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Battle in the Brine:

Salinity modifies the effect of resource availability on competition within phytoplankton communities

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Tiivistelmä - Referat - Abstract

Pro gradu -tutkielmani käsittelee suolapitoisuuden vaikutusta kasviplanktonien keskinäisessä kilpailussa. Suolapitoisuudella on tärkeä merkitys Itämeren eliöyhteisön rakenteeseen, ja se vaikuttaa myös ravinteiden imeytymiseen nopeuttamalla eliöiden metaboliaa. Tämän perusteella on oletettavissa, että suolan ja ravinteiden interaktio muuttaa kasviplanktonien ravinnekilpailua määrittäviä piirteitä. Tämä tutkimusaihe on erittäin tärkeä, koska Itämeren ylemmän vesikerroksen suolapitoisuuden ennustetaan laskevan tulevaisuudessa ilmastonmuutoksen myötä. Tutkimme muutoksia perustamissamme eliöyhteisöissä, jotka koostuivat kymmenestä eri kasviplanktonlajista. Kasvatimme niitä vesissä, joissa suola-, (0, 5, 12 ja 24) typpi-, fosfori- (N:P = 2, 10, 16 and 80) ja valopitoisuus (10 and 130 µmol photon m^{-2} s^{-1}) vaihtelivat. Tutkimuksessa havaitsin, että kaikki nämä vaikuttavat sekä eliöyhteisön ravinnekilpailuun liittyviin tekijöihin että kasviplanktonlajien olemassaoloon ja prosentuaaliseen määrään populaatiossa. Rajoitettu valomäärä esti eliöyhteisön kasvua riippumatta suolapitoisuudesta, mutta mahdollisti *Phaeodactylum tricornutum* -piilevän hallitsevuuden. Kirkkaassa valossa eliöyhteisön kasvu oli nopeampaa, mutta veden suolapitoisuus oli merkittävämpi tekijä. Kun suolan määrä oli korkein, havaittiin ravinnepuutteiden (typen ja/tai fosforin) kielteisimmät seuraukset sekä kasvuun että monimuotoisuuteen. Makeassa vedessä, jossa oli eniten *Monoraphidium* sp. -viherlevää typen puute ei estänyt eliöyhteisön kasvua, mutta fosforin puute vaikutti kielteisesti eliöyhteisön selviämiseen. Suolapitoisuuden lasku vähensi hiilen osuutta eliöyhteisössä (C:N ja C:P). Tämä vaikutus on päinvastainen kuin yleisimmät arvioidut ilmastonmuutoksen seuraukset. Tulokset osoittavat, että suolan ja ravinnepuutteen määrällä on yhteys kasviplanktonin eliöyhteisön toimintaan. Tutkimukseni perusteella on todennäköistä, että mahdollinen suolapitoisuuden lasku muuttaa ulapan ekosysteemiä Itämeressä.

Avainsanat - Nyckelord
Itämeri, suolapitoisuus, monimuotoisuus, piirre, ilmastonmuutos, kasviplankton, kilpailu

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Tiivistelmä - Referat - Abstract

My master's thesis aims to determine the effect of salinity on phytoplankton traits related to nutrient acquisition, and particularly how this interacts with resource availability. Salinity is an important driver structuring phytoplankton communities in the Baltic Sea. Salinity can also influence nutrient uptake by increasing metabolic rates required for osmotic adjustment. Thus, interaction between salinity and nutrient availability is expected to change community structure by altering phytoplankton traits determining resource competition. This is a particularly relevant area of study for the Baltic Sea due to predicted future freshening of the sea's upper layer. We performed a microcosm experiment using artificial communities of 10 diverse phytoplankton species grown under different combinations of salinity (0, 5, 12 and 24), Nitrogen to Phosphorus molar ratio (N:P ratio = 2, 10, 16 and 80) and light (10 and 130 µmol photon m^{-2} s^{-1}) conditions. A three-way interaction among these environmental parameters influenced phytoplankton traits associated with resource competition, as well as the presence and proportions of phytoplankton taxa. Light limitation inhibited community growth under all salinity conditions, but allowed diatom *Phaeodactylum tricornutum* to dominate. Community growth rate was higher under high light, but also more variable between salinity conditions. The strongest negative effects of nutrient limitation (N, P, and both nutrients together), both on growth rate and taxonomic diversity, were observed in the highest salinity treatment. In the freshwater treatment with the highest proportion of green algae *Monoraphidium* sp., N-limitation did not inhibit phytoplankton community growth and P-limitation had a more profound negative effect on community performance. Decreasing salinity appeared to decrease community C:N and C:P ratios. This shift is in opposition to the increasing C:N and C:P predicted as a consequence of other climate change-related drivers. Our results emphasise the importance of a trade-off between salinity and resource limitation in functioning of phytoplankton communities and suggest that future freshening of the Baltic Sea is likely to modify phytoplankton community composition and performance.

Keywords
Salinity, phytoplankton, Baltic Sea, traits, climate change, competition, diversity

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1 Introduction: Human induced environmental pressures on the Baltic Sea

Human-induced environmental pressures including climate change, eutrophication and resource overexploitation exert strong influence on the structure and function of marine ecosystems (Suikkanen et al., 2013). One marine ecosystem that is considerably vulnerable to such changes is the Baltic Sea – a large, shallow, semi-enclosed brackish water body draining into the North Sea (The BACC II Author Team, 2015) (*Figure 1.1*). The Baltic Sea is permanently stratified, with a seasonal temperature cycle resulting in a three-layer structure in summer. Permanent salinity stratification into two layers is formed by freshwater discharge from land to surface waters and limited lateral movement of saline waters from the North Sea deeper below the halocline, the vertical zone where salinity changes rapidly with depth in the water column. The Baltic Sea halocline has a typical depth of 60-80 m, but is somewhat shallower in southern basins (Liblik & Lips, 2019; Schneider & Müller, 2018; The BACC II Author Team, 2015). This strong stratification limits interaction between upper and lower water layers (The BACC II Author Team, 2015).

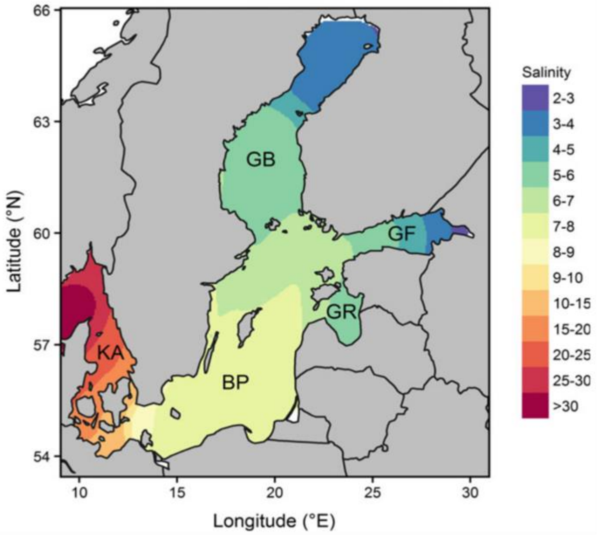


Figure 1.1 Surface water salinity distribution and major sub-regions of the Baltic Sea: *BP* Baltic Proper, *GB* Gulf of Bothnia, *GF* Gulf of Finland, *GR* Gulf of Riga, *KA* Kattegat (from Schneider & Müller, 2018, Fig 3.1).

The Baltic Sea is considered particularly vulnerable to climate change due to its low mean water depth of only 54 m, as well as large temporal variations in stratification and currents compared with other seas (Meier et al., 2012). Furthermore, many Baltic Sea organisms live currently at the lower edge of their salinity tolerance range, placing them at risk of mortality in response to environmental changes such as freshening (Olli et al., 2019). Due to its unique early history of multiple stressors driving ecosystem deterioration and requiring cross-border environmental management, the Baltic Sea can be considered a 'time machine', allowing us a glimpse into the future to study impacts of - as well as potential solutions for - anthropogenic coastal disturbance, which may become more widespread in the future (Reusch et al., 2018).

The Baltic Sea is warming. The upper layer of the Baltic has warmed by 0.03-0.06 °C per year, and the sub-halocline deep layer has warmed by 0.04-0.06 °C per year, with a total warming trend of +1.07 °C over 35 years (Liblik & Lips, 2019). This warming is predicted to continue through the twenty-first century, with summer sea-surface temperature expected to increase by 2 °C in southern parts of the Baltic and by 4 °C in the north (The BACC II Author Team, 2015). This could have important ramifications for phytoplankton communities, as temperature has been found to modify responses to other abiotic factors such as nutrient supply and salinity (Larson & Belovsky, 2013).

Nutrient inputs to the Baltic, of both nitrogen (N) and phosphorus (P), have also increased considerably in the last 150 years due to human activity in the Baltic Region, though there has been a decrease in inputs since the 1980s due to pollution reduction actions (The BACC II Author Team, 2015). Nitrogen deposition in the Baltic drives eutrophication and depleting oxygen levels, causing the frequency of cyanobacteria blooms to increase, despite nutrient reductions, due to warming waters (Reusch et al., 2018). Depending on nutrient loads, oxygen concentrations in Baltic deep water are predicted to decrease, promoted by shifts in oxygen saturation and turnover rates of biogeochemical processes due to climate warming (Meier et al., 2012). Furthermore, reduced water transparency may occur due to increased concentrations of organic material, and rising atmospheric CO_2 concentrations may lead to increased acidification (Meier et al., 2012; Reusch et al., 2018).

Climate models have predicted reduced salinity for areas of the Baltic in the future (Meier et al., 2012). Climate change has impacted, and will continue to impact, ocean salinity worldwide. There are documented signs of an intensifying global water cycle over the past 50 years due to enhanced surface water fluxes, evaporation, and precipitation; a substantial further intensification of 16-24% is also predicted for a future world warmed by 2-3 °C (Durack et al., 2012). Sea surface salinity changes show a pattern of increasing salinities in evaporation-dominated midlatitudes and decreasing salinities in rainfall-dominated tropical atmospheric convergence zones and polar regions, with salty ocean regions getting saltier compared to the global mean and fresh regions getting fresher (Durack et al., 2012). Salinity shifts with climate change could produce filtering effects, eliminating species with narrow resource constraints and potentially disrupting ecosystem functions performed by these species (Basset et al., 2013).

Salinity changes in the Baltic Sea during the twentieth century were highly variable, but there are indications that the duration of precipitation periods, as well as the risk of extreme precipitation events, increased (Lehmann et al., 2021; The BACC II Author Team, 2015). While there is no clear long-term trend for Baltic mean salinity, increasing salinity has been detected below the halocline (0.02 to 0.04 g $kg^{-1}year^{-1}$), likely due to increased lateral import from the North Sea. Freshening (-0.005 to -0.014g kg^{-1} $year^{-1}$) has also been reported for the upper layer, resulting from reduced vertical salt fluxes from deeper layers and/or increased freshwater flux from atmosphere and land (Lehmann et al., 2021; Liblik & Lips, 2019). Surface salinity varies interannually, as well as between regions of the Baltic, depending on atmospheric forcing and/or river runoff (Lehmann et al., 2021). Vuorinen et al. (2015) modelled the river runoff effects on Baltic Sea salinity, based on four climate change scenarios between 1960 and 2100. Three out of four of their increasing runoff scenarios project low salinity of less than seven for the entire Baltic Sea surface water around 2060 (Vuorinen et al., 2015). Precipitation is projected to increase across the Baltic, particularly during winter (The BACC II Author Team, 2015). In turn, the higher net precipitation over the Baltic Sea catchment area is projected to increase river runoff by 15-22% (Meier et al., 2012).

Multi-model ensemble simulations for the Baltic Sea - using IPCC greenhouse gas emission scenarios A1B or A2 - found that water temperatures at the end of this century may be warmer and salinities and oxygen conditions may be lower than ever measured since 1850 (Meier et al., 2012). Therefore, it is imperative that we predict the effects of such changes on organisms, so that we can respond accordingly. Such research also has global implications due to projected future changes in marine environmental conditions throughout the Earth’s oceans.

Trait-based approaches are one way that we can consider the response of ecosystems to environmental change without focusing on specific species (Litchman & Klausmeier, 2008). They represent a strategy to build mechanistic understanding of how environmental stressors shape community organization, which is becoming increasingly important to understand how communities will be affected by anthropogenic change (Litchman et al., 2010; Thomas et al., 2017). Trait-based approaches have already been used to consider competition for nutrients and how this is affected by temperature (Edwards et al., 2016; Litchman et al., 2015; Thomas et al., 2017). However, the interaction between changing salinity and nutrient-related traits has not yet been thoroughly explored.

This thesis aims to consider the effect of salinity on phytoplankton traits related to nutrient acquisition, and particularly how this interacts with resource availability. A microcosm experiment is applied using artificial communities of 10 diverse phytoplankton taxa cultured under four different nutrient levels, two light conditions, and along a gradient of four different salinities. A summary of background literature, covering theory and prior research into Baltic Sea phytoplankton, coexistence of phytoplankton and competition for resources, as well as implications of predicted salinity change in the Baltic Sea, is presented in Chapter 2. Chapter 3 outlines study aims and hypotheses generated for the experiment, which are informed by this literature. The methodology applied in our microcosm study is presented in Chapter 4, and results from this study are presented in Chapter 5. In Chapter 6, I discuss the results, with consideration of their implications for the Baltic Sea in the future, and present final conclusions.

2 Theoretical background

Pelagic environments like the Baltic Sea show several unique ecosystem features, including a high level of mixing, high interaction between organisms, and short generation time of plankton (Ptacnik et al., 2010). The short generation time of plankton, as well as their small size, large populations and ease of manipulation in the laboratory, make them an excellent model system for addressing fundamental ecological questions in a relatively short time (Litchman & Klausmeier, 2008; Ptacnik et al., 2010). Phytoplankton are also extremely important players in global biogeochemical cycling and in regulating atmospheric CO_2 (Falkowski, 1998).

2.1 Plankton in the Baltic Sea

Baltic Sea phytoplankton communities show a pattern of seasonal succession driven by stratification, light, and nutrient dynamics. In spring, stabilizing thermal surface layer stratification and improving light conditions result in a bloom dominated by diatoms and dinoflagellates (Gasiūnaitė et al., 2005; The BACC II Author Team, 2015). This spring bloom shows a marked overlap between diatoms and dinoflagellates, but in parts of the northern Baltic, particularly the Gulf of Finland, an ongoing climate change-driven shift appears to be tipping the composition from diatom dominance towards more dinoflagellates (Klais et al., 2011, 2013; Spilling et al., 2018).

Dominance changes between the diatoms and dinoflagellates that make up the Baltic spring bloom are expected to continue as a result of climate change in the future (Spilling et al., 2018; The BACC II Author Team, 2015). Temperature and ice conditions inform proportions of these taxa in spring, as warm winters favour dinoflagellates. As such, dinoflagellate populations have increased in the central and northern Baltic at the expense of declining diatom biomass (Klais et al., 2011, 2013; Suikkanen et al., 2007, 2013; The BACC II Author Team, 2015). However, dinoflagellate population changes in response to larger scale climatic changes in the Baltic appear to be moderated by local hydrogeography, weather and community composition (Klais et al., 2011, 2013; The BACC II Author Team, 2015).

Projected temperature increases and corresponding stratification in the Baltic are expected to change seasonal succession of phytoplankton in general. This includes more cyanobacteria, with a shift towards smaller sized organisms in the plankton community (The BACC II Author Team, 2015). Suikkanen et al. (2007, 2013) reported an increase in the summer biomass of cyanobacteria, chrysophytes and chlorophytes in the Baltic from 1979-2008. Over the same period, the biomass of cryptophytes decreased. The most important factors driving these changes were rising summer sea surface temperatures, declining salinity and an increased ratio of dissolved inorganic nitrogen to phosphorus. These changes were also associated with an increase in rotifers and a decrease in total zooplankton, cladoceran and copepod abundance in some areas (Jansson et al., 2020; Suikkanen et al., 2013), suggesting phytoplankton assembly changes have important implications for the entire pelagic food web.

2.2 Resources and phytoplankton diversity

2.2.1 Nutrient supply and phytoplankton communities

Nutrient supply is an important determinant of phytoplankton community structure. For example, community evenness of phytoplankton from hypersaline lakes was found to decrease with nutrient enrichment, as selected species were favoured by nutrient enrichment and became dominant (Larson & Belovsky, 2013). These results are consistent with observations in other aquatic autotroph communities that nutrient enrichment increases species richness but decreases community evenness (Hillebrand et al., 2007). Phytoplankton are essential parts of most important global biogeochemical cycles, and thus understanding of how phytoplankton take up and allocate available nutrients is also crucial for understanding connections between these cycles in the past, present and future (Bonachela et al., 2016).

Phosphorus (P) and nitrogen (N) are considered the key limiting elements in water bodies (Redfield, 1958; Tilman et al., 1982). Phosphorus is a critical nutrient implicated in cell growth, and is recognised as an important driver of algal ecology in freshwater and marine systems (Dyhrman, 2016), while nitrogen is an important component of the photosynthetic apparatus in phytoplankton cells (Raven & Giordano, 2016). Phytoplankton primary productivity - conversion of inorganic matter into biomass using solar radiation - in Northern Hemisphere high latitude oceanic waters is often co-limited by N and P, as shown by models, remote sensing, and in situ data (Arteaga et al., 2014).

2.2.2 Nutrients in the Baltic Sea

Winter and spring availability of inorganic nutrients differs between sub-basins of the Baltic Sea, although most areas are N-limited (The BACC II Author Team, 2015) (*Table 2.1*). A study of long-term phytoplankton and physicochemical monitoring data from different regions of the Baltic Sea by Gasiūnaitė et al. (2005) found that phytoplankton community composition in the Baltic is sensitive to nutrient concentrations, with maximum summer biomass significantly correlated with winter dissolved inorganic nitrogen (DIN) and phosphorus (DIP) concentrations.

Table 2.1 Winter nutrient concentrations and nutrient limitation in Baltic Sea sub-basins (mean values from 2000-2005) (adapted from The BACC II Author Team, 2015, Table 18.2)

Basin	Winter DIN (mmol m^{-3})	Winter DIP (mmol m^{-3})	DIN:DIP (mol:mol)	Spring limitation
Kattegat	6.46	0.56	11.5	N
Bornholm Basin	3.30	0.60	5.5	N
Gotland Sea	3.50	0.60	5.8	N
Bothnian Sea	3.07	0.20	15.4	N
Bothnian Bay	6.79	0.06	113	P
Gulf of Riga	11.45	0.93	12.3	P/N
Gulf of Finland	7.02	1.25	5.6	N

Increased freshwater and nutrient discharge have the potential to increase primary production and phytoplankton biomass in areas where production is limited by availability of inorganic nutrients (The BACC II Author Team, 2015). Suikkanen et al. (2007) observed a trend of increasing winter DIN:DIP ratio in the North Baltic Proper, as well as increases in winter DIN concentrations and DIN:SiO4 in the Gulf of Finland, along with a parallel increase in chlorophyll *a* concentrations, between 1979 and 2008.

2.2.3 The Redfield ratio and phytoplankton elemental stoichiometry

The Redfield N:P ratio of 16:1, derived from analysis of empirical oceanic data (Redfield, 1934, 1958), is often used as a benchmark to separate N-limitation from P-limitation, and to represent stoichiometry in global-level biogeochemical models (Bonachela et al., 2016). Redfield derived an average oceanic C:N:P atomic ratio of 106:16:1, similar to the average found in most phytoplankton when nutrients are not limiting (Redfield, 1934). He concluded that this ratio reflects the influence of phytoplankton nitrogen-fixation adjusting oceanic N:P stoichiometry (Klausmeier et al., 2004; Redfield, 1958). Working under the assumption that N:P ratios in water purely reflect decomposition or synthesis of organic matter, the ratio is assumed to reflect proportions of nitrogen and phosphorus in the plankton community as a whole (Redfield, 1934). The ratio's use as a benchmark to separate N- and P- limitation assumes phytoplankton are N-limited when N:P<16, and P-limited when N:P>16 (Geider & La Roche, 2002).

However, marine elemental stoichiometry varies spatially and temporally at the ecosystem level (Bonachela et al., 2016). Differences in community elemental composition reflect a number of factors: interspecific variability between algal species that differ in their C:N:P requirements under optimal growth conditions, as well as physiological acclimation to growth under N- or P- limitation, and within-species genetic variability (Bonachela et al., 2016; Geider & La Roche, 2002; Klausmeier et al., 2004; Yvon-Durocher et al., 2015). Phytoplankton have distinct resource-utilization strategies, which incorporate both nutrient-uptake rates and cellular allocation (Bonachela et al., 2016). The N:P ratio of algal and cyanobacterial cells is very plastic under nutrient limitation in both the field and laboratory cultures. Growth under nutrient limitation causes intraspecific physiological variability, which can override variability between species (Geider & La Roche, 2002). Nutrient-limited cells display wider C:N:P stoichiometry ranges than cells in nutrient-replete conditions (Geider & La Roche, 2002). C:N, C:P and N:P all vary considerably across species in nutrient-replete cultures, although C:N shows less variation than N:P and C:P (Bonachela et al., 2016; Geider & La Roche, 2002; Quigg et al., 2011). Different phytoplankton taxa are characterised by distinct C:N:P stoichiometry under nutrient-replete conditions (Geider & La Roche, 2002). The elemental composition of nutrient-replete phytoplankton is influenced by physicochemical factors including dissolved nutrient ratios, day length, irradiance, salinity and temperature (Bonachela et al., 2016; Geider & La Roche, 2002). Eukaryotic phytoplankton appear to have higher stoichiometric plasticity than prokaryotes. This may be due to the larger cell size of eukaryotes, as well as their ability to quickly regulate gene expression in response to changes in their environment (Tanioka & Matsumoto, 2020).

C:N:P stoichiometry differs between phytoplankton taxa, reflecting different macromolecule distribution and availability. The Redfield N:P ratio thus represents an average of species-specific ratios, rather than a global biochemical optimum (Klausmeier et al., 2004). Nuance is therefore lost in the averaging nature of the Redfield ratio. As a consequence of this, current research has shifted to consider consequences of cellular stoichiometry changes, taking into consideration the way that diverse phytoplankton stoichiometry affects global biogeochemical cycles via dynamic feedback mechanisms between phytoplankton and their environment (Bonachela et al., 2016).

2.2.4 Interaction of nutrients and light

The amount of available light is also of key interest when considering phytoplankton, as growth is often co-limited by availability of nutrients and light in the oceans (Geider & La Roche, 2002). Similar to nutrients, different phytoplankton species have different light requirements and some of them are better adapted to low light conditions than others (Brauer et al., 2012; Huisman & Weissing, 1994). Light availability has consequences for elemental composition of taxa, as well as their photosynthetic apparatus. A meta-analysis performed by Tanioka & Matsumoto (2020) synthesized responses of marine phytoplankton C:P and C:N ratios to major environmental drivers including irradiance. They found a significant and consistent trend where an increase in available light increased both C:P and N:P. The effect of light on nutrient requirements is predicted to be particularly significant in cyanobacteria, due to the high N-requirement of their light-harvesting phycobilisomes (Geider & La Roche, 2002).

2.3 Competition and Coexistence between plankton

The high diversity of plankton communities in homogenous marine environments with few limiting resources lead Hutchinson (1961) to propose ‘The Paradox of the Plankton’: the puzzle of high apparent redundancy in communities where taxa have similar resource requirements and uptake methods (Hutchinson, 1961). Multispecies interactions depend on the relationship between species’ resource requirements and resource consumption characteristics (Huisman & Weissing, 2001). Species showing diverse resource acquisition traits, and exhibiting higher trade-offs, have a higher likelihood of coexistence (Chesson, 2000; Huisman & Weissing, 2001; Ptacnik et al., 2010).

Chesson (2000) defines mechanisms promoting coexistence between species as either ‘equalizing’, minimizing average fitness differences between species, or ‘stabilizing’, increasing negative intraspecific interactions relative to interspecific. A species is generally not considered capable of competitively excluding another species if the effect that it has on itself is more than its effect on the other species. However, asymmetries in resources can cause fitness differences that counteract stabilizing effects and cause competitive exclusion, unless an equalizing mechanism is present that can reduce this fitness difference (Chesson, 2000).

2.3.1 Trait-based approaches and trade-offs

Traits are well-defined and measurable properties of organisms that can be compared across species, with a functional trait defined as a trait that strongly influences organismal performance (Mcgill et al., 2006). A study of traits encompasses numerous morphological, physiological, behavioural and life history traits, serving a range of functions (Litchman & Klausmeier, 2008) (*Figure 2.1*). Many traits, such as nutrient and light utilization, as well as predator resistance, are highly correlated with cell size, hence cell size is considered a ‘master trait’ shaping phytoplankton ecological niches (Litchman et al., 2010; Litchman & Klausmeier, 2008). Major parameters of nutrient uptake and growth scale with cell size (Litchman et al., 2007).

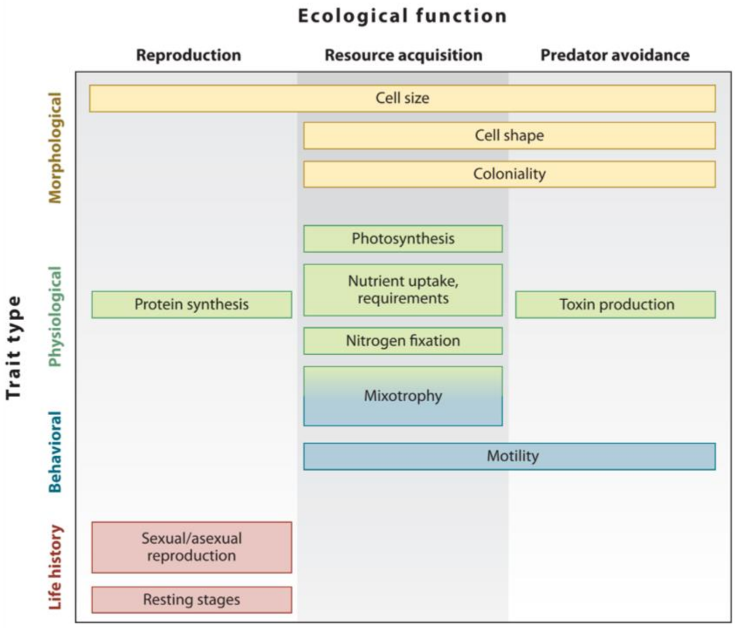


Figure 2.1 Typology of phytoplankton functional traits (from Litchman & Klausmeier, 2008, Figure 1).

Traits may be correlated in a way that constitutes a trade-off if their increase or decrease has opposing effects on fitness (Litchman et al., 2015). Trade-offs between acquisition of different resources will likely affect both probability of coexistence, as well as species traits that determine production of biomass (Hillebrand et al., 2014). Trade-offs result in diversification of ecological strategies and may facilitate coexistence of multiple species (Litchman et al., 2007).

2.3.2 Resource competition models

Species coexistence has been extensively explored through resource competition, an important theory relating competition for limiting nutrients to biodiversity and community structure (Tilman, 1980). A resource is a factor whose availability is positively associated with population growth rates, and which is consumed by the population (Tilman, 1980). Resource competition models are models that link the population dynamics of competing species with the dynamics of the resources that these species are competing for. An attractive feature of resource competition models is that they use the biological traits of species to predict the result of competition, making them more generalisable than species-specific models (Huisman & Weissing, 2001).

In his classic paper, Tilman (1980) outlines a graphical-mechanistic approach to consider competition for resources and predation. Tilman et al. (1982) review this resource competition theory, applying it to questions of equilibrium vs nonequilibrium approaches, seasonal succession, and multitrophic level effects in phytoplankton communities. The model incorporates estimates of resource supply, mortality, and consumption rates, as well as growth responses, to predict the equilibrium outcome of interspecific resource competition for two resources (Tilman, 1980). R^* is the key parameter for predicting the long-term equilibrium outcome of competition among species limited by the same resource. R^* is defined as the essential resource level (R) below which a species cannot sustain its population size. A population is expected to increase if $R > R^*$, decrease if $R < R^*$, and remain stationary if $R = R^*$. R^* captures the ability of a species to extract resources at a low concentration, and to grow and reproduce rapidly enough at low resource levels to compensate for mortality (Chesson, 2000). R^* can be modified by other factors (e.g. predation), with species experiencing lower mortality rates having a lower R* than other species (Chesson, 2000). The species with the lowest R^* value is considered the superior competitor and expected to displace all other competitors (Chesson, 2000; Tilman et al., 1982).

The zero net growth isocline (ZNGI) is the growth isocline at which reproductive rate is equal to mortality rate (Tilman, 1980). A habitat where the resource supply point falls inside the ZNGI will fail to provide sufficient resources to maintain a stable population (Tilman, 1980). If the ZNGI of two species do not touch or cross, no two-species equilibrium is possible. In this case, is expected that one species will be competitively dominant and displace the other by reducing the concentration of nutrients to its comparatively lower R^* where it has enough resources to maintain a stable population but the other does not (Tilman et al., 1982) (*Figure 2.2a*). A point where the ZNGI of two species touch or cross is a resource equilibrium point at which the species can potentially coexist (*Figure 2.2b*). For such an equilibrium to be stable, each species must consume relatively more of the resource that limits its own growth rate, and the amounts of each resource consumed by individuals of the same species may change only slightly in response to small changes in resource availability (Tilman, 1980). Species B may still competitively displace another species A by consuming excess quantities of a resource and thus displacing A from a region of resource supply space in which both species could coexist if B consumed less of this resource, a competitive strategy termed ‘hoarding’ or ‘luxury consumption’ (Tilman, 1980).

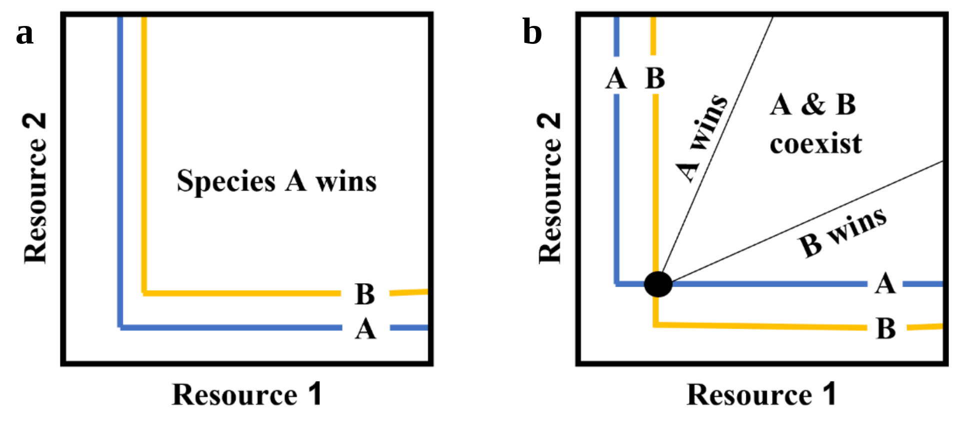


Figure 2.2 Elements of Resource Competition Theory. Lines = the zero net growth isocline (ZNGI) of hypothetical species A and B. **a.** If the ZNGI of two species do not touch or cross, no two species equilibrium is possible. **b.** A point (black dot) where the ZNGI of two species touch or cross is a resource equilibrium point at which the species can potentially coexist (adapted from Tilman, 1982, Figure 1 E and F).

For a pair of essential resources, such as N and P, the growth rate of a species will be determined by the resource that is more limiting (Tilman, 1980). Different ratios of potentially limiting resources have different effects on community structure, with the ratio at which the two nutrients are supplied determining which of the two nutrients limits phytoplankton growth, a condition referred to as the 'Resource Ratio Hypothesis' (Tilman et al., 1982) (*Figure 2.3a*). Coexistence of species is restricted to intermediate resource ratios, where there is a high probability of both resources being limiting (Tilman et al., 1982).

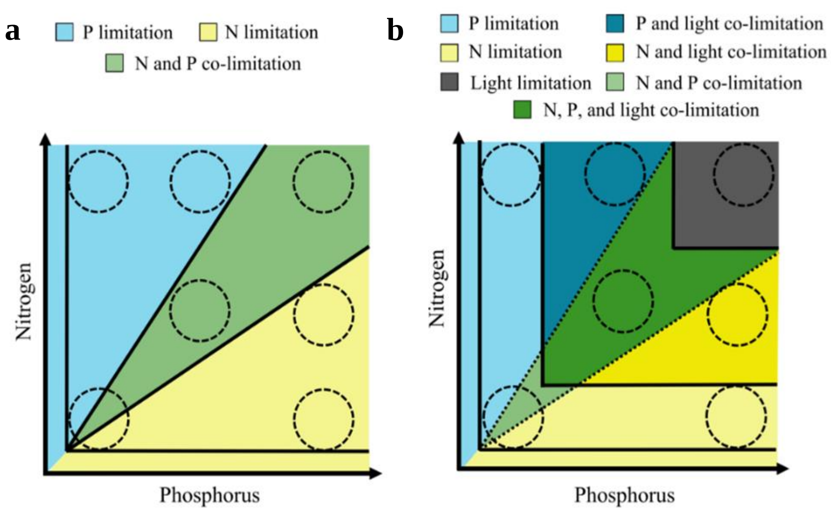


Figure 2.3 Models of resource limitation as a function of nitrogen and phosphorus, as well as light **a.** The Resource Ratio Hypothesis predicts that resource limitation depends on nitrogen and phosphorus ratios. **b.** The Nutrient Load Hypothesis incorporates light as a third limiting resource, with resource limitation depending on absolute values of nitrogen and phosphorus loads in addition to their ratio (from Burson et al., 2018, Fig 1).

The resource competition model can be modified to explain competition between different kinds of resources, greater than two resources or competitors, spatial and temporal heterogeneity, and seasonal succession (Tilman et al., 1982). It has been extensively tested with competition experiments using phytoplankton species (Huisman & Weissing, 2001, and references therein).

2.3.3 Models for utilization of nutrients and light

Models predict that the superior competitor for light will be the species with the lowest critical light intensity, I^*_{out}, defined as the light intensity measured at the bottom of a well-mixed water column where species can just maintain a stationary population under light-limited conditions. I^*_{out} resembles R^* in that a species will decrease below this value, remain stationary at this value, and increase above this value (Huisman & Weissing, 1994). Huisman & Weissing (1994; 1995) present a graphical isocline approach analogous to earlier resource-based approaches like that of Tilman (1980; 1982) (*Figure 2.2*) for modelling competition between nutrients and light. The model predicts that if species compete for light and a single nutrient in a mixed water column, stable coexistence of two species is possible if one species is limited by the nutrient and the other by light. The pattern depends on light supply, as well as background turbidity and mixing depth (Huisman & Weissing, 1994). Consistent with earlier models, their results find that two species may stably coexist provided that each species consumes relatively more of the resource that most limits its own growth (Huisman & Weissing, 1995). However, in contrast to resource-based approaches, Huisman & Weissing (1995)'s model predicts that absolute supply rates of nutrient and light matter for the outcome of competition, not only their ratio, as higher nutrient availability leads to greater biomass and therefore lower light penetration (self-shading effect). Thus, the Resource Ratio Hypothesis does not apply when species compete for both nutrients and light (Huisman & Weissing, 1994, 1995).

Brauer et al. (2012) extend the classical resource competition model for two nutrients, incorporating light as an additional resource. They conclude that their results support a 'Nutrient Load Hypothesis' (*Figure 2.3b*), which predicts that nutrient ratios determine species composition in low nutrient environments while nutrient loads are key to species composition when there are high nutrient levels (Brauer et al., 2012). At low to intermediate nutrient loads, the nutrient ratio determines which of the two nutrients that species are competing for will limit growth. In contrast, under high nutrient loads, when community biomass increases and competitive interactions shift towards competition for light, absolute nutrient loads determine the transition from nutrient limitation to colimitation by nutrients and light, as well as the transition from colimitation to light limitation (Brauer et al., 2012). Simulation results indicate that this trend holds for competition between three species, with stable coexistence of all three species if each consumes most of the resource for which it is the inferior competitor (Brauer et al., 2012). Species consuming most of the resource for which they are the best competitor will monopolise this resource when limited, leading to competitive exclusion where the winner depends on initial conditions, while species consuming most of the resource for which they are the intermediate competitor end up in nontransitive interactions showing species oscillations (Brauer et al., 2012; Huisman & Weissing, 1999). Intermediate nutrient loads will not necessarily lead to high diversity, but can produce alternative stable states in species composition. Small changes in nutrient loads or initial species could modify final outcomes of competition, making species composition at intermediate nutrient loads difficult to predict (Brauer et al., 2012).

2.3.4 Extensions to traditional resource competition models

Traditional resource competition models predict that, as only one species can have the highest requirement for a given resource, the number of coexisting species cannot exceed the number of limiting resources and they are thus unable to entirely account for the Paradox of the Plankton (Huisman & Weissing, 1995, 2001). However, the addition of spatial heterogeneity in the pelagic environment, with individual algal cells or colonies experiencing different average rates of resource supply, could allow more species to coexist than there are limiting resources (Tilman et al., 1982). Resource fluctuations and non-linear responses from species also facilitate a larger number of species coexisting on one or two limiting resources (Tilman et al., 1982).

Huisman & Weissing (1999)'s solution for the Paradox of the Plankton is based on the dynamics of competition, suggesting that oscillations and chaotic fluctuations in species abundances - sometimes generated when species compete for three or more resources - can allow the coexistence of many species on a handful of resources. Once a plankton community is complex enough to generate its own non-equilibrium dynamics, the number of coexisting species can greatly exceed the number of limiting resources (Huisman & Weissing, 1999, 2001). Phytoplankton communities limited by multiple resources appear to be widespread, lending support to this theory (Huisman & Weissing, 2001). Huisman & Weissing (2001) predict that competition generates: stable coexistence when species consume most of the resource they require most, oscillations and chaos if species consume resources they have intermediate requirements for, and competitive exclusion when species consume most of the resources they have the lowest requirements for, with the winner depending on initial conditions.

2.4 Salinity

2.4.1 Salinity in the Baltic Sea

Salinity is a particularly important factor in the Baltic Sea, where most species are of marine or freshwater origin and live at the edge of their salinity tolerances (The BACC II Author Team, 2015). Cross-colonization of protists in marine and freshwater is physiologically restricted by high energetic costs related to osmoregulation (Hoef-Emden, 2014; Olli et al., 2019; Suescún-Bolívar & Thomé, 2015). Specialist species in transitional waters, particularly in terms of temperature and salinity requirements, will be constrained by climate changes and are considered at higher risk of population decline and extinction compared with generalists (Basset et al., 2013). With Baltic Sea communities adapted to current salinity levels, declining salinity levels are expected to lead to ecological stress for organisms adapted to higher salinities, and thus negative impacts on their ecosystem functions (Lehmann et al., 2021). Research indicates that salinity plays a key part in structuring Baltic Sea phytoplankton communities. Olli et al. (2019) report higher alpha diversity - mean local species diversity - at the marine end of the Baltic Sea salinity gradient. They also describe a threshold type community change pattern for phytoplankton in the Baltic, with community structure changing abruptly at salinities between 7-9, coinciding with a species richness minimum. This distinct transition zone between two relatively homogenous assemblages suggests a repelling edge effect, with communities on either side of this salinity band experiencing stressful conditions (Olli et al., 2019).

2.4.2 Salinity, Resource Uptake and Competition

Salinity has been found to influence resource uptake by increasing metabolic rates required for osmotic adjustment (Finkel et al., 2010). Protists in a hypotonic freshwater environment need to control influx of water, achieved by adaptations such as a rigid cell wall and contractile vacuoles, which pump out excess water. However, protists in marine or brackish water accumulate osmolytes, which counter the high osmotic pressure of their environment (Hoef-Emden, 2014; Welsh, 2000). The synthesis of these ‘osmoprotectants’, such as glycerol, comes from carbon fixation through photosynthesis or from degradation of energy-storage molecules (Hoef-Emden, 2014; Suescún-Bolívar & Thomé, 2015; Welsh, 2000). Compatible solutes can play alternative roles in cells as intracellular nutrient (C and N) and energy reserves, with synthesis of N-containing solutes having the potential to enable luxury uptake of nitrogen by microalgae. Osmoprotectants may also be involved in protecting cells against other environmental stressors, including extreme temperatures (Raven & Giordano, 2016; Welsh, 2000). Hoef-Emden (2014) reported nutrient-dependent effects on the range of osmotolerance for cryptophytes, with a *Rhodomonas* sp. unable to grow in higher N and P medium. They propose that strains restricted to freshwater may be unable to produce intracellular osmolytes, while strains restricted to marine environments may not be able to down-regulate their production. Thus, changing community structure and distribution of traits determining resource competition is expected between different salinity and nutrient conditions, similar to those that have been observed for temperature (Edwards et al., 2016; Hofmann et al., 2019; Marañón et al., 2018; Thomas et al., 2017). Such results have not been reported so far. Major syntheses of research exploring environmental drivers influencing marine phytoplankton resource acquisition and stoichiometry (eg. Tanioka & Matsumoto, 2020) have considered inorganic resource levels, irradiance, and temperature, but overlooked the effect of salinity.

3 Research aims and hypotheses

The overall aim of our study was to identify which groups of phytoplankton will benefit from potential future freshening of the Baltic Sea. Within this project, my thesis focused on answering:

How does resource availability affect phytoplankton trait distribution along a salinity gradient?

I hypothesised that changing salinity would modify the response of phytoplankton communities to resource limitation, because osmotic stress affects nutrient acquisition. I expected that phytoplankton community composition and traits would reflect nutrient preferences/competitive abilities of taxa present and patterns of dominance, but that this may be modified by salinity. Eukaryotic plankton, especially diatoms, are typically considered to be the superior P competitors, while cyanobacteria - particularly filamentous N-fixing forms - outcompete others for light and N (Brauer et al., 2012; Yang & Jin 2008, and references therein). Green algae are considered weak competitors, but are common in freshwater, and are capable of dominating phytoplankton communities at high N:P (Yang & Jin, 2008). Furthermore, it has been suggested that a small cell size provides a competitive advantage under nutrient limitation (Burson et al., 2018). Thus, I expected that taxa with smaller cells would dominate under nutrient limited conditions, as well as low salinity. In contrast, taxa with high surface-area-to-volume-ratio such as diatoms should dominate under low light conditions, because they can maximise light harvesting (*Table 3.1*).

Table 3.1 Predicted effect of starting nutrient conditions, as well as anticipated effect of freshening, on phytoplankton community structure

Nutrient condition	Predicted dominating taxa	Predicted effect of freshening
High light, high N:P	Diatoms	Increasing green algae and diatoms, declining cryptophytes
Low light, high N:P	Diatoms	Increasing green algae and diatoms, declining cryptophytes
High light, N:P = 16	Diatoms	Increasing green algae
Low light, N:P =16	Diatoms	Increasing green algae
High light, low N:P	Diatoms	Increased smaller cells, increasing cyanobacteria
Low light, low N:P	Cyanobacteria	Increased smaller cells, increasing cyanobacteria

4 Materials and methods

4.1 Experimental design

To answer my research question, we set up a full factorial microcosm experiment with artificial phytoplankton assemblages (10 species in polyculture) exposed to four salinity treatments (0, 5, 12, and 24), two light intensity treatments (10 and 130 µmol photon m^{-2} sec^{-1}), and four nutrient treatments varying in N:P ratios (2, 10, 16, and 80). Treatments were replicated three times, which resulted in 96 experimental units.

Table 4.1 Nutrients added to media for different nutrient treatments

N:P ratio	**NO_3**	**PO_4**
16	80 µmol/L	5 µmol/L
80	80 µmol/L	1 µmol/L
2	10 µmol/L	5 µmol/L
10	10 µmol/L	1 µmol/L

To establish salinity treatments, natural seawater (salinity 5.2) collected from Storfjärden was modified by the addition of Aquarium Systems *Instant Ocean* (Sarrebourg, France) (accounting for projected salinity changes with the addition of algal taxa in their culture media) to salinity levels of 12 and 24. Natural seawater represented the 5 condition, while MilliQ water was used for the 0 condition. Seawater was vacuum filtered through Sartobran 300 sterile filter capsules (Sartorius, Göttingen, Germany) with pore sizes 0.45 and 0.2 µm.

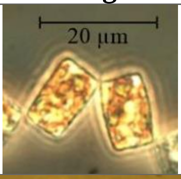
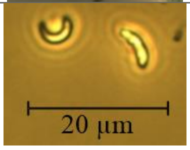
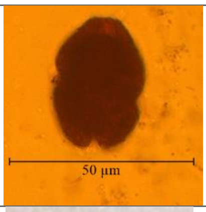
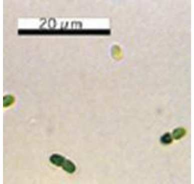
Salinity treatments were further subdivided into four nutrient levels via the addition of different amounts of NO_3 and PO_4 (*Table 4.1*) to create modified f/2 media (Guillard, 1975). Amounts of Si, trace metal solution and vitamin solution were kept constant. Six replicates of 480 mL water, 2.1 mL f/2 media and 0.6 mL Si were created in 600 mL sterilized, non-treated, tissue culture flasks with vent caps (VWR, PA, USA) for each nutrient level and salinity, producing a total of 96 replicates. Samples were weighed to verify comparable volumes and all experimental work was performed under sterile conditions.

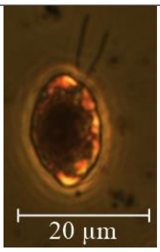
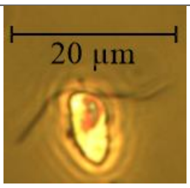
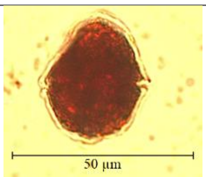
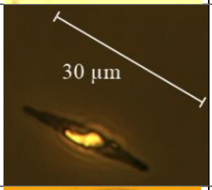
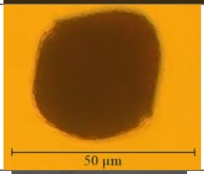
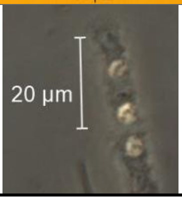
The experiment was performed in a climate chamber at constant 16 °C and a 16:8 hour light:dark cycle. Half of the experimental units received 10 µmol $m^{-2}s^{-1}$ light (light limited conditions). The other replicates formed the high light intensity group and received 130 µmol m^{-2}s^{-1} of light. Positions of the flasks in the climate chamber were randomised to account for artifacts related to shading and differences in air circulation.

4.2 Creation of artificial algal communities

Based on previous experiments with algal monocultures (Iris Orizar, unpublished), we selected 10 species from the FINMARI algal culture collection (*Table 4.2*), which were most compatible with experimental requirements and able to grow at all four salinity levels. These species were also selected to maximise trait diversity, showing considerable phylogenetic divergence, different cell sizes, mobility, and pigment composition. All selected taxa are known to coexist in nature. Prior to the experiment, monocultures of all phytoplankton taxa were grown at salinity 5, 16 °C, and 130 µmol m^{-2}s^{-1} of light (16:8 hour light:dark cycle). To create an artificial phytoplankton community, monocultures were mixed in equal proportions based on their *in vivo* fluorescence, added to experimental units, and placed into a climate chamber as described above.

Table 4.2 Taxa included in artificial algal communities

Species	Image	Strain	Origin	Description
Diatoma tenuis		DDTBB5	Tvärminne	Common cold-water pennate diatom (Spilling, 2007).
Monoraphidium sp.		TV70	Tvärminne	Solitary, elongated symmetrical green algae (Chlorophyta) with spirally twisted ends and lacking mucilage (Komarek, 1974; Komárková-Legnerová, 1969).
Levanderina fissa		GFF1101	Åland/ Föglö	Unarmoured dinoflagellate with ovoid cells, varying from spherical to slightly laterally or dorsoventrally compressed, and containing a peridinin-type plastid (Moestrup et al., 2014)
Synechococcus sp.	 (University of Sevilla, 2006)	TV65	Tvärminne	Cosmopolitan cyanobacterial genus. Basically solitary, but capable of forming small groups in summer (Kuosa, 1991; Li, 1998).

Rhodomonas sp.		Crypto07-B1	Tvärminne	Cosmopolitan, free-swimming ovoid cryptophyte, containing a chloroplast with the phycobiliprotein Cr-phycoerythrin 545 (Guiry, 2021). Important member of the main part of the spring bloom phytoplankton biomass in the northern Baltic (Suikkanen et al., 2011).
Diacronema lutheri		TV3	Tvärminne	Brackish water unicellular haptophyte, round with yellow-green plastid containing chlorophylls a and c. Common component of near coastal phytoplankton communities (Bendif et al., 2011).
Kryptoperidinium foliaceum		KFF0901	Åland/ Föglö	Binucleate dinoflagellate with cells that are flattened dorsoventrally, containing a 'tertiary' plastid of diatom origin (Figueroa et al., 2009).
Phaeodactylum tricornutum		TV335	Tvärminne	Raphid, pennate diatom found in marine coastal habitats, and known to be able to adapt to unstable environments (Martino et al., 2007).
Alexandrium ostenfeldii		AOF0940	Åland/ Föglö	Bloom-forming dinoflagellate, with a plastid containing the pigment peridinin, capable of producing toxins (Anderson et al., 2012).
Skeletonema marinoi		C1407	Bothnian Sea	Centric diatom; cells form long chains (Sarno et al., 2005). Important member of the main part of the spring bloom phytoplankton biomass in the northern Baltic (Suikkanen et al., 2011).

4.3 Monitoring of phytoplankton growth and sampling

In vivo fluorescence of chlorophyll *a* in phytoplankton polycultures was measured less than 24 hours after creation of the artificial algal communities with a Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies Inc., Santa Clara, CA, USA) for excitation 440 nm and emission 680 nm and was designated as F_{t1}.

Fluorescence was measured for each experimental unit every second day until termination. Treatment groups (all three replicates) were terminated when chlorophyll *a* fluorescence at 480 nm wavelength indicated that communities had reached stationary phase for three consecutive sampling days, or showed a trend of decline (Figure S1, Appendix).

After termination, 4 ml sample was reserved for flow cytometry to measure abundance of *Synechococcus* sp.; samples were fixed with 36% formaldehyde (2% final solution), vortexed and stored at -80 °C. 16 ml samples for chlorophyll *a*, particulate carbon, nitrogen and phosphorus (POC, PON, and POP) were vacuum filtered through 25 mm acid washed and combusted glass microfiber filters (Whatman GF/F, GE Healthcare Inc., Chicago, IL, USA), and 48 ml was filtered through a 25 mm syringe filter with 0.2 µm cellulose acetate membrane (VWR, Radnor, PA, USA) for dissolved nutrient analysis. All nutrient and chlorophyll *a* samples were stored in -20 °C in the dark until analysis. 100 ml of cultures were preserved for analysis of phytoplankton community composition with 1 ml acidic Lugol's iodine solution, which has been determined to be the most suitable fixative for Baltic Sea phytoplankton (Hällfors et al., 1979; HELCOM, 2017).

4.4 Laboratory analyses

4.4.1 Analysis of chlorophyll *a* concentrations

To analyse chlorophyll *a* concentration, chlorophyll was extracted via addition of 10 ml 94% ethanol, sonication for five minutes in a Branson 2510 Ultrasonic Cleaner (Emerson Electric Co., St. Louis, MO, USA) and then storage at 4 °C over 24 hours. Chlorophyll *a* concentration was measured using a Cary Eclipse Fluorescence Spectrophotometer with excitation 430 nm and emission 670 nm, with two technical replicates per sample. Samples were diluted as required through the addition of 94% ethanol. Relative fluorescence was converted to chlorophyll *a* concentration via comparison to commercial standards. To measure phaeophytin *a* concentration, 0.25 M HCl was added to extracted chlorophyll and left for an hour to denature, then measured as above.

4.4.2 Particulate Organic Phosphorus (POP)

Analysis of POP was performed after Koistinen et al. (2020). In brief, samples are combusted at 450 °C for four hours to decompose phosphorus compounds, polyphosphate is hydrolysed to orthophosphate and then analysis is based on the antimony-molybdate method, where orthophosphate reacts with molybdate and antimony to form an antimony phosphomolybdate complex that is reduced by ascorbic acid to form a blue colour, for which intensity can be measured spectrophotometrically at 880 nm. The POP calibration curve based on my working standards is provided in the Appendix (*Figure S2*).

4.4.3 Particulate Organic Carbon (POC) and Particulate Organic Nitrogen (PON)

POC and PON concentrations were measured using an elemental analyser (Thermo Scientific EA Flash 2000, Thermo Fisher Scientific, Waltham, MA, USA) at the LMU Munich. Prior to this, filters with samples were dried at 60 °C for 24 hours, folded into tin caps and stored in a desiccator until analysis.

4.4.4 Dissolved nutrients

Analyses of nitrate and nitrite (NO_3+NO_2), ammonium (NH_4), phosphate (PO_4) and dissolved silicate (SiO_4) in the water samples were performed at the Tvärminne Zoological Station using a continuous flow autoanalyzer (AAII) according to Hansen & Koroleff (1999).

4.5 Analysis of community composition

Phytoplankton samples were gently agitated to homogenize contents and then settled in 3 ml Utermöhl chambers for a minimum of four hours on a horizontal surface, and under an overturned plastic box to protect them from temperature changes and drafts. Samples were counted using a DM IRB inverted microscope (Leica, Wetzlar, Germany) with 500X magnification. Counting followed best practices outlined in HELCOM (2017). For non-dominant taxa, counting was performed for a minimum of 64 fields of view (FOV) or until 300 cells for each taxon were reached. The dominant taxon was counted to a minimum of 400 cells. Because of timing constraints, only one replicate per experimental unit was counted for this thesis (total number = 32 samples).

Density of cells per mL was calculated for each taxon for each sample using the following equation:

$$abundance\ [cells\ ml^{-1}] = \frac{n\ \times A}{(a * V)}$$

Where n is the number of cells counted, A represents the entire area of the Utermöhl chamber in mm^2, a is the total area counted in mm^2, and V is the settled volume in ml.

4.5.1 Flow cytometry

Synechococcus sp. abundance (cells ml^{-1}) was measured with a BD Accuri C6 flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) equipped with a blue (488 nm) and red (640 nm) excitation lasers. Counts of *Synechococcus* sp. were determined in the phycoerythrin fluorescence detection channel (585/40 nm).

4.6 Calculation of growth rate and maximum fluorescence (Pmax)

In vivo chlorophyll *a* fluorescence was used to estimate the maximum fluorescence (Pmax) and growth rate of phytoplankton. Growth rate (μ) was calculated based on fluorescence at day 1 of the experiment and at the end of the exponential stage as per the equation:

$$\mu = \frac{ln\left(\frac{F_{t2}}{F_{t1}}\right)}{t2 - t1}$$

Where F_{t1} is the fluorescence of the sample on the first day of exponential growth (we defined this as fluorescence for the first day of the experiment, see above), F_{t2} is fluorescence of the sample on the last day of exponential growth, t_1 is the first day of exponential growth (which for our samples was 1), and *t2* is the day of the experiment judged empirically to be the end of the exponential phase.

Pmax was calculated as the maximum fluorescence for the sample during the duration of the experiment.

4.7 Statistical analysis

All statistical analyses were performed in Rstudio using R version 4.1.1 (R Core Team, 2021) and plots were created using R-package ggplot2 3.3.2 (Wickham, 2016).

Three-way ANOVA was performed on each of the response variables (rv) - growth rate, Pmax and chlorophyll *a* concentration - as a function of light, salinity and N:P with the form: rv~light*salinity*NP (*Tables S1-S3*). Because there was a significant light treatment effect, the dataset was separated into two light conditions (see below) and two-way ANOVA was performed for each light condition on each rv as a function of salinity and N:P with the form: rv~salinity*NP, to allow for comparison between light saturated and light limited conditions (*Tables S4-S9*). Three-way ANOVA was also performed on the whole dataset (not split by light condition) for the response variables (rv) POP, POC, PON, C:N, C:P and N:P as a function of light, salinity and initial N:P of media with the form: rv~light*salinity*NP, to analyse resource use (*Tables S10-S15*). Validity of ANOVA as a statistical approach for the data was determined by scatter plots to identify outliers, as well as plotting histogram of residuals, residuals vs fitted values, normal Q-Q plot, and scale location plot (between square root of standardized residuals versus fitted values), which revealed no clear violations of ANOVA assumptions of normality and homogeneity of variance. Our experimental design furthermore ensured observations were independent.

Differences in phytoplankton community composition between samples were calculated using Bray-Curtis dissimilarity indices in R-package *vegan* 2.5-7 (Oksanen et al., 2020). Bray-Curtis dissimilarity (Bray & Curtis, 1957; Chao et al., 2005) accounts for relative abundances of species.

5 Results

5.1 Community traits

All three phytoplankton community traits of growth rate (µ), maximum fluorescence (Pmax) and chlorophyll *a* concentration (chl *a*) showed a significant three-way interaction between light, N:P and salinity (*Table 5.1*, *Tables S1-S3*). The three traits showed different overall magnitudes between the high and low light conditions: growth rate tended to be higher for all groups in the higher light condition, while Pmax and chlorophyll *a* were higher under low light (*Figure 5.1, Figure S1*). Hence, data for each light condition were analysed separately to explore the effects of salinity and nutrients on the phytoplankton community traits under different light availability.

Table 5.1 Results of three-way ANOVA for the interaction between light, N:P and salinity influencing the response variables of growth rate (µ), Pmax and chlorophyll *a* concentration (chl *a*). Factor interaction degrees of freedom 9, residual degrees of freedom 64. (See *Tables S1-S3* for full ANOVA results)

	F	**p**
µ	5.14	< 0.001
Pmax	7.52	< 0.001
chl *a*	3.98	0.00045

Table 5.2 Results of two-way ANOVA for the interaction between N:P and salinity influencing the response variables of growth rate (µ), Pmax and chlorophyll *a* concentration (chl *a*) at high (130 µmolm^{-2}s^{-1}) and low (10 µmolm^{-2}s^{-}1) light levels. Factor interaction degrees of freedom 9, residual degrees of freedom 32. (See *Tables S4-S9* for full ANOVA results)

	F	**p**
µ		
High light	5.31	0.0002
Low light	3.58	0.004
Pmax		
High light	3.99	0.002
Low light	6.14	< 0.0001
chl *a*		
High light	2.81	0.01
Low light	4.3	0.0009

5.1.1 Growth rate

There was a significant interaction between N:P and salinity influencing phytoplankton growth rate under both light conditions (*Table 5.2*, *Tables S4-S5*). Growth rate was higher under high light conditions and appeared to vary more between salinity levels within each nutrient condition compared to low light (*Figure 5.1a*). The relationship between salinity and growth rate within each nutrient condition was not linear. A faster community growth rate was observed under high light at 0 and 12 salinities compared to other salinity conditions, but considerable variation was also observed at least at 12 salinity, particularly under higher N:P. For 0 salinity, N-limitation (N:P = 2) did not inhibit phytoplankton growth, but P-limitation (N:P = 80) had a negative effect on community performance. Conversely, low μ variability was observed under low light conditions and slowest μ was observed at 0 salinity.

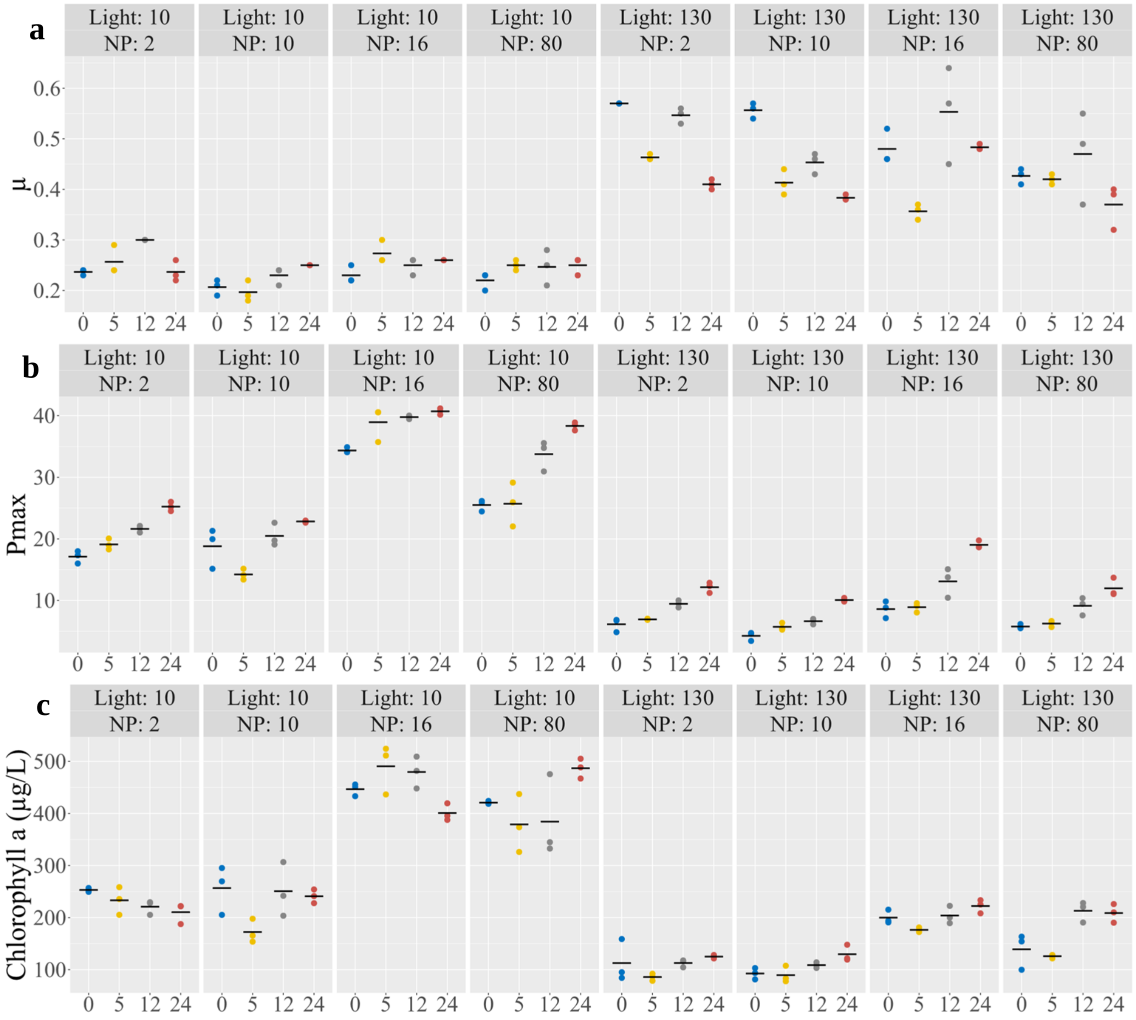


Figure 5.1 Measured **a.** growth rate (μ) **b.** Pmax and **c.** total chlorophyll *a* concentration (μg/L) for communities at different nutrient (N:P ratio), light (μmol $m^{-2}s^{-}1$), and salinity (x-axis) levels. Points = individual replicates, black bar = group mean.

5.1.2 Pmax and chlorophyll *a*

The interaction between nutrient and salinity conditions was found to significantly affect the maximum fluorescence (Pmax) and chlorophyll *a* concentration of the community in both light conditions (*Table 5.2*, *Tables S6-S9*). Both Pmax and chl *a* increased with salinity in all nutrient conditions under the high light condition (*Figure 5.1bc*). However, only Pmax was found to increase with salinity under low light conditions, while chl *a* concentration showed higher variability along the salinity gradient. In addition, higher Pmax, chl *a* and phaeophytin *a* (phe *a*) concentrations were observed for groups with higher N:P ratio, with phe *a* following chl *a* quite closely (*Figure 5.1bc, Figure S3*).

5.1.3 Residual and particulate nutrients

Dissolved nitrate and phosphate were almost entirely depleted in all light and nutrient experimental groups by the time the community reached stationary phase of growth or began to decline, and experimental units were terminated (*Figure S4ac*). However, higher amounts of residual nutrients remained at lower salinity levels in most treatments, and this trend was also seen for ammonia (*Figure S4b*). Dissolved silica (*Figure S4d*) was lower in treatments with high abundance of diatom taxa, particularly *Diatoma tennuis* (*Figure S5cf*).

As expected, particulate organic phosphorus (POP) concentrations were highly dependent on starting phosphate concentration, i.e. POP was low in P-limited media. However, this appears to vary between salinity levels, with the lowest POP concentrations at 0 salinity and a clearer trend of increasing POP with salinity under nutrient replete and light-saturated conditions (*Figure 5.2a*). A three-way ANOVA for POP found a significant interaction only between salinity and starting N:P ($F_{9,64}$=2.15, p=0.037) (*Table S10*). Particulate organic carbon (POC) showed a generally increasing trend with salinity (*Figure 5.2b*); a three-way ANOVA found significant interactions between light and salinity ($F_{3,64}$=3.85, p=0.013), light and starting N:P ($F_{3,64}$=5.51, p=0.002) and salinity and starting N:P ($F_{9,64}$=11.77, p<0.0001), but did not find a significant three-way interaction effect ($F_{9,64}$=1.35, p=0.23) (*Table S11*). Particulate organic nitrogen (PON) results were highly variable (*Figure 5.2c*), with a significant three-way interaction between light, starting N:P and salinity ($F_{9,64}$=2.14, p=0.039) (*Table S12*).

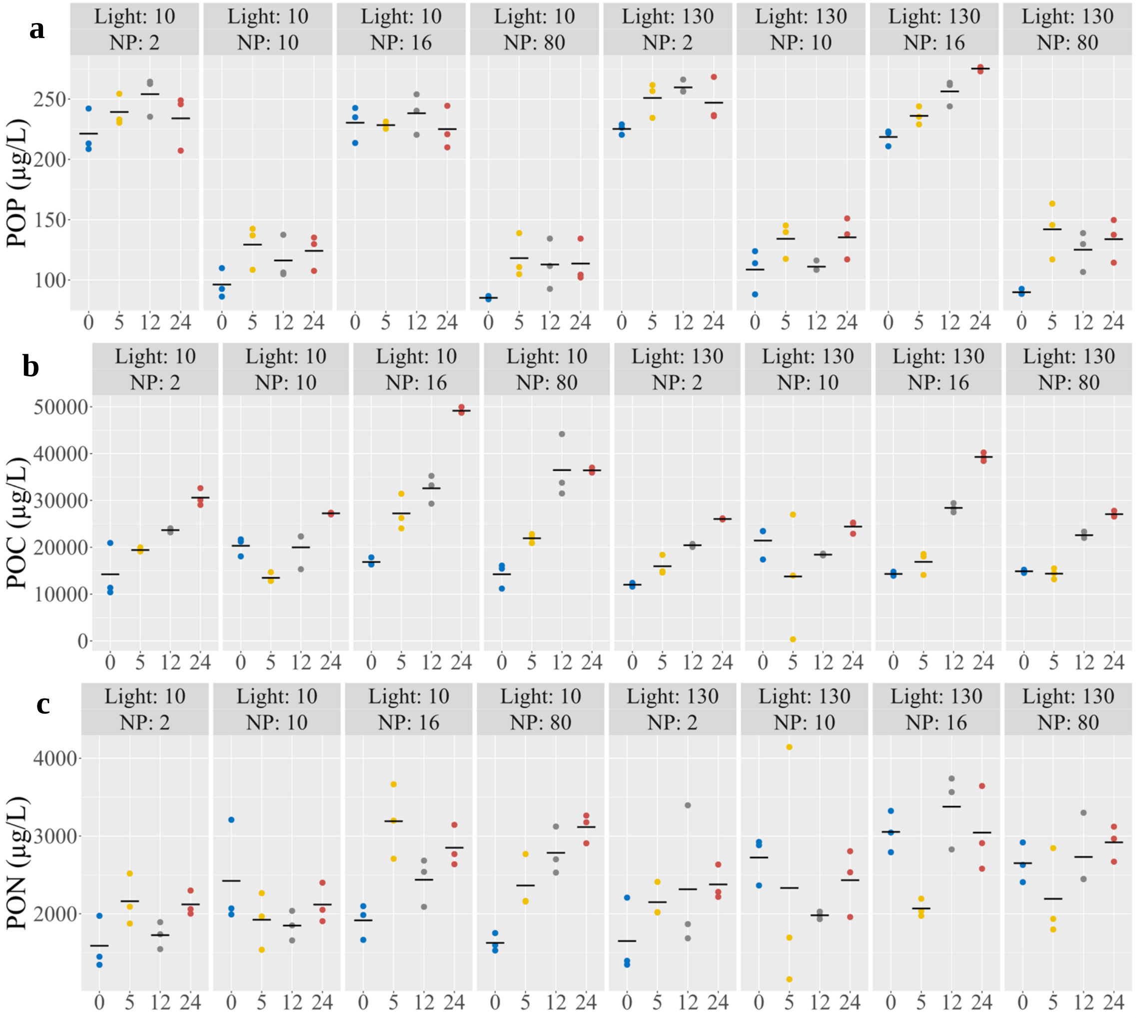


Figure 5.2 Measured **a.** Particulate Organic Phosphorus (POP) **b.** Particulate Organic Carbon (POC) and **c.** Particulate Organic Nitrogen (PON), for communities at different nutrient (N:P ratio), light (µmol $m^{-2}s^{-}1$), and salinity (x-axis) levels. Points = individual replicates, black bar = group mean.

For stoichiometric ratios of particulate carbon (C), phosphorus (P) and nitrogen (N), particulate C:P, C:N and N:P ratios all showed nonlinear trends for salinity within nutrient conditions. Highest C:P and C:N generally occurred at highest salinity (*Figure 5.3ac*), and lower particulate N:P occurred at higher starting phosphate concentrations (N:P 2 and 16 treatments) (*Figure 5.3b*). A three-way ANOVA found a similar result for C:P as POC: significant interactions between light and salinity ($F_{3,64}$=2.77, p=0.048), light and starting N:P ($F_{3,64}$=5.77, p=0.0015) and salinity and starting N:P ($F_{9,64}$=4.66, p<0.0001), but non-significant three-way interaction between the explanatory variables ($F_{9,64}$=1.19, p=0.32) (*Table S13*). For both particulate C:N and N:P ratios, a three-way ANOVA only found a significant interaction between salinity and starting N:P (C:N $F_{3,64}$=2.51, p=0.01; N:P $F_{3,64}$=2.17, p=0.0355) (*Tables S14, S15*).

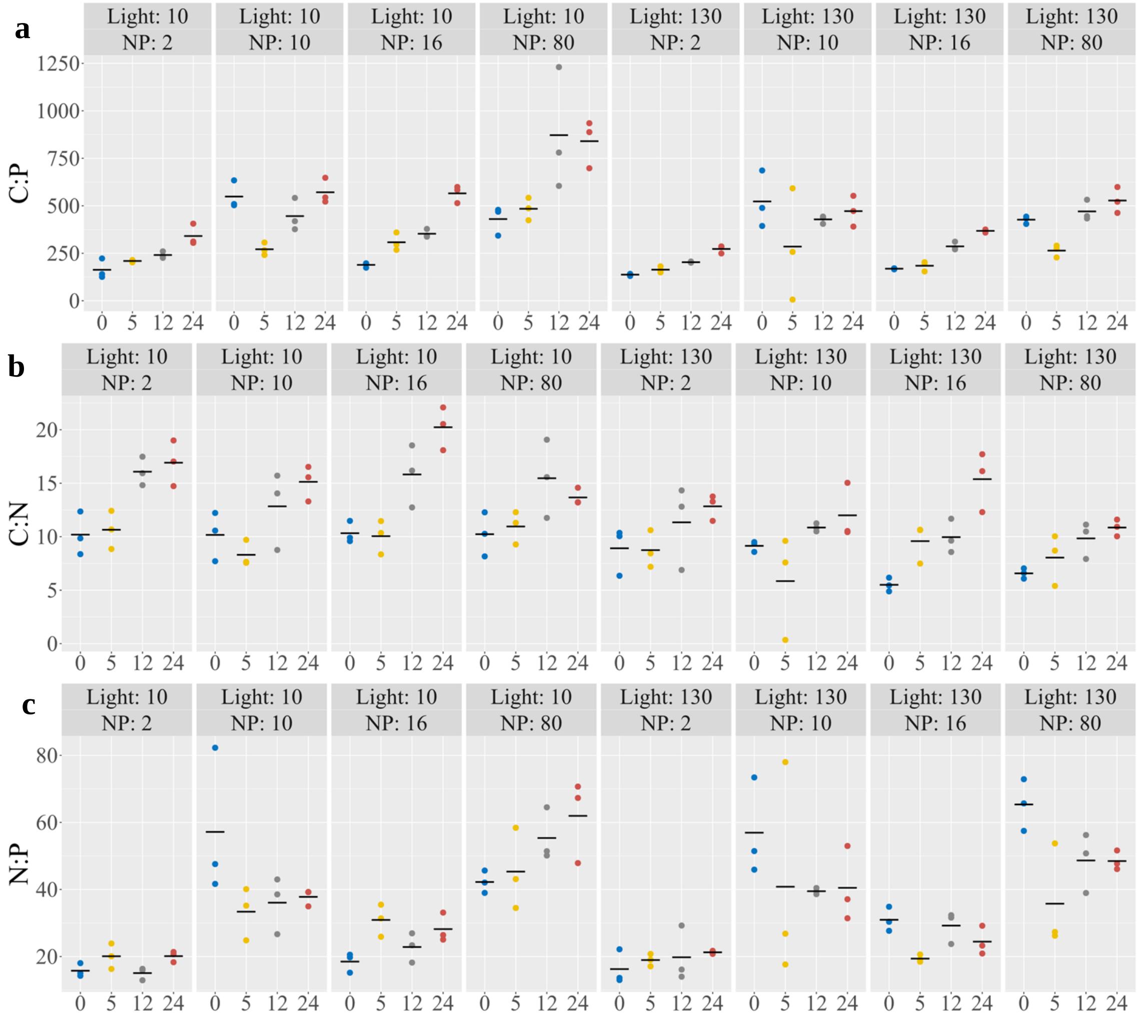


Figure 5.3 Calculated **a.** POC:POP (C:P) **b.** POC:PON (C:N) **c.** PON:POP (N:P), for communities at different nutrient (N:P ratio), light (µmol $m^{-2}s^{-}1$), and salinity (x-axis) levels. Points = individual replicates, black bar = group mean.

5.2 Community composition

Communities under different light and nutrient conditions showed different absolute and relative amounts of the 10 included taxa, as well as cysts of dinoflagellates and *Rhodomonas* sp. (*Figure 5.4*, *Figure S8*). *Phaeodactylum tricornutum* was dominant in nearly all communities, and highly represented at N:P ratio of 16. *P. tricornutum* showed increased dominance under low light conditions, and higher salinities, but did not show a consistent trend for overall abundance with changing salinity within nutrient conditions (*Figure S8f*). In contrast, *Monoraphidium* sp. generally decreased with salinity within each nutrient level, especially at higher light levels (*Figure S8e*). *Synechococcus* sp. did not show a consistent trend along the salinity gradients within treatments but did vary considerably between salinities and was generally more abundant at higher salinities, also showing greater abundance under high light conditions and N:P 16 (*Figure S8g*). *Diatoma tenuis* abundance was higher at intermediate salinities 5 and 12 and lowest at salinity 24, appearing similar between light levels (*Figure S8c*). *Diacronema lutheri* abundance was higher at intermediate salinities under low light and these treatments also increased at limited phosphorus concentrations (N:P 10 and 80), but this trend was absent under high light conditions (*Figure S8d*). *Rhodomonas* sp. was poorly represented at low salinities, particularly under low light levels, and most abundant at salinity 12 under most conditions, although highest *Rhodomonas* sp. values were found at salinity 24 for low light levels at limited phosphorus concentration (N:P 10 and 80) (*Figure S8a*). The highest number of cysts occurred at salinity 12 followed by salinity 5 under high light, while under low light a relatively higher number of cysts was produced at salinity 24, particularly at higher N:P (*Figure S8b*). Although their overall counts were low, more dinoflagellates were found at higher light levels compared to other treatments, particularly at limited phosphorus concentrations (N:P 10 and 80). Dinoflagellate counts did not appear to show a trend following salinity levels, although under low light dinoflagellates were only observed at intermediate salinities 5 and 12 and low N:P (*Figure S8h*). No living *Skeletonema marinoi* cells were observed in any of the treatment samples.

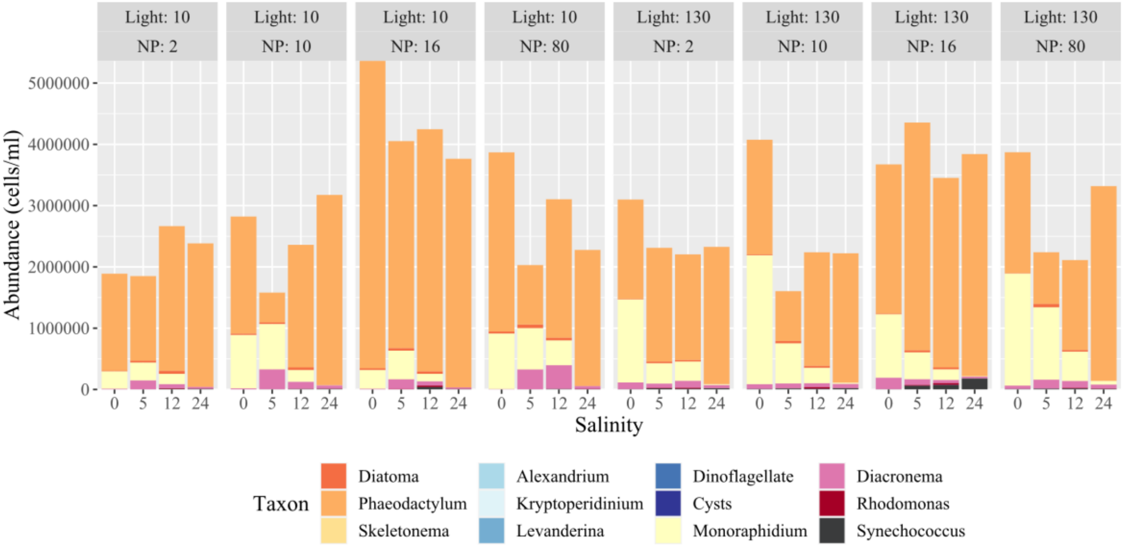


Figure 5.4: Absolute abundance of microalgal taxa (cells ml^{-1}) for communities at different nutrient (N:P ratio), light (µmol m^{-2}s^{-1}), and salinity (x-axis) levels. Taxa are referred to by only genus for convenience.

A non-metric multidimensional scaling (NMDS) plot based on Bray-Curtis dissimilarity values calculated for the absolute estimated counts of microalgal taxa between the different light, nutrient, and salinity conditions (*Figure 5.5*) highlights differences in community composition between experimental treatments. Results reiterated trends observed in *Figure 5.4*, with clear separation of groups experiencing 0 and 24 salinity levels, influenced particularly by the opposing trends of *Rhodomonas* sp. (as well as *P. tricornutum* and *Synechococcus* sp.) and *Monoraphidium* sp. with salinity. Salinity 5 and 12 groups were not as easily separated, aside from those under high light that had higher numbers of dinoflagellates. Low and high light conditions at 0 salinity can be somewhat separated based on higher levels of *Monoraphidium* sp. and *D. lutheri* at higher light levels. No strong parallel trends were observed between starting nutrient levels and dissimilarity between groups, except for under 0 salinity, where there is a split between high and low N:P ratios, which appears to be largely driven by proportions of *P. tricornutum* and *Monoraphidium* sp.

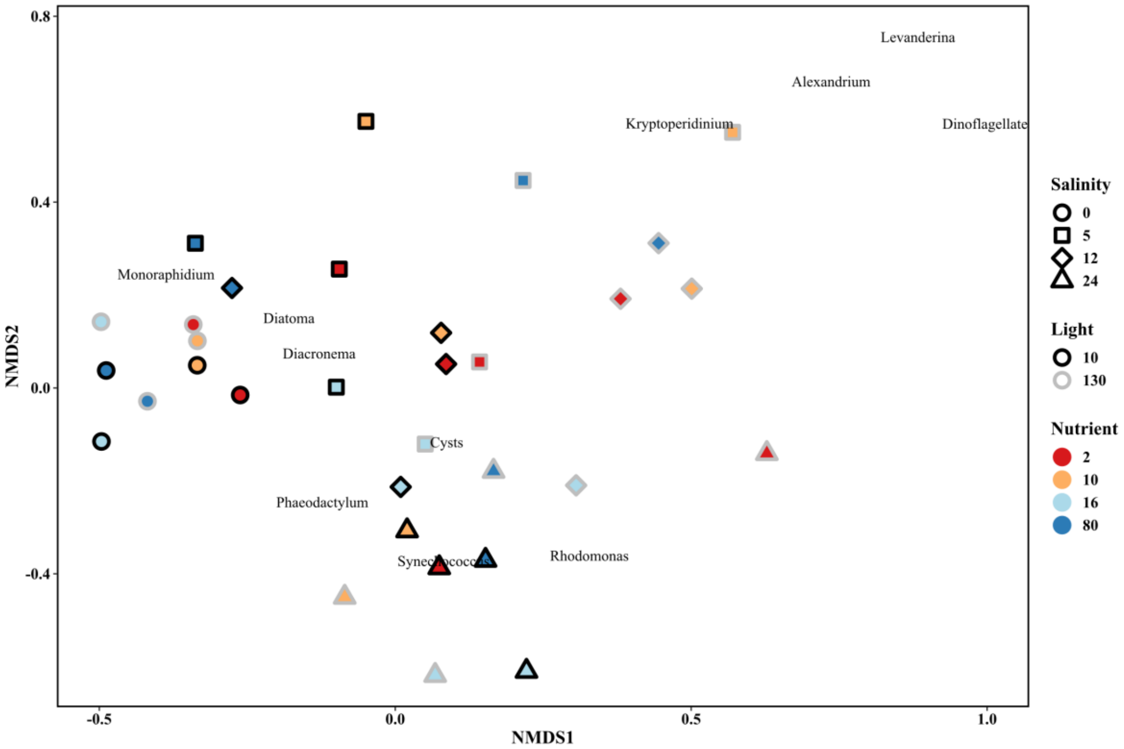


Figure 5.5: Results of NMDS performed on Bray-Curtis dissimilarity values calculated for absolute estimated counts of microalgal taxa in communities at different salinity, light (μmol m^{-2}s^{-1}), and nutrient (N:P ratio) levels. Stress: 0.1. Taxa are referred to by only genus for convenience.

6 Discussion

Climate-driven changes in ocean salinity are likely to influence phytoplankton community trait composition, with consequences for marine ecosystem functions and services. Our microcosm experiment with artificial phytoplankton communities revealed a three-way interaction between light, N:P and salinity influencing community composition as well as community traits related to species competition for nutrients. Within the two light conditions, the effects of nutrient limitation on phytoplankton composition and community traits varied between salinity levels, but this response was not linear. In this chapter, I consider what these results might reveal about the effect of salinity on phytoplankton competition for nutrients and light, as well as its consequences for marine ecosystems, focussing particularly on the Baltic Sea and predicted freshening in its upper layer.

6.1 Light

The influence of light availability upon phytoplankton communities was apparent through its strong effect on growth rate, Pmax and chlorophyll *a* concentration, with light-limitation inhibiting growth under all nutrient and salinity conditions (*Figure 5.1*). Conversely, communities under light limitation showed higher carrying capacity, as estimated by Pmax, which is used as a measure of the maximum yield that can be reached for given resource supply. However, Pmax in our experiment was estimated based on the fluorescence of chlorophyll *a*, not cell counts. This makes interpretation of my results more difficult because cellular chlorophyll content increases under low light conditions, contributing to measured Pmax (Litchman & Klausmeier, 2008). A typical photoacclimation response causes cells grown in low light to have higher chlorophyll *a* content, as the amount of light harvesting pigments is increased to compensate for lower excitation levels (Seppälä, 2009). Nonetheless, cell counts and POC, which is also used as a proxy for phytoplankton biomass, indicate that total biomass of phytoplankton communities was in-fact higher under light limitation. Community traits estimated from chlorophyll *a* fluorescence do not incorporate *Synechococcus* sp., because cyanobacteria show low chlorophyll *a* specific fluorescence, which cannot be used for their detection in the presence of eukaryotic species (Seppälä, 2009), although the low abundance of the cyanobacterium in nearly all treatments means this is unlikely to affect my conclusions.

Light limitation increased the dominance of *Phaeodactylum tricornutum*, particularly due to reduced levels of *Monoraphidium* sp., but absolute counts of *P. tricornutum* were nonetheless higher under limited light for many treatments (*Figure S8f*). Counts of *Diatoma tenuis* were also higher under limited light (*Figure S8c*), emphasizing the ability of diatoms to maximise light harvesting under low light levels due to the high surface area:volume ratio of their cells. Furthermore, abundance of larger cells such as dinoflagellates was lower in limited light conditions, while smaller phytoplankton such as *Diacronema lutheri* were generally more abundant under limited light levels, at least for the middle two salinity treatments (*Figure S8d*). Small cells may show more effective utilization of low light levels due to a reduced packaging effect, which refers to self-shading of light-capturing pigments (Litchman et al., 2010; Seppälä, 2009). However, the fact that smaller celled *Synechococcus* sp. and *Monoraphidium* sp. were less abundant under low light conditions suggests more than cell size was at play behind the success of *D. lutheri* in these treatments. A study published this year of an under-ice algal bloom in the Arctic reported dominance of mixotrophic brackish water haptophytes in the light-limited, nutrient-poor freshwater-influenced water under 1 m thick sea ice (Søgaard et al., 2021), emphasising the ability of haptophytes like *D. lutheri* to perform well under these conditions. Contrary to previous studies, such as by Yang & Jin (2008) and de Tesanos Pinto & Litchman (2010), who observed that the overriding effect of light limitation was able to dominate N:P effects in structuring communities, there still appeared to be an effect of both N:P and salinity on phytoplankton community composition and traits in our experiment, even under light limitation, although the light effect was admittedly dominant.

6.2 Salinity and community composition

We found an interaction between salinity and N:P, which influenced phytoplankton community composition and traits in both light conditions. The ability of microalgae to span both marine and freshwater environments is modified by osmoregulation costs (Hoef-Emden, 2014; Olli et al., 2011; Suescún-Bolívar & Thomé, 2015), and the consequences of these energetic costs may be reflected in the modified community growth rates, carrying capacities and compositions that we observed between salinity treatments in our study. While the phytoplankton species included in our experiment are all capable of surviving at all four salinity levels, their abundances in mixed communities varied, likely informed by both energetic and metabolic requirements of osmotic adjustment as well as dynamics of competition with other taxa. For example, *Rhodomonas* sp. showed considerably lower abundance at 0 salinity, and also at 5 salinity for most treatments. This is unsurprising, as cryptophytes are mostly found in marine and brackish environments, with the prevalence of marine/brackish species, as well as the predominantly marine lifestyles of red algae from which their plastids originated, suggesting that the common ancestor of cryptophytes was a marine or brackish water species (Hoef-Emden, 2014). A competition experiment performed by de Tezanos Pinto & Litchman (2010) with freshwater phytoplankton also found cryptomonads including *Rhodomonas* to be inferior competitors to diatoms and cyanobacteria under all treatment conditions of varying N:P and light levels. Associated with low temperatures and high salinities, cryptophytes have shown significant decreasing trends in all areas of the Baltic (Suikkanen et al., 2013). My results indicate that this decline may continue if the upper layer freshens in the future.

My NMDS analysis indicates salinity was more influential for community composition than light or nutrients (*Figure 5.5*), although the effect of nutrient levels is nonetheless apparent, for example through generally higher cell counts for many taxa at higher N:P and lower starting phosphorus conditions. Hillebrand & Lehmpfuhl (2011) found that freshwater species richness and especially evenness decreased with increasing P supply under highly nutrient limited conditions. They propose that this reflects patterns of reduced niche dimensionality, due to the additional nutrient supplied in these imbalanced ratios, increasing probability of species being limited by the same resource (Hillebrand & Lehmpfuhl, 2011). Dinoflagellates show high uptake affinity and assimilation rate of P, likely due to their large genomes rich in P (Kremp et al., 2008; Spilling et al., 2014), and this may partly explain higher dinoflagellate abundance under P-limited conditions.

While overall abundance and proportions of phytoplankton taxa varied between treatments, phytoplankton composition and dominance patterns remained largely consistent. *Phaeodactylum tricornutum* was the dominant taxon in most treatments; this could be attributed to *P. tricornutum*'s ability to adapt to changing environmental conditions (Martino et al., 2007), which also may explain the lack of clear trend for *P. tricornutum* abundance along salinity gradients. The dominance of *P. tricornutum* in our artificial communities warns of the potential for 'stress specialists' like *P. tricornutum* to dominate under deviant salinity conditions when they experience competitive release from species unable to tolerate physiologically stressful conditions (Larson & Belovsky, 2013). Fairly consistent community dominance patterns between treatments agree with work by Kremp et al. (2008), who used mesocosms to study the effect of variable nutrient additions and nutrient supply ratios on species composition for phytoplankton communities in the coastal northern Baltic Sea. They found that variations in dissolved elemental ratios did not have major effects on phytoplankton community composition. In their study, additions of N and P - as well as increased irradiance - stimulated diatom growth relative to dinoflagellates, but did not alter the outcome of competition between diatoms and dinoflagellates when the latter were initially dominant (Kremp et al., 2008). Thus, modified nutrients may change taxa proportions, but appear less likely to change overall outcomes of phytoplankton interspecific competition.

The only exception to the trend of *P. tricornutum* dominance was under high light conditions and freshwater, where *Monoraphidium* sp. was able to dominate under most nutrient conditions except N:P of 16. Suikkanen et al. (2007) observed an increase in the biomasses of chlorophytes across their whole study area in the Baltic from 1979-2011, linking this change with decreasing salinity due to the freshwater origins of many green algae. Although they are typically considered poor competitors for nutrients, it appears that lower salinity can give green algae an advantage, indicating the potential for freshening marine waters to modify typical phytoplankton competition dynamics.

As dinoflagellates are considered inferior competitors relative to diatoms if they are at high concentrations (Klais et al., 2011; Kremp et al., 2008; Suikkanen et al., 2011), it is unsurprising that dinoflagellates were poorly represented in final community compositions, given that starting amounts of each taxon in the community were approximately the same. Dinoflagellates show significantly lower maximum growth rates compared with diatoms (Litchman et al., 2007). Thus, the population growth of *Alexandrium* typically results in moderate biomass levels and co-occurrence with other species, rather than monospecific blooms (Anderson et al., 2012). Diatom and dinoflagellate nutrient uptake can be considered to typify r- and K-strategies respectively (Klais et al., 2011); dinoflagellates are favoured under conditions of crowding and low resources, and their larger cell sizes and specialised competitive strategies (such as allelopathy) relative to high growth rate and nutrient uptake all typify K-selection (Kremp et al., 2008). Dinoflagellate allelopathy may even have consequences for non-dinoflagellate taxa. Suikkanen et al. (2011) studied allelopathic effects of three dinoflagellates on five typical spring bloom diatoms and one cryptophyte. They found that dinoflagellates exerted significant negative effects on the cell numbers of the diatom species, with *Skeletonema marinoi* the most affected, but that their effects on cryptophyte *Rhodomonas* sp. were mostly positive. They suggest that *Rhodomonas* sp. benefited from organic matter released by dinoflagellates or slightly higher nutrient controls, and propose the lack of negative effect on this taxon may indicate that chemicals produced by Baltic vernal dinoflagellates are targeted to specific competitors such as diatoms (Suikkanen et al., 2011). Dinoflagellates are inferior competitors when it comes to growth rate, but show a suite of adaptive strategies, which enable them to persist in the Baltic Sea and elsewhere.

Considering dinoflagellates as K-strategists is complicated, however, as they produce many ‘offspring’ in the form of cysts, of which only a few will germinate. This could be considered an r-selection strategy (Anderson et al., 2012). All three dinoflagellates included in our study, as well as *Rhodomonas sp,* produce cysts, and the abundance of cysts was higher than dinoflagellate cells in nearly all treatments. Formation and spreading of cysts are behind the global expansion of dinoflagellate blooms in relatively shallow coastal waters (Klais et al., 2011). Thus, cyst-production may represent one way that dinoflagellates and *Rhodomonas* sp. could be able to persist in the Baltic despite projected freshening.

6.3 Salinity and community traits

In addition to community composition, community traits also varied with salinity, likely reflecting both interspecific community composition as well as intraspecific plasticity. Varying chl *a* concentrations, modified by both N:P and salinity, illustrate how pigments and stoichiometry interact, with the effect of light limitation apparently dependent on nutrient availability – mainly N supply. Decreased chl *a* under N limitation has been noted previously, attributed to N content of chlorophylls and associated with decreased photosynthesis during N stress (Seppälä, 2009, and references therein). The apparent influence of salinity on pigments is also consistent with findings that plankton fluorescence is affected by the physiological state of cells (Seppälä, 2009). It is interesting to note higher community concentrations of phaeophytin *a* relative to chlorophyll *a* under lower salinity (*Figure S3*), particularly under high light conditions, perhaps indicating poorer physiological conditions of communities under low salinity. Growth rate exhibited a non-linear response to salinity within nutrient treatments. This is indicative of a complex interaction between environmental drivers, which is worthy of further consideration and study.

6.4 Salinity and particulate nutrients

Salinity and starting N:P had strong effects on phytoplankton community stoichiometry (*Figure 5.2*), likely reflecting taxon-specific osmoregulation strategies informing phytoplankton nutrient requirements. POP was found to be highly dependent on starting dissolved P concentrations, pointing to the key importance of P for phytoplankton communities. It is interesting to note that a salinity trend was only clearly visible under nutrient replete and light saturated conditions, suggesting that nutrient availability had a stronger effect than salinity on POP. Global particulate C:P varies as a linear function of ambient phosphorus concentrations, while C:N only varies with ambient nitrate concentrations when nitrate is most scarce (Galbraith & Martiny, 2015). Galbraith & Martiny (2015) link this pattern to adjustment of the phytoplankton community to local nutrient availability, with phytoplankton showing greater flexibility of C:P due to P being a less abundant cellular component than N. Conversely, POC in our experiment showed a strong effect of salinity, reflecting community composition, with highest POC under high salinity conditions dominated by *P. tricornutum*. Spilling et al. (2014) investigated effects of phytoplankton community composition on spring bloom carbon flows and nutrient stoichiometry in multiyear mesocosm experiments, and found that community structure significantly affected C accumulation, with highest POC build-up in diatom-dominated communities. PON exhibited high variability and was driven by an interaction between all three variables. The stronger influence of light on PON compared to POC and POP makes sense considering the relationship between N content and concentration of photosynthetic pigments.

The effect of environmental drivers on particulate nutrients translates into changes in stoichiometric ratios, with our results suggesting that decreased salinity may decrease phytoplankton C:N and C:P ratios (*Figure 5*.3). This is an opposite trend to that expected with future P and N decrease, iron and light increase and warming. Tanioka & Matsumoto (2020) predict that combined effects of these drivers will lead to increased C:P and C:N due to increase of C:P with decreased P availability, light and warming, and increased C:N from decreased P and N availability and increased light. Elemental stoichiometry varies both between and within taxa, under high and low nutrient levels (Tanioka & Matsumoto, 2020). Phytoplankton may respond to environmental drivers by changing cellular allocation of volume between pools rich in P (RNA/DNA, polyphosphates and phospholipids, often associated with the biosynthetic apparatus), N (proteins and pigments associated with light-harvesting) and C (Carbohydrates and lipids, associated with energy storage reserves) (Moreno & Martiny, 2018; Tanioka & Matsumoto, 2020). The Maximum Growth Rate Hypothesis predicts that P concentrations should increase with increasing growth rate, with more P required to make P-rich components of the biosynthetic apparatus (Klausmeier et al., 2004; Moreno & Martiny, 2018; Rees & Raven, 2021). Rees & Raven (2011) report a strong positive relationship between maximum growth rate of all photosynthetic organisms and their P content, although they found that the growth rate hypothesis is only a partial explanation for the relationship between maximum growth rate and P content, as RNA does not account for most of the P-containing constituents. Our results do not reveal particularly parallel trends in community growth rate and C:P, suggesting more complex dynamics are at play. Increasing C:P with increasing salinity may reflect increasing taxonomic diversity at higher salinity levels. This is consistent with predictions by Ptacnik et al. (2010) that increasing diversity in phytoplankton communities will increase C:P ratios of autotrophs, due to higher trait variance in light acquisition than inorganic nutrient resource acquisition traits maximising light use and thus carbon fixation. Variation in N:P, such as was observed in our results, has been mostly linked to greater plasticity in cellular N:P relative to C:N, resulting in N:P increasing as overall macronutrient concentrations decrease, and N:P changes occurring mostly due to changes in PO_4 concentration (Galbraith & Martiny, 2015).

Taxonomic composition is considered the best predictor of bulk particulate organic matter composition in global oceans (Lomas et al., 2021; Sharoni & Halevy, 2020; Weber & Deutsch, 2010). Cyanobacteria thrive in nutrient-poor environments and typically have

N:P ratios in culture greater than the Redfield Ratio (Bertilsson et al., 2003; Lomas et al., 2021; Martiny et al., 2013; Sharoni & Halevy, 2020), while diatoms thrive in nutrient rich environments and typically have N:P ratios lower than the Redfield ratio (Lomas et al., 2021; Martiny et al., 2013; Weber & Deutsch, 2010). A review of laboratory culture results found a mean N:P for diatoms of 14 (n=27 taxa), 17 for dinoflagellates and green algae (n=33), and 26 for cyanobacteria (n=25) (Sharoni & Halevy, 2020). There are noticeable parallels between taxonomic composition and community N:P ratio in our experimental communities, with higher N:P in freshwater conditions dominated by *Monoraphidium* sp. and lower in communities with more complete *P. tricornutum* dominance.

6.5 Consequences

Climate change has the potential to increase or decrease environmental dimensionality through changed element cycles and altered biodiversity (Ptacnik et al., 2010). The results of our study point to a number of potential consequences arising from predicted future freshening of the Baltic Sea upper layer.

Lower predicted diversity in lower salinity treatments, with loss of dinoflagellates and cryptophyte *Rhodomonas* sp., raises questions about the stability and resilience of future Baltic Sea phytoplankton communities. Steudel et al. (2012) measured biomass of microalgae grown in microcosms along two stress gradients of heat and salinity, and compared their results with 13 published case studies measuring biodiversity-ecosystem functioning relationships under different environmental conditions. They found that positive effects of biodiversity on ecosystem functioning decreased with increasing stress in absolute terms, but that increasing stress had a stronger relative effect on low-diversity communities. This emphasises the need to maintain high levels of biodiversity as insurance, which buffers against impacts of changing environmental conditions. However, for the Baltic Sea it may be useful to consider the Estuarine Quality Paradox, which refers to the challenge of using diversity as a quality indicator for detecting anthropogenic stress in transitional water ecosystems. The functioning of these ecosystems despite often low biodiversity goes against traditional conceptions of biodiversity-ecosystem functioning relationships, necessitating that greater emphasis is placed on ecosystem function rather than structure to assess human impacts on these systems (Elliott & Quintino, 2007). Flöder & Hillebrand (2012) tested the diversity-stability hypothesis, which suggests more diverse communities show higher stability and productivity, on freshwater phytoplankton communities experiencing a combination of simulated grazing and pH reduction stressors. They conclude that the relationship between biodiversity and stability from disturbance depends on how traits related to resistance and recovery are distributed in the community (Flöder & Hillebrand, 2012). They found that fast recovery of dominant green alga *Scenedesmus obliquus* prevented long-term disturbance effects on final community biomass, and that communities with *S. obliquus* present showed slower recovery rates and lower final biomass when they were more diverse, as the strong performance of *S. obliquus* was diluted by more species being present. Thus, the stability of communities responding to multiple disturbances depends on how sensitive species are to the stressors, as well as the population dynamics of a well-performing dominant species. This enforces the importance of both richness and evenness of communities when it comes to their stability. Thus, population dynamics of dominant taxa, which in the Baltic spring bloom are diatoms and dinoflagellates, may determine the response of the entire community to stress.

Although the results of our experiment do not reveal so much about the fate of cyanobacteria in the Baltic Sea, aside from indicating that *Synechococcus* sp. are inferior competitors when starting with similar biomass (particularly under nutrient limited conditions), nutrients and their effects on biodiversity are nonetheless key to understanding mechanisms behind eutrophication and its consequences (Hillebrand et al., 2014), a problem of considerable importance to the Baltic. Trait-based approaches are also crucial for explaining, predicting, and preventing harmful eukaryotic algal bloom occurrence in diverse ecosystems, due to shared plastic traits of many bloom-forming algae, such as *Alexandrium ostenfeldii* (Litchman et al., 2010). Initial abundance can determine the outcome of competition, illustrated by the expansion of cyst-producing dinoflagellates in areas of the ocean (Klais et al., 2011). When species are consuming most of the resource for which they are the superior competitor, theory expects that they will monopolise this resource when it is limited, leading to competitive exclusion, with the winner depending on initial conditions (Brauer et al., 2012; Huisman & Weissing, 1999). Experiments exploring the relationship between competitive ability of potentially harmful cyanobacteria and dinoflagellates, their initial biomass, and nutrient and salinity levels, could prove a useful strategy for anticipating harmful algal blooms in areas of the Baltic Sea based on nutrient and salinity levels in the future.

Our results indicate that future freshening of the Baltic Sea upper layer during spring may favour diatoms, a trend that is opposite to current observations of increasing dinoflagellate proportion in the spring bloom due to warmer winters. Diatoms and dinoflagellates appear to be functional surrogates in the Baltic, capable of blooming in spring and using up winter inorganic nutrient pools (Klais et al., 2011; Spilling et al., 2018). Diatoms and dinoflagellates also show comparable nutrient requirements, excluding diatoms' need for silica, and provide similar ecosystem services regarding nutrient uptake and biomass production (Klais et al., 2011; Kremp et al., 2008). Nonetheless, the relative abundance of diatoms and dinoflagellates is expected to have consequences for nutrient cycling in the Baltic Sea. Diatoms sink to the seafloor intact relative to dinoflagellates, which disintegrate and release nutrients into the productive surface layer, although their cysts resist degradation and their organic matter is not readily available to the benthic system when they settle to the bottom (Heiskanen, 1998; Kremp et al., 2008; Spilling et al., 2014, 2018). Thus, patterns of nutrient cycling in the northern Baltic may be modified by changes in bloom dominance patterns, with ecosystem-wide implications (Kremp et al., 2008). The fate of organic matter produced in the spring bloom during highest annual primary production and sinking of organic matter to the seafloor is crucial for understanding material fluxes and how they affect ecosystem functioning. Increasing dominance of dinoflagellates with high encystment efficiency relative to diatoms could increase retention and burial of organic matter in sediment, with the potential to actually reduce eutrophication in areas of the Baltic (Spilling et al., 2018). For diatom dominance, nutrient drawdown to the bottom is efficient and reduces nutrient stocks for summertime in the euphotic zone. However, more organic material may remain in the surface layer in the case of dinoflagellate dominance, supporting recycling through the microbial loop (Spilling et al., 2018).

Dominant taxa also shape their environment through the uptake and accumulation of nutrients, which is neatly demonstrated by our results for residual nutrients, with nearly all nutrients taken up prior to decline of phytoplankton biomass. In addition to their strong relationship with silica, which could be seen in the negative trend between residual silica and diatom abundance in our results, diatoms have been noted to show higher carbon-specific nitrate uptake rates relative to dinoflagellates and chlorophytes, while green algae show intermediate values for nitrate-dependent growth and uptake, and dinoflagellates show relatively low maximum uptake rates and high half-saturation constants for nitrate uptake (Litchman et al., 2007). Green algae generally show higher affinity for ammonium uptake (Litchman et al., 2007), however, higher residual ammonia remained in *Monoraphidium* sp.-dominated freshwater treatments of our experiment. The question remains as to whether the higher ammonia, and to some extent phosphate and nitrate, in freshwater treatments is related to community utilization or chemical properties such as more efficient ion transport in more saline water. Further research into the interaction between ion transport and phytoplankton physiology might help to interpret our findings for residual nutrients, and thus elucidate the way that changing taxonomic compositions in the future might modify nutrient levels in the Baltic Sea.

When the results of individual taxa resource utilization traits are upscaled, effects of salinity change on plankton stoichiometry could have global consequences (Bonachela et al., 2016; Litchman et al., 2015). Our results indicate the potential for the freshening of the Baltic Sea upper layer to lower community C:P. Stoichiometry changes can alter regional efficiency of the biological pump, with P supplied to high C:P regions leading to greater export of carbon than P supplied to low C:P regions (Martiny et al., 2013; Moreno et al., 2018). Thus, changes in phytoplankton elemental stoichiometry have the potential to fundamentally change carbon partitioning between the atmosphere and the ocean, with lower carbon export from surface waters in lower C:P regions (Moreno et al., 2018).

Predicted changes in phytoplankton community composition and stoichiometry also have consequences for Baltic Sea food webs. Observed changes in Baltic Sea phytoplankton communities indicate a shift at the base of the food web from higher quality food (such as cryptophytes) towards more microbial, less energy-efficient food webs of lower food quality and smaller organisms (such as N-fixing cyanobacteria). Combined with the direct effects of stressors such as warming and freshening, this could create a strong disadvantage for mesozooplankton and lead to decreased availability of energy for grazing zooplankton and fish (Suikkanen et al., 2013). Nutrient deficiency in environments can reduce energy transfer efficiency at the base of food webs by altering phytoplankton biomass and chemical composition, which affects their quality as food for consumers (Bi & Sommer, 2020). Changes in community composition and species richness can alter stoichiometric interactions in food webs (Hillebrand et al., 2014). Recent studies have identified a close link between zooplankton stoichiometric ratios and the stoichiometry of their algal prey, with low food quantities and nutrient deficient diets (lower prey C:N ratios) resulting in lower egg production by copepod *Acartia tonsa* (Bi & Sommer, 2020). N and P deficiency in environments can reduce trophic transfer efficiencies of C from phytoplankton to zooplankton, decreasing zooplankton production and changing structure and functions of marine food webs (Bi & Sommer, 2020). Our results suggest that freshening could lower C:P and C:N ratios in phytoplankton communities, weakening the negative effects of N and P fertilisation, increasing irradiance and warming, but further work is required to determine the net effect of diverse climate change-related drivers on Baltic food webs in the future.

6.6 More than salinity

Our study emphasises the influence of salinity on resource utilisation traits, however it incorporates only a subset of the environmental change-associated drivers that are likely to influence future phytoplankton communities. For example, ocean acidification, due to increasing atmospheric CO_2, is also a major concern for the Baltic (The BACC II Author Team, 2015). In addition, site-specific hydrodynamics, likely to be modified by changing patterns of precipitation and runoff, have already been identified as important predictors of the presence of some phytoplankton taxa in the Baltic (Gasiūnaitė et al., 2005). Fluctuations in nutrient supply also influence phytoplankton competition and community structure (Chesson, 2000). Yang & Jin (2008) found that under fluctuating light intensity, densities of cyanobacteria, green algae and diatoms were lower in both monoculture and mixed culture relative to conditions of constant light intensity, suggesting frequent fluctuations in light intensity are disadvantageous for all three groups of bloom-forming algae that are expected to benefit from future Baltic freshening.

Although its effect could not be incorporated into our study design, predation can also both directly and indirectly structure phytoplankton communities. A generalist predator can be considered a single limiting factor with analogous impacts to limitation by a single resource (Chesson, 2000). Predators can promote species coexistence if each species has its own specialist predator, or else if specialist natural enemies control each other's density (Chesson, 2000). Hillebrand & Lehmpfuhl (2011) included two ciliate species as consumers in half of their metacommunities of freshwater phytoplankton, and found that consumer presence reduced overall biomass and richness, with biomass removal increasing with increasing resource supply. Differential nutrient regeneration and nutrient patchiness produced by zooplankton may be even more important effects of zooplankton on phytoplankton communities than direct grazing, as if some nutrients are regenerated more rapidly than others, zooplankton may change both nutrient supply rates and ratios (Tilman et al., 1982). Under high phytoplankton mortality, such as due to high grazing, a greater cellular allocation is expected for assembly machinery, resulting in a lower N:P ratio, due to less allocation to N rich resource acquisition machinery (Klausmeier et al., 2004). Work by Clio Hall et al. (in preparation) using mesocosms to explore salinity effects on bacteria, nanoflagellates, phytoplankton and zooplankton will help connect our findings with their consequences for higher trophic levels in the Baltic Sea pelagic food web.

Research suggests that changing temperatures in the future are also likely to change phytoplankton communities, at least in part by altering their traits that relate to resource competition (Edwards et al., 2016). There appear to be interactions between temperature and N:P ratio influencing these traits (Edwards et al., 2016; Hofmann et al., 2019; Marañón et al., 2018; Thomas et al., 2017). Temperature increase is expected to result in higher C:P, and N:P (Moreno et al., 2018; Yvon-Durocher et al., 2015), thus exerting an opposite predicted influence on community stoichiometry to that predicted by this study for freshening. Although our microcosm experiment took place under the reasonably high temperature (for the Baltic) of 16 °C, further research is required to understand the interaction between salinity and temperature and their apparent opposing trends for phytoplankton communities, as well as how they interact with other important drivers.

Furthermore, other phytoplankton traits, which were not directly considered in this thesis, could influence communities in a changing ocean. Many eukaryotic phytoplankton exhibit mixotrophy, including all three dinoflagellates included in this study (Anderson et al., 2012; Figueroa et al., 2009; Moestrup et al., 2014) and haptophyte *Diacronema* *lutheri* (Bock et al., 2021). Along with other adaptive competitive strategies, including motility and allelopathy, mixotrophy can compensate for low nutrient uptake affinities in dinoflagellates relative to other common phytoplankton (Suikkanen et al., 2011). Dominance of mixotrophic haptophytes has been noted in the nutrient-depleted and freshwater-influenced environments under-ice blooms in the Arctic, and this lead Søgaard et al. (2021) to predict that future freshening could drive a shift in dominant algal species to mixotrophs. Mixotrophy provides a competitive advantage in light- and nutrient-limited environments, by supplying additional nitrogen and organic carbon as an energy source (Søgaard et al., 2021). Mixotrophy may also provide a stabilizing effect on ecosystem functions by providing alternative pathways capable of buffering the effect of species loss (Ptacnik et al., 2010).

The *Synechococcus* sp. included in our experimental communities is not a nitrogen-fixing cyanobacterium, thus our study is unable to consider the effect of freshening on nitrogen fixation in the Baltic. Freshwater origins of several species - tendency to thrive in warm waters, and the favouring of N-fixing by high P (and low N:P) levels - support the predicted increase of N-fixing cyanobacteria in the Baltic in line with anticipated environmental changes (Suikkanen et al., 2013). Increase of nitrogen-fixing cyanobacteria abundance could result in increased supply of nitrogen to nitrogen-limited phytoplankton, thereby promoting coexistence (Agawin et al., 2007; The BACC II Author Team, 2015). N fixers have high N:P ratios due to higher light-harvesting machinery required to power N-fixation relative to more P-rich cell assembly machinery (Klausmeier et al., 2004), thus an increase in N-fixing cyanobacteria might increase the N:P ratio in the Baltic. It is important to include the effects of N-fixation when considering changes in cyanobacterial abundance, and their consequences, in the future Baltic. Both mixotrophy and N/fixation represent additional factors influencing phytoplankton nutrient uptake, competition and cycling, which were not captured by our study design but are worthy of future consideration when predicting how salinity change will affect pelagic ecosystems.

Resource uptake of phytoplankton strongly depends on cell size, which is considered a ‘master trait’. Thus, inclusion of cell size in models of resource utilization and competition is important (Litchman and Klausmeier, 2008). When counting cells, I observed considerable variation in cell size, which is consistent with previous studies of these taxa (eg. (Figueroa et al., 2009; Moestrup et al., 2014). Larger cell size is associated with resistance to grazers, and greater maximum uptake rates per cell, as well as potentially larger nutrient storage capacity relative to minimum requirements, providing an advantage under conditions of fluctuating nutrient supply (Edwards et al., 2011; Litchman et al., 2007, 2009, 2010). Particulate C:N and C:P also tend to decline as volume increases (Edwards et al., 2011). In addition to size, cell shape of marine phytoplankton appears to be an important trait that is correlated with global taxonomic richness (Ryabov et al., 2021). While a study of cell size and shape did not fit into the scope of this thesis, I hope that work continued by Iris Orizar on this topic will elucidate potential connections between resource-related traits considered here.

The considerable variation we observed in community traits under different environmental drivers can be attributed to both interspecific taxonomic variation as well as intraspecific plasticity. For a single genotype, traits may vary based on growth history and environmental conditions and show phenotypic plasticity (Litchman et al., 2015). Plasticity, arising from different gene expression within a species, increases diversity of trait values and responds to the environment on the scale of days (Litchman et al., 2010), with the temporal scale of our microcosm experiment focussing on this short-term plastic response by phytoplankton taxa. *Phaeodactylum tricornutum*'s ability to adapt to changing environmental conditions has been linked to high intraspecies morphological variability (Martino et al., 2007), which I also observed in our experiment. An oval morphology in *P. tricornutum,* affiliated with higher aggregation into clusters surrounded by mucilage, is associated with potentially higher survival in sub optimal conditions including low salinity, low light conditions and nutrient depletion (Martino et al., 2007). Shifts in cellular stoichiometry may occur in the short term through phenotypic plasticity, but then be extended and reinforced through genotypic adaptation if they prove to be selectively advantageous (Yvon-Durocher et al., 2015). With resource utilization traits in microbes evolving under competition (Litchman et al., 2015), I consider the question of how resource utilization traits in phytoplankton evolve under both competition and changing environmental drivers an intriguing avenue of future study.

6.7 Conclusions

Understanding the responses of marine food webs to environmental changes is of critical importance to predict the structure and functioning of marine ecosystems under future ocean scenarios (Bi & Sommer, 2020). I set out to explore how resource availability affects phytoplankton trait distribution along a salinity gradient, hypothesising that changing salinity would modify response of phytoplankton communities to resource limitation. The results of our experiment are in line with these expectations, with both phytoplankton taxa counts and nutrient-related community traits varying between light and nutrient conditions as well as between salinity levels within these treatments. Competitive strategies of dominant taxa, such as the high surface-area-to-volume-ratio of diatoms capable of maximising light absorption, did inform community composition and traits as predicted. However, complex interactions between light, nutrients and salinity, indicate trade-offs between these drivers and complicate the drawing of direct parallels between cell traits and competitive dominance. Future freshening in areas of the Baltic Sea may be more beneficial for green algae and diatoms than dinoflagellates, although additional competitive strategies possessed by dinoflagellates may nonetheless facilitate their persistence. A combined understanding of salinity, additional environmental drivers, and how they interact, as well as the full suite of key phytoplankton traits they can modify, will help to model phytoplankton communities in future marine ecosystems including the Baltic Sea. They will also enable us to predict important consequences of these changes for pelagic food webs and cycling of elements in the world’s oceans.

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9 Appendix

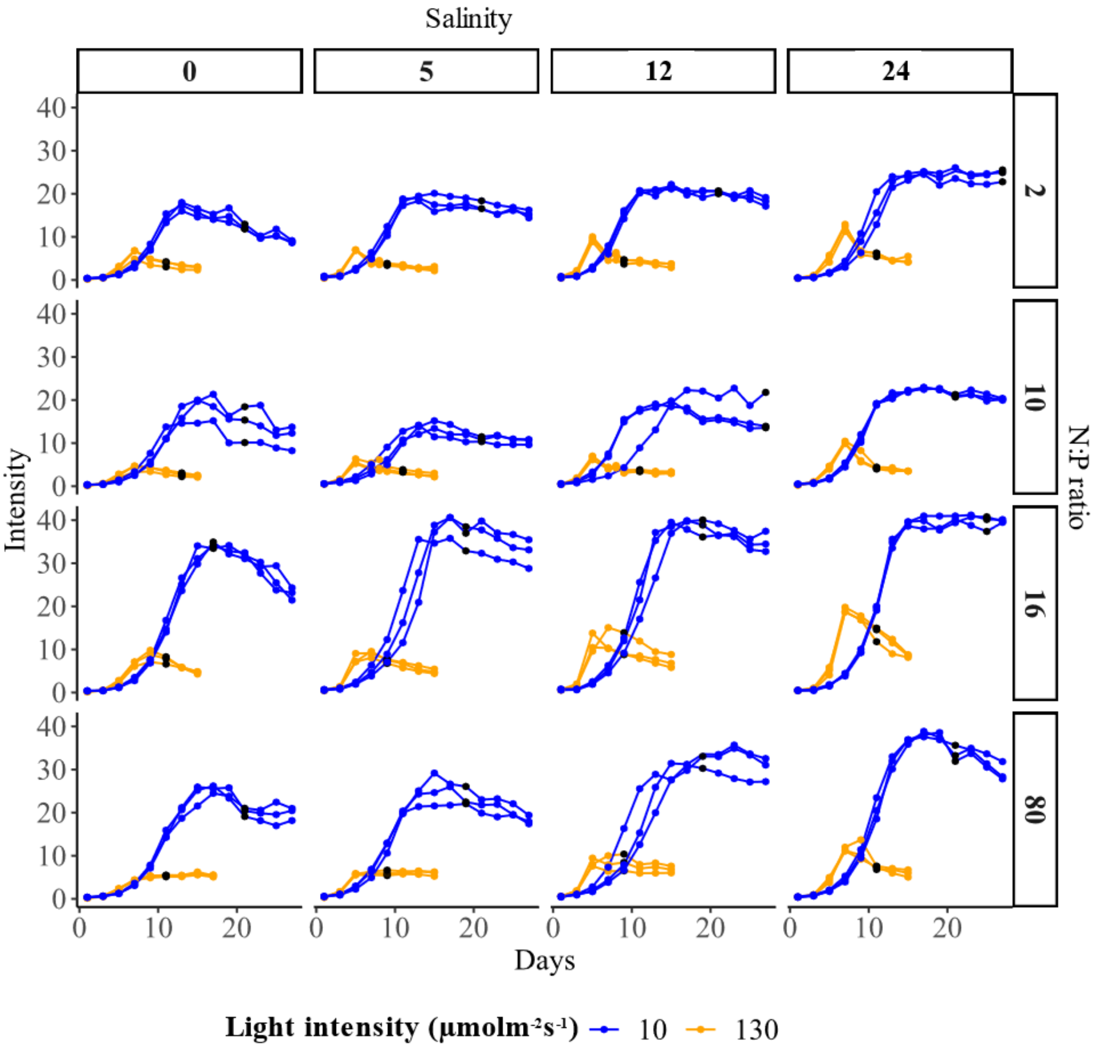

Figure S1 Fluorescence intensity at 480 nm of samples for the duration of the experiment. Black dot = day of experimental group termination.

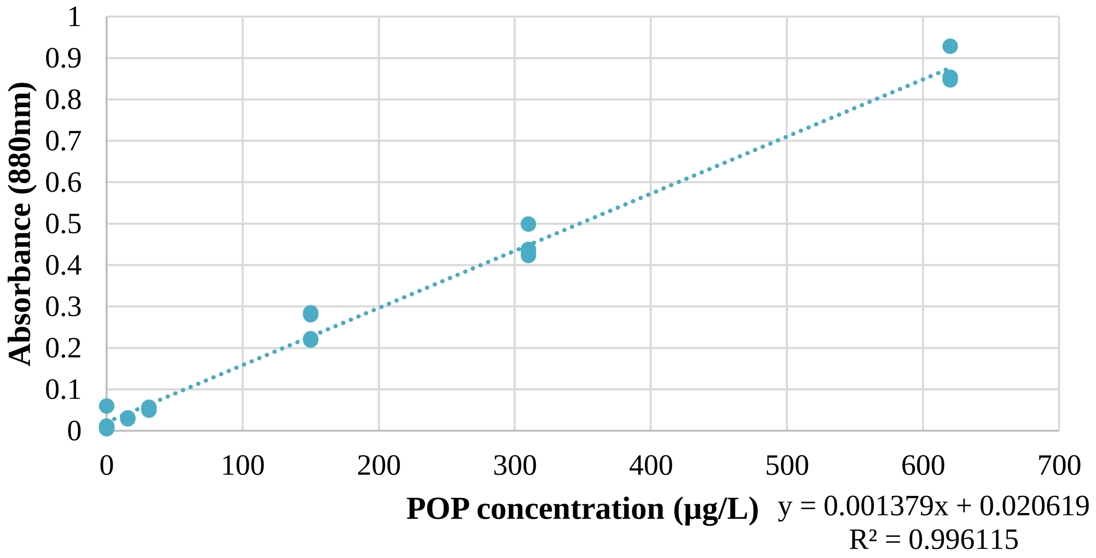

Figure S2 Particulate organic phosphorus (POP) standards calibration curve

Table S1 Full three-way ANOVA results for growth rate

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
light	1	1.12450	1.12450	1268.5323	< 2.2e-16 ***
sal_f	3	0.04926	0.01642	18.5237	9.221e-09 ***
NP	3	0.03334	0.01111	12.5354	1.518e-06 ***
light:sal_f	3	0.06549	0.02183	24.6279	1.032e-10 ***
light:NP	3	0.01255	0.00418	4.7203	0.00488 **
sal_f:NP	9	0.03858	0.00429	4.8363	6.156e-05 ***
light:sal_f:NP	9	0.04100	0.00456	5.1392	3.100e-05 ***
Residuals	64	0.05673	0.00089		
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1					

Table S2 Full three-way ANOVA results for Pmax

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
light	1	8013.8	8013.8	4097.1112	< 2.2e-16 ***
sal_f	3	861.2	287.1	146.7600	< 2.2e-16 ***
NP	3	2267.7	755.9	386.4576 <	2.2e-16 ***
light:sal_f	3	11.8	3.9	2.0062	0.121940
light:NP	3	905.4	301.8	154.3049	< 2.2e-16 ***
sal_f:NP	9	63.7	7.1	3.6161	0.001078 **
light:sal_f:NP	9	132.3	14.7	7.5163	2.061e-07 ***
Residuals	64	125.2	2.0		
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1					

Table S3 Full three-way ANOVA results for chlorophyll *a* concentration

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
light	1	832726	832726	1059.5221	< 2.2e-16 ***
sal_f	3	15709	5236	6.6624	0.0005508 ***
NP	3	501287	167096	212.6050	< 2.2e-16 ***
light:sal_f	3	7921	2640	3.3596	0.0240715 *
light:NP	3	97397	32466	41.3077	5.668e-15 ***
sal_f:NP	9	29287	3254	4.1404	0.0003093 ***
light:sal_f:NP	9	28151	3128	3.9797	0.0004522 ***
Residuals	64	50300	786		
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1					

Table S4 Full two-way ANOVA results for growth rate under high light

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
sal_f	3	0.107406	0.035802	24.8698	1.698e-08 ***
NP	3	0.036173	0.012058	8.3758	0.0002982 ***
sal_f:NP	9	0.068852	0.007650	5.3142	0.0001893 ***
Residuals	32	0.046067	0.001440		
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1					

Table S5 Full two-way ANOVA results for growth rate under low light

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
sal_f	3	0.0073500	0.0024500	7.3500	0.0006998 ***
NP	3	0.0097167	0.0032389	9.7167	0.0001040 ***
sal_f:NP	9	0.0107333	0.0011926	3.5778	0.0035588 **
Residuals	32	0.0106667	0.0003333		
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1					

Table S6 Full two-way ANOVA results for Pmax under high light

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
sal_f	3	371.35	123.783	118.7644	< 2.2e-16 ***
NP	3	212.10	70.701	67.8341	5.824e-14 ***
sal_f:NP	9	37.40	4.155	3.9868	0.001714 **
Residuals	32	33.35	1.042		
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1					

Table S7 Full two-way ANOVA results for Pmax under low light

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
sal_f	3	501.59	167.20	58.2638	4.615e-13 ***
NP	3	2961.02	987.01	343.9462	< 2.2e-16 ***
sal_f:NP	9	158.57	17.62	6.1398	5.426e-05 ***
Residuals	32	91.83	2.87		
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1					

Table S8 Full two-way ANOVA results for chlorophyll *a* concentration under high light

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
sal_f	3	19628	6542.7	21.2021	9.482e-08 ***
NP	3	80050	26683.2	86.4687	1.955e-15 ***
sal_f:NP	9	7805	867.2	2.8103	0.01493 *
Residuals	32	9875	308.6		
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1					

Table S9 Full two-way ANOVA results for chlorophyll *a* concentration under low light

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
sal_f	3	4002	1334	1.0559	0.381425
NP	3	518634	172878	136.8463	< 2.2e-16 ***
sal_f:NP	9	49633	5515	4.3654	0.000891 ***
Residuals	32	40426	1263		
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1					

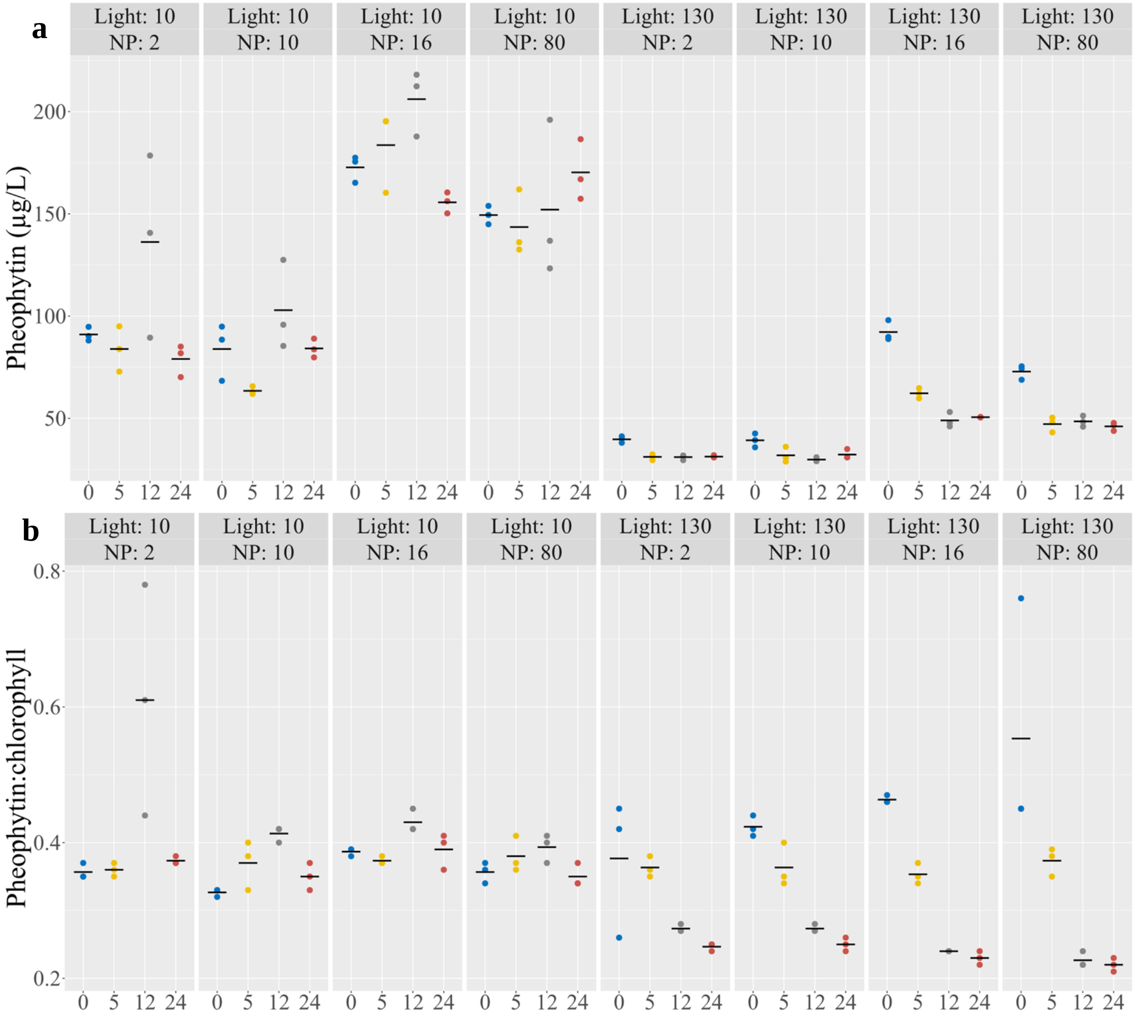

Figure S3 Measured **a.** total pheophytin concentration (µg/L) and **b.** total pheophytin:chlorophyll *a* ratio, for communities at different nutrient (N:P ratio), light (µmol m^{-2}s^{-}1), and salinity (x-axis), levels. Points = individual replicates, black bar = group mean.

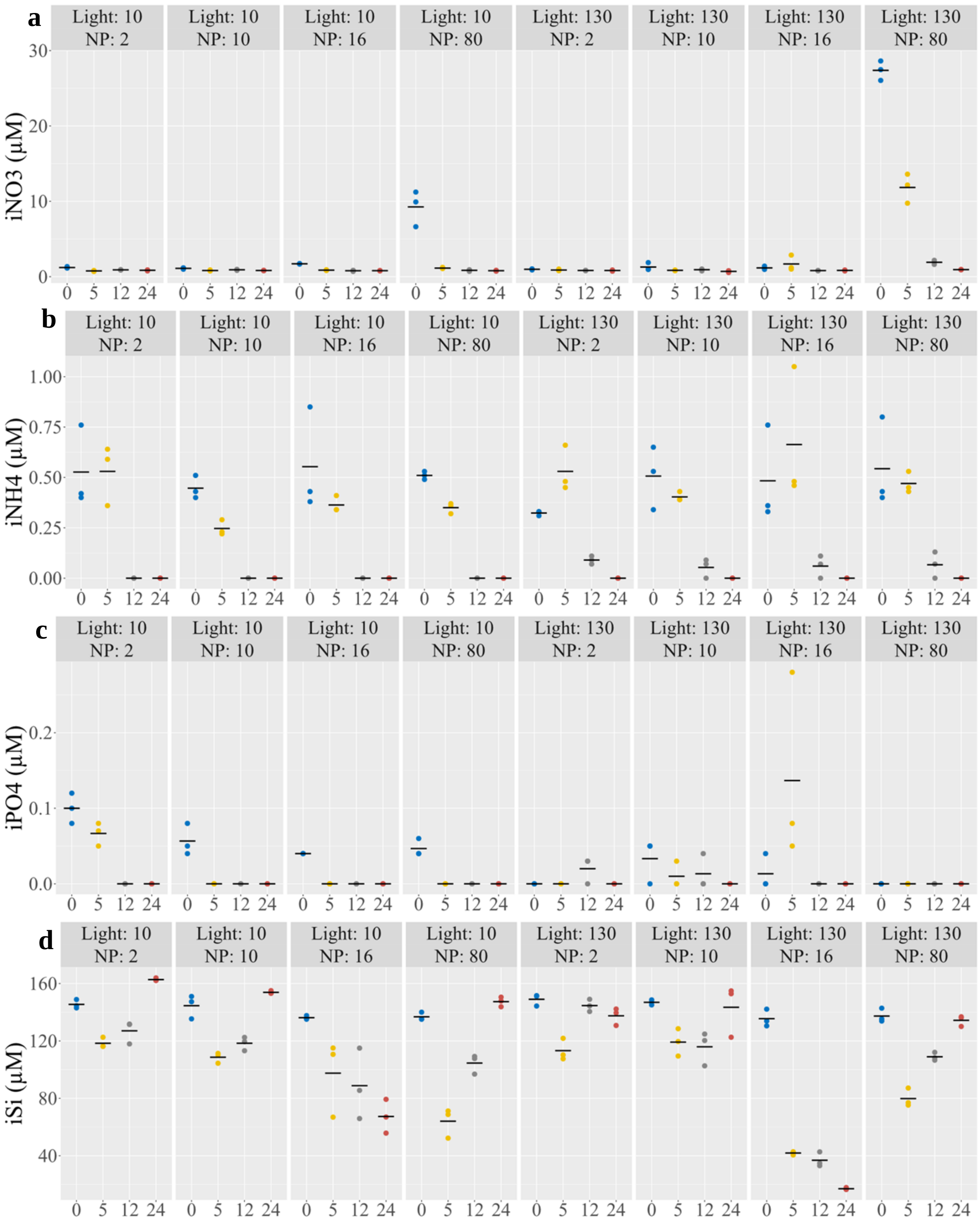

Figure S4 Measured **a.** residual nitrate iNO3 (µmol/L) **b.** residual ammonia iNH4 (µmol/L) **c.** residual phosphate iPO4 (µmol/L) and **d.** residual silica iSi (µmol/L), for communities at different nutrient (N:P ratio), light (µmol $m^{-2}s^{-}1$), and salinity (x-axis), levels. Points = individual replicates, black bar = group mean.

Figure S5 Absolute estimated counts of microalgal groups (a: *Rhodomonas* sp., b: cysts c: *Diatoma tenuis*, d: *Diacronema lutheri*, e: *Monoraphidium* sp., f: *Phaeodactylum tricornutum*, g: *Synechococcus* sp., h: dinoflagellates [Dinoflagellate = unidentified dinoflagellate cell]) for communities at different nutrient (N:P ratio), light (μmol $m^{-2}s^{-}1$), and salinity (x-axes) levels. Taxa are referred to by only genus for convenience.

Table S10 Full three-way ANOVA results for POP

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
light	1	3126	3126	14.0814	0.0003796 ***
sal_f	3	11809	3936	17.7338	1.731e-08 ***
NP	3	361920	120640	543.5143	< 2.2e-16 ***
light:sal_f	3	1479	493	2.2206	0.0942614 .
light:NP	3	455	152	0.6837	0.5652508
sal_f:NP	9	4298	478	2.1515	0.0374539 *
light:sal_f:NP	9	2245	249	1.1240	0.3592947
Residuals	64	14206	222		
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1					

Table S11 Full three-way ANOVA results for POC

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
light	1	507481594	507481594	47.1938	3.147e-09 ***
sal_f	3	4103439522	1367813174	127.2012	< 2.2e-16 ***
NP	3	1037276888	345758963	32.1542	8.505e-13 ***
light:sal_f	3	124362271	41454090	3.8551	0.013386 *
light:NP	3	177734787	59244929	5.5095	0.001982 **
sal_f:NP	9	1139089008	126565445	11.7701	1.106e-10 ***
light:sal_f:NP	9	130480953	14497884	1.3482	0.230639
Residuals	64	688201217	10753144		
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1					

Table S12 Full three-way ANOVA results for PON

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
light	1	1356221	1356221	6.1839	0.01551 *
sal_f	3	2323362	774454	3.5312	0.01963 *
NP	3	7680265	2560088	11.6731	3.393e-06 ***
light:sal_f	3	2423734	807911	3.6838	0.01639 *
light:NP	3	76488	25496	0.1163	0.95027
sal_f:NP	9	4355358	483929	2.2065	0.03284 *
light:sal_f:NP	9	4221442	469049	2.1387	0.03861 *
Residuals	64	14036151	219315		
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1					

Table S13 Full three-way ANOVA results for C:P

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
light	1	255300	255300	28.6578	1.247e-06 ***
sal_f	3	699785	233262	26.1840	3.602e-11 ***
NP	3	1488815	496272	55.7072	< 2.2e-16 ***
light:sal_f	3	74158	24719	2.7748	0.048429 *
light:NP	3	154324	51441	5.7744	0.001471 **
sal_f:NP	9	373647	41516	4.6603	9.214e-05 ***
light:sal_f:NP	9	95401	10600	1.1899	0.316883
Residuals	64	570149	8909		
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1					

Table S14 Full three-way ANOVA results for C:N

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
light	1	249.65	249.647	52.5065	6.872e-10 ***
sal_f	3	582.70	194.234	40.8519	7.147e-15 ***
NP	3	48.56	16.186	3.4043	0.02283 *
light:sal_f	3	23.69	7.898	1.6612	0.18420
light:NP	3	12.55	4.184	0.8800	0.45625
sal_f:NP	9	107.33	11.925	2.5081	0.01586 *
light:sal_f:NP	9	26.07	2.896	0.6091	0.78476
Residuals	64	304.29	4.755		
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1					

Table S15 Full three-way ANOVA results for N:P

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
light	1	22.5	22.5	0.2385	0.62699
sal_f	3	692.3	230.8	2.4444	0.07202 .
NP	3	15816.0	5272.0	55.8405	< 2e-16 ***
light:sal_f	3	631.1	210.4	2.2281	0.09342 .
light:NP	3	75.4	25.1	0.2662	0.84951
sal_f:NP	9	1846.9	205.2	2.1736	0.03553 *
light:sal_f:NP	9	1213.1	134.8	1.4277	0.19525
Residuals	64	6042.3	94.4		
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1					