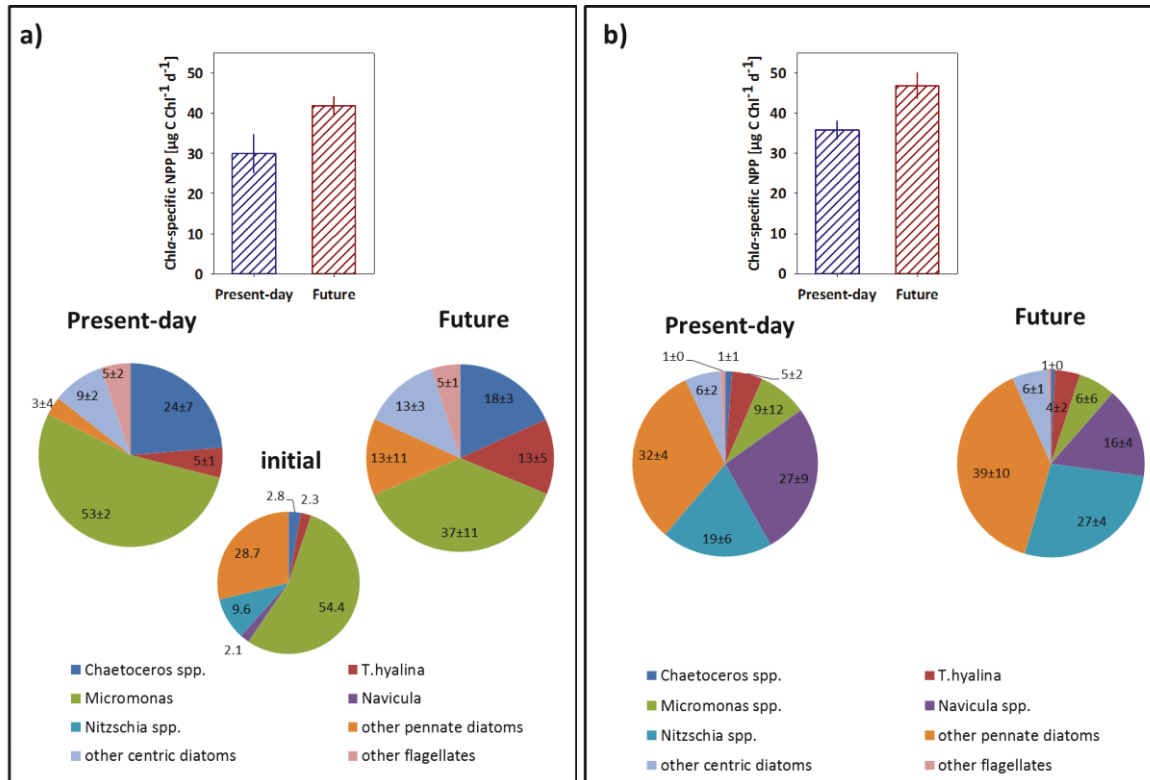
# Appendix

## Appendix 1

## Additional Data community incubation experiments 2014 and 2016



**Figure A1: Species composition and Chl *a*-normalized Net Primary Productivity (NPP) for the extreme treatments *present-day* and *future* of the community incubations a) 2014 (KFa) and b) 2016 (KFb) at the final time-points, which served as selection environments for the isolates investigated in Wolf et al. (2018) and *Publication II*, respectively. Numbers in the pie charts denote the percentage contribution of each species to the total cell count. The initial species composition could only be assessed of experiment KFa. Bars show final mean NPP of the community incubations with the standard deviation of the biological replicates. Please note that when tested against each other (student's t-test), NPP of the two treatments were significantly different within KFa and KFb, while the effect of *acidification* alone within the temperature levels of all experiments in *Publication I* was only significant under the lowest investigated temperature . Details including methods can be found in *Publication I*.**

*Although the present-day and future treatment in both experiments diverged strongly (KFa: 3°C, 400µatm and 6°C 1000µatm at 30µE and 150 µE irradiance, KFb: 2°C, 400µatm and 7°C 1000µatm both at 50µE irradiance) and were expected to present a substantially different selection environment, the species composition was not significantly different in either of them. The species shift compared to the initial community as depicted in a) is likely due to the bottle-effects of the laboratory conditions and occurred independently of the manipulation of temperature and CO₂ in all replicate bottles. This suggests that the majority of species within the communities were similarly competitive under the applied treatments. As shown in Publication III and Appendix 4, the allelic composition of T. hyalina populations was different between experiments KFa and KFb but not between the present-day and future treatments within them.*

## Appendix 2

## Environmental variability in the Kongsfjord and spring bloom in 2016
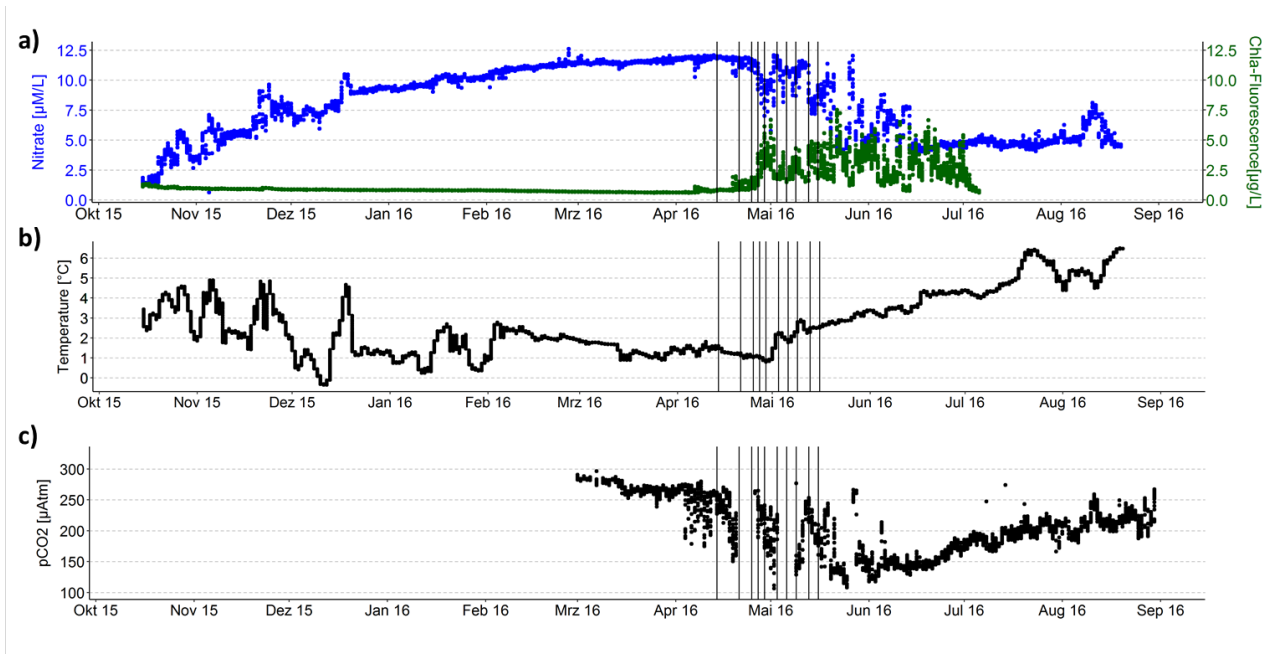


**Figure A2.1:** *Environmental variability in the Kongsfjord as measured by on-site time-series. Vertical solid lines denote date of DNA sampling for microsatellite PoolSeq barcoding. a) Nitrate concentrations [µM] and nominal Chl a-Fluorescence [µg L$^{-1}$] as well as b) temperature [°C] for the season 2015-2015 as measured by autonomous sensors moored in the fjord close to the coast between stations KB2 and KB3 at 25m ('Brandal', Smoła et al. (in prep.); Nitrate was measured by a SUNA-V2 nitrate sensor (Satlantic) and calibrated based on discrete samples from a RAS water sampler. Fluorescence and temperature were measured by a SBE16+ CTD (SeaBird Scientific). c) pCO$_2$ [µAtm] of spring and summer 2016 as measured by the 'Ferry Box' station sensors from a near-shore seawater inlet (HZG time series database; http://tsdata.hzg.de). Discrete pH measurements between March and September were in the range of 8.07 and 8.26. Please not that pCO$_2$ values are in raw version and not quality controlled.*
*Monitoring of some key environmental parameters show very well how nitrate concentrations and Chl a levels are correlated. Over the winter months, nitrate accumulated to some 12  5µM NO$_3$ L$^{-1}$, but was quickly drawn down as soon as the spring bloom started and Chl a began to rise. A stable state (likely of regenerated nutrients) established in the later summer at some 5µM NO$_3$ L$^{-1}$. Temperature in the spring bloom phase between March and June was approximately between 0.8 and 4°C, pCO$_2$ between 100 and 300 µAtm. Both ranges differ substantially from the treatments applied in my experiment as warming and high CO$_2$.*
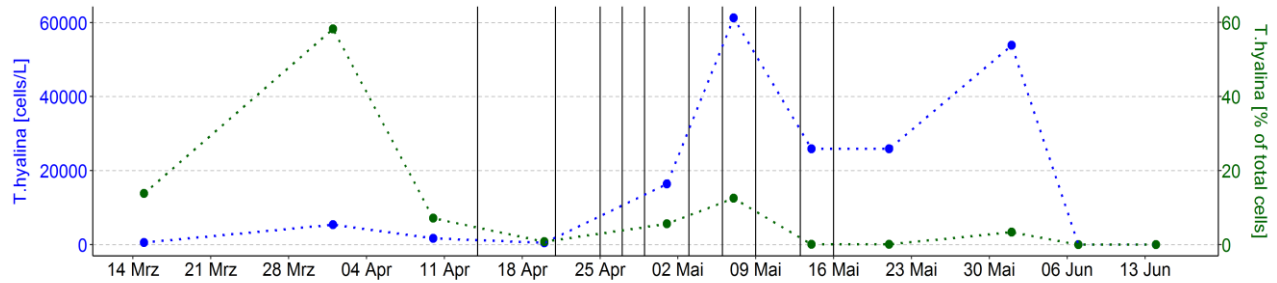
**Figure A2.2:** *Development of T. hyalina cell abundance throughout the spring bloom 2016 as counted manually under a light microscope in samples from a mooring deployed close to the coast between stations KB2 and KB3 ('Brandal', Smoła et al. (in prep.)). Absolute number of T. hyalina cells per liter (blue) and as percentage of the total count of phytoplankton cells per 10mL (green). Vertical solid lines denote date of DNA sampling for microsatellite PoolSeq barcoding.*

*The absolute population size of T. hyalina reached its peak in early may but declined in mid-May, which coincides with a drop in nitrate concentrations. Notably, this is also the timeframe in which microsatellite poolseq barcoding revealed a shift in allelic composition. Afterwards, towards the end of May, the population increased again until it crashed entirely in early June. In relative terms, the contribution of T. hyalina to the total cell count was largest at the beginning of the bloom and played only a minor role once massive Chl a accumulation started. Please note, however, that this is relative cell number, while T. hyalina contributes some 100 times the amount of biomass per cell compared e.g. to picoplankton like Micromonas sp., which are often abundant in cell counts.*

*Smoła, Z. T., Wiktor, J. M., Hoppe, C. J. M., Cottier, Finlo, Greenacre, Michael, Salter, Ian, Scholz, Daniel, Berge, J. and Leu, E. (in prep.). Seasonal succession of phytoplankton communities in two contrasting fjords: how does Arctic environment control bloom phenology.*

## Appendix 3

## Variable physiological strategies of strains from the same population
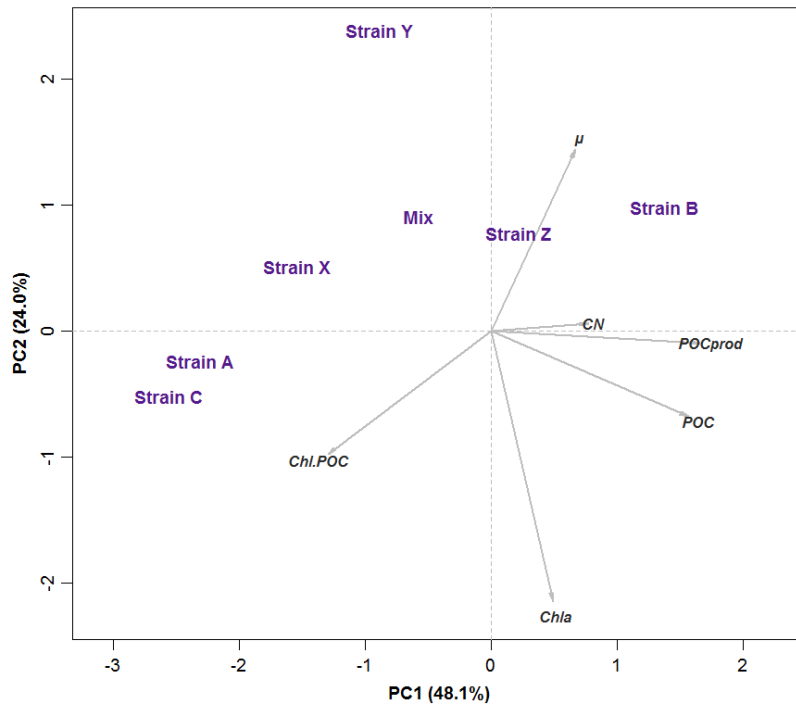


**Figure A3: Principal component analysis of the mean effect size between present-day and future treatments for six strains in monoculture isolated from experiment KFb, as well as the multi-strain incubation ('Mix') including all of them together. The effect size of the following ecophysiological parameters was included in this analysis: growth rate (μ), Chl *a* and POC quota, POC production as well as ratios of C:N and Chl *a*:POC. Details can be found in *Publication II*.**
*All strains displayed different adjustments of their ecophysiological traits under changed conditions, suggesting that several potential adaptive strategies were present. Some strains responded more similarly than others (e.g. strain A and C), which may hint towards similar physiological strategies being adopted by different genotypes. It is noteworthy that strain Y, the dominant strain within the multi-strain culture, is depicted at a larger ordination distance to the others, mainly due to strongly reduced Chl a quota and elevated growth.*

## Appendix 4

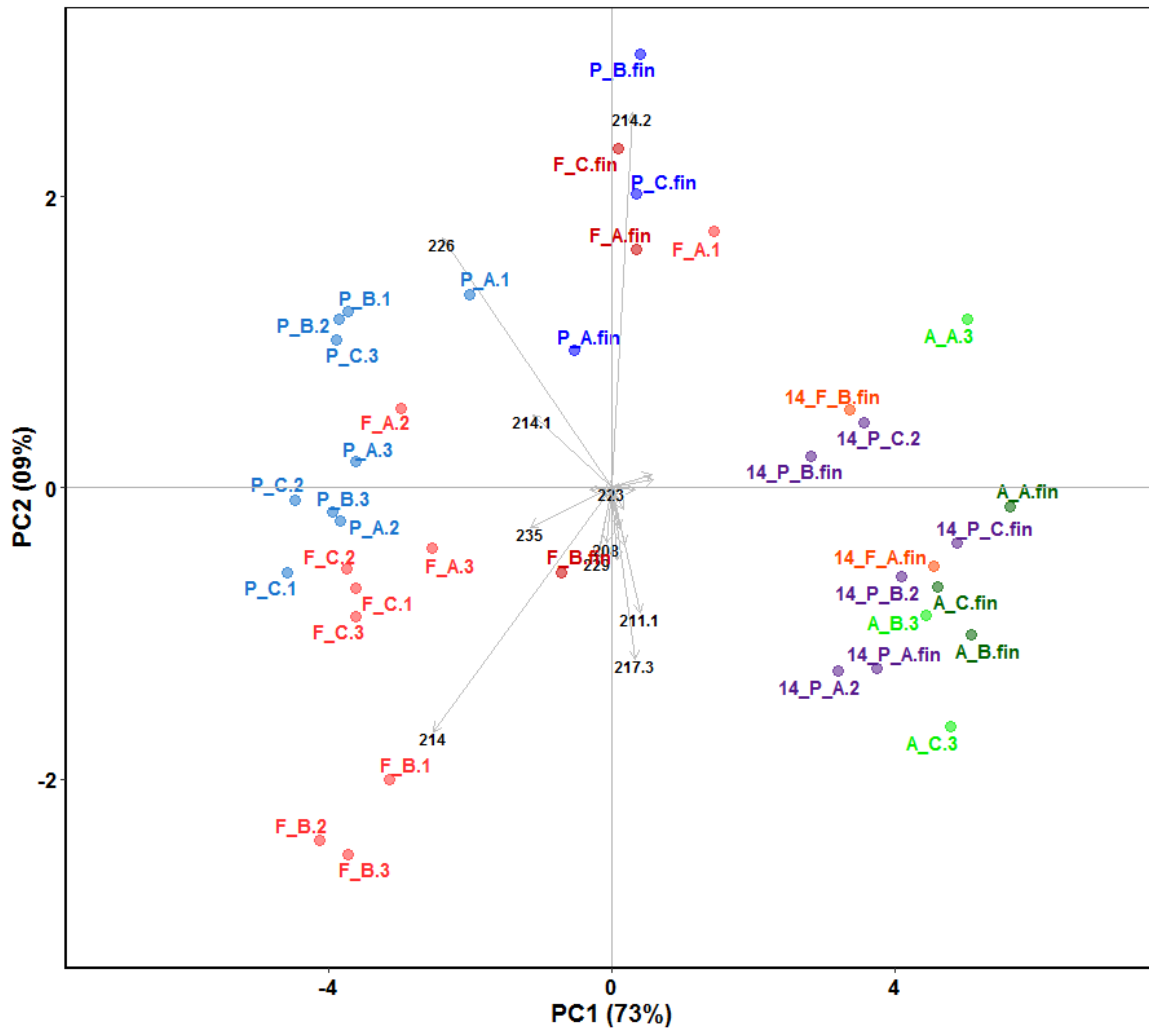### Microsatellite poolSeq barcoding of community incubations 2016 and 2014



**Figure A4: Principal component analysis of allele frequencies within samples from the community incubation experiments KFa (14_P: *present-day*: purple, 14_F: *future*: orange) and KFb (P: *present-day*: blue, F: *future*: red, A: *acidification*: green) as measured by microsatellite poolSeq barcoding using primer ThKF3. *t1-tfin* describe the time-point of sampling within the experiment. Black numbers refer to the allele lengths, which were used as underlying variables for PCA. Only the 10 most influential ones are identified here. Different allele versions of the same lengths (homoplasy) have unique names. Details can be found in *Publication III*.**

*The samples of the two experiments KFa and KFb form two distinct clusters (initiated with populations from 2014 and 2016, respectively). While the samples of the present-day and future treatments in both experiments do to not cluster separately, those of the acidification treatment in KFb (i.e. where NPP decreased as well) are clearly different to the others from this experiment. Interestingly, the allele composition of KFa appears to resemble that of incubations under the acidification treatment.*

## Declaration

Erklärung

Hiermit erkläre ich, dass ich die Doktorarbeit mit dem Titel:

**Adaptive potential of the Arctic diatom *Thalassiosira hyalina* to climate change: intraspecific diversity, plasticity and population dynamics**

selbstständig verfasst und geschrieben habe und außer den angegebenen Quellen keine weiteren Hilfsmittel verwendet habe.

Bei dieser veröffentlichen Version meiner Dissertation handelt es sich um eine überarbeitete, aber inhaltlich unveränderte Version der Doktorarbeit.

Bremen, 10.04.2019

……………………………………………………………………………

Klara Wolf