

**Missing Links –
Experimental Evaluation of Phytoplankton Growth
and Succession**

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Somewhere, something incredible is waiting to be known
Carl Sagan

Für meine Eltern

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General Introduction

As autotrophic primary producers, phytoplankton species are the basis of the oceans' pelagic foodwebs. Despite their small size, they account for a major proportion of global primary production and oxygen (Field et al. 1998, Sommer 1998). On a geological time scale, it was the photosynthesis of phytoplankton that led to atmospheric oxygen allowing the evolution of terrestrial life. During photosynthetic processes, they take up large quantities of the greenhouse gas CO₂ and thus serve as climate buffer.

Planktonic species can not choose their environment actively and are subjected to the hydrographic forces resulting from winds, waves and currents which also affect the availability of resources. Using resources effectively is therefore an important factor for survival and reproduction. Since energy is also a limited resource, it is impossible to optimize adaptation to all potentially limiting factors. Therefore, species have to be either specialists with respect to the utilisation of a resource or generalists that can use many resources but never outcompete a specialist once that resource in particular becomes limiting to more than one species (Sommer 1998).

As stated by Liebig's "Law of the Minimum" (1855), the resource that has the lowest concentration in relationship to an organism's minimal requirements will generally become the limiting factor for growth. For phytoplankton species, resources known to become limiting are the elements nitrogen, phosphorus and, for diatoms, silicate. Apart from macronutrients, trace elements such as iron can also be limiting factors (Kolber et al. 1994, Frost 1996). While in freshwater systems phosphorus generally is regarded as a limiting factor, this role is played by nitrogen in the oceans. These assumptions are simplified however, and there is evidence that in marine systems, phosphorus concentrations can become limiting as well (Wetzel 1983, Sommer 1998, Dittami 2006). Light can also be a limiting resource either by seasonal variations in day length or vertical gradients (Sommer 1998, Litchman 2000), especially in poorly mixed systems (Klausmeier & Litchman 2001).

Under non limiting conditions, species with high growth rates dominate. Once a resource becomes limiting however, they will be replaced by better competitors for

this particular factor, until the one with the lowest requirement persists. Theoretically, this would lead to a coexistence of a maximum number of species as there are limiting resources (Tilman 1982). However, the basis of this theory is the assumption of spatially and temporally stable conditions without changes in abiotic conditions and biotic interactions. Introducing a single other factor, e.g. a predator, could allow for coexistence of two phytoplankton species competing for a single resource. At low resource concentrations, the better competitor will dominate and at high concentrations the grazing resistant species. At intermediate levels, both can coexist or, if the timing of the establishment of the system matters, show founder control, i.e. the outcome of competition depends on the initial abundances of the competitors (Holt et al. 1994, Leibold 1996, Grover & Holt 1998, Klausmeier & Litchman, pers. information). These theoretical approaches are however contrasted by the coexistence of sometimes hundreds of phytoplankton species and strains, a question already raised by Hutchinson (1961). Even extended models including grazers rely on otherwise stable conditions without further disturbance. In nature, this stability is never reached long enough for the complete exclusion of an inferior competitor. Fluctuations can occur on various scales from annual cycles in e.g. temperature, daily changes e.g. in light or small spatial scales e.g. variations in nutrient concentrations caused by a copepod's excretion (Sommer 1998).

As the basis of food webs, phytoplankton cells are heavily grazed upon by many different taxa such as protozoans, crustaceans and even fish. The impact of grazing is especially seen each year in the clear water phase following spring blooms (Bautista et al 1992). Despite their small size, algae are capable of adaptations and defence mechanisms to avoid predation. These include morphological changes such as colony formation (Wiltshire & Lampert 1999), spikes and variations in cell size as well as physiological responses such as higher growth rates (Larsson & Dodson 1993) and the production of toxins (Kirchner et al. 1995, Iannora et al. 1996, Ban et al. 1997, Miralto et al. 1999, Pohnert et al. 2002, Pohnert 2005). It has been argued that there have to be some costs in the development of these defences that might lead to lesser competitive abilities, but they have not yet been found.

Another part of the foodweb playing an important role in algal life are viruses and bacteria. Viruses lead to higher mortalities, but are also recyclers of important micro nutrients such as iron (Brussaard 2004). Bacteria are not only decomposers of

detritus as has been assumed in early publications (Strickland 1965). While nutrient recycling is a very important process, the role of bacteria can also range from competition under nutrient depleted conditions (Guerrini et al. 1998), parasitism (Fukami et al. 1997, Lovejoy et al. 1998) to symbiosis (Stewart et al. 1997, Croft et al. 2005) e.g. by taking up algal exudates (Myklestad et al. 1972, Darley 1977, Nalewajko et al 1980, Bell 1984) and in turn synthesizing vitamins (Donderski & Strzelczyk 1987, Croft et al. 2005) and other growth promoting factors (Stewart et al 1997). These relationships can be close also in spatial terms with bacteria living attached to algae or in the so called “phycosphere” (Bell & Mitchell 1972), the area surrounding a phytoplankton cell. The close coupling between algae and bacteria can also be found in bacterial abundance and community structure (Sapp et al. 2006), as these follow the seasonal patterns of phytoplankton developments (Billen et al. 1990, Gerds et al. 2004).

The North Sea is a marginal sea of the North Atlantic Ocean. The south eastern part, the German Bight, is a shallow basin with an average depth of 20 m. It is a highly dynamic region characterised by spatial and seasonal heterogeneity. The main influences on the oceanographical structure are tides, wind and temperature as well as long-term effects of climate events in the North Atlantic region (Bundesamt für Seeschiffahrt und Hydrographie 2005). Although the cycles of the latter may be slow, they also affect every aspect of abiotic factors and biotic interactions. The influence of the North Atlantic Oscillation (NAO) in particular on meteorological factors such as rainfall, wind speed and direction which in turn modulate hydrographical conditions, can be linked to phytoplankton abundances and shifts in the algal communities (Ueyama & Monger 2005, Breton et al. 2006, Sharples et al. 2006).

At Helgoland, about 60 km off the German coast, mean water temperatures vary between 2 °C in winter and 18°C in summer. Over the past decades, a warming trend has been observed with an increase of the annual mean temperature by 1.13 °C (Wiltshire & Manly 2004). Solar heating and prolonged sunshine hours (up to 17 hrs day⁻¹ during summer) are also responsible for stratification processes. The stability of these layers is also influenced by vertical currents generating shear processes and wind mixing. If stratification occurs, it is therefore found during summer, while in late autumn and winter periods of mixing occur (van Haren & Howarth 2004). The salinity

varies between 23.7 and 34.8, with an average of 32.3 (Helgoland Roads Long-term Data 1991-2001, <http://www.pangaea.de>). Low values are usually measured when freshwater plumes from the rivers Elbe and Weser reach Helgoland. They not only affect the salinity but also nutrient concentrations, as rivers are responsible for a large proportion of nutrient input. Therefore, events of higher nutrient concentrations but lower salinities can be observed. The freshwater input can also be seen in phytoplankton composition when living cells of freshwater species e.g. *Scenedesmus* sp. are found in the samples. The concentrations of the major nutrients follow a seasonal pattern with the highest concentrations in winter and the lowest in late spring after the phytoplankton bloom. The onset of the spring bloom, expressed by the proxy “mean diatom day” usually occurs between the middle of February and the middle of March (Wiltshire & Manly 2004). The bloom is dominated by Bacillariophyceae with important species coming from the genera *Chaetoceros*, *Coscinodiscus*, *Guinardia*, *Odontella*, *Leptocylindrus*, *Rhizosolenia*, *Skeletonema*, *Thalassionema* and *Thalassiosira* (Hoppenrath 2004). They are followed by *Prymnesiophyceae* (e.g. *Phaeocystis* sp.). During summer, the phytoplankton is dominated by dinoflagellate genera such as *Alexandrium*, *Ceratium* and *Dinophysis*. Zooplankton, mainly copepods, follow the seasonal pattern of the diatoms, with herbivorous species dominating during spring, carnivorous grazers during summer followed by species feeding on detritus (Greve et al. 2004).

The backbone of ecological research at Helgoland is the Helgoland Roads Long-term Data Series. Since 1962, samples have been taken work-daily at the “Kabeltonne” location between the main island and the “Düne” (Franke et al. 2004, Fig. 1).



Fig. 1: Location of the “Kabeltonne” sampling site

The physical and chemical parameters measured include temperature, salinity, concentrations of major dissolved inorganic nutrients and secci depth. Phytoplankton samples of the surface water are counted at the species level when possible. Additionally, net samples are inspected quantitatively for an inventory of phytoplankton species (Hoppenrath 2004). In addition to the phytoplankton, the microbial community has also been studied since 1962 (Gerdt et al. 2004). In 1974, zooplankton samples were added to the program (Franke et al. 2004).

During the 40 years of sampling, natural variability has led to background noise and interpreting the data is a complex matter. Changes in personnel, technical advances, revisions, e.g. of an algae's taxonomical position, or mistakes in transferring data from the original counts to electronic systems have left their marks on the data, as well. While the dataset has recently undergone a rigorous quality control with respect to these factors (Wiltshire & Dürselen 2004), a certain amount of scatter remains.

Therefore, it is difficult to analyse the data beyond general trends and patterns because so far no background knowledge of physiological requirements and morphological reactions to abiotic and biotic factors of the phytoplankton species at Helgoland existed.

The difficulties in interpreting the data archived at Helgoland are exemplary for all other in situ data. Phytoplankton species have been important research objects for more than 100 years and many quantitative and qualitative studies from ecosystems of every ocean exist as well as an equal amount of laboratory data. However, comparisons between studies and transferring results from one system to explain the findings in another one are difficult. Even data from the same system might not be entirely comparable because of natural shifts and fluctuations if the sampling occurred at different times as well as differences in sampling techniques, analyses and statistical approaches. Therefore, a set of comparable data of both field studies and laboratory experiments is needed to evaluate the in situ observations in a system. This would also be beneficial for modelling purposes since the accuracy of predictions is directly linked to the precision of the data entered and present models based on e.g. on the data of the Helgoland Roads Series so far require sophisticated methods to reconstruct underlying patterns on a species level (Wirtz & Wiltshire 2005).

Based on the Long-term data and starting from the observation of the spring bloom 2004, the aim of this thesis was to investigate the factors and mechanisms that regulate phytoplankton succession and interactions with other trophic levels. Key species were isolated and studied in laboratory experiments to assess their basic demands regarding nutrients and temperature, their reactions to physical stress, simulated grazer presence and the role of bacteria in their ecology. Several uni- and multivariate statistical methods were used to find underlying patterns, common traits and significant differences. Comparability of the data was ensured by using freshly isolated algae and, in one experiment, water from the spring bloom 2004. The results were then compared to the existing data to see whether they could explain the natural succession.

In Chapter 2, the observations and analyses of the spring bloom 2004 with regards to the succession of six key species are presented. Correlation matrices, regression trees and redundancy analyses were used to identify the relative importance of abiotic and biotic factors on abundance, cell size, colony size and photosynthetic efficiency.

Chapter 3 focuses on the influence of the physical stress of turbulence and chemical signals from grazers on growth rates and chain lengths of two key species, *Thalassiosira rotula* and *Thalassionema nitzschioides*. The effects of two different but commonly used media on the results of the experiments were also tested as well as differences between clones in the case of *T. rotula*.

In Chapter 4, long-term developments in batch cultures over eight weeks were followed for seven phytoplankton species. Abiotic parameters measured were the nutrients NO_2^- , NO_3^- , PO_4^{3-} and SiO_4^{2-} , surface tension and pH. The development of the algal population was assessed by counting living, degrading and dead cells, measuring photosynthetic efficiency, cell sizes and chain length. Additional data on bacterial abundances was used to investigate possible interactions.

In Chapter 5, data on the basic nutrient requirements, the effect of temperature and the influence of simulated grazer presence on growth rates, cell size and photosynthetic efficiency of three diatom key species are presented. The results of these experiments were compared to the Helgoland Roads Long-term Series and the observations from the spring bloom 2004.

Chapter 6 presents a discussion of the results and their relevance for the understanding of the phytoplankton succession at Helgoland.

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A Plankton Puzzle: Observations during a Spring Bloom Event

In this study, we present observations from the spring bloom 2004 at Helgoland Roads. Temperature, salinity, Secci-depth and nutrients were measured and phytoplankton cell counts were made as part of the Helgoland Long-term Series. Additionally, cell size, colony size and photosynthetic efficiency of the algae were measured. To obtain an insight into the patterns behind the phytoplankton succession, these results were combined with zooplankton data from samples taken at the same time and large scale hydrodynamic and meteorological data. For pattern recognition, correlations, classification trees and RDA were used. The results show the water temperature to be the overall most important factor behind community parameters such as diversity but also for the abundance of most species studied. Apart from common algal patterns such as the relationship with temperature, classification trees and RDA revealed many, sometimes subtle, different responses to factors like wind, currents, nutrients and grazers.

Introduction

Food webs represent complex trophic interactions in communities influenced by numerous parameters on various spatial and temporal scales. These range from global cycles like seasonality and its changes in e.g. temperature and day length, medium scaled influences like meteorological developments and currents to local parameters as for example salinity and nutrient concentrations (Edwards et al. 2002, Smetacek et al. 2002, Beaugrand 2004). While each species reacts differently to those parameters, they also mediate interactions between trophic levels (Beaugrand & Reid 2003). While this allows for flexibility of food webs and explains the differences observed between years, it further complicates research on the pathways of matter and energy flow as well as other interactions of trophic levels, e.g. grazing pressure provoking defence mechanisms. Therefore, analysing patterns, interactions and abiotic parameters encompassing several trophic levels with reasonably robust results beyond the general trends remains difficult. Hence, many studies focus either on one or two aspects of food web structure and the influence of abiotic parameters or describe trends in ecology. Taking a look at zooplankton and meteorological and hydrological factors, there are for example studies on the relationship of mesozooplankton abundance in relation to temperature, salinity and wind velocity (e.g. Paffenhöfer & Flagg 2002, Józefczuk et al. 2003), feeding performance of polychaete larvae with regards to temperature and water viscosity (Bolton & Havenhand 2005), the survival of copepod nauplii in connection with temperature and salinity (Chinnery & Williams 2004) and reports on the role of currents for the distribution of fish larvae (Pepin & Helbig 1997, Proctor et al. 1998, Helbig & Pepin 2002). On the phytoplankton side, there are also many publications investigating the effects of chemical and hydrological parameters on phytoplankton species and communities (e.g. Joordens et al 2001, Marty et al. 2002, Mei et al. 2002) but also papers, where the impact of grazing is investigated alongside abiotic impacts. Studies are conducted on small scales, e.g. investigating the effect of turbulence and interactions with predators on the abundance of *C. tripos* (Havskum et al. 2005) or on foodwebs and ecosystem levels. Studies considering large scale influences are less abundant but rising in numbers. In 2002, Philips et al. investigated the factors controlling phytoplankton abundance in a lagoon choosing nutrient limitation, salinity, light availability, temperature, wind, hydraulic flushing and grazing as driving influences. In Arhonditsis et al. (2004), the patterns of succession were analysed

using nutrient concentrations, solar radiation, air and water temperature, wind speed, cladoceran and total zooplankton biomass as well as large-scale climatic processes as the El-Niño Southern Oscillation and the Pacific Decadal Oscillation. Climatic effects were also taken into account by Hansen et al. 2005, describing the primary production on the Faroe Shelf and its transmission to higher trophic levels. In all of these cases, climatic and hydrological parameters had great influence on the food webs.

Because of the Helgoland Roads Long-term Series, the plankton community at Helgoland is well documented. Alongside phytoplankton counts to species level, secchi-depth, temperature, salinity and nutrients have been measured work-daily since 1962. In 1974, zooplankton counts were added. While this time series provides an excellent background for studies on general trends like global warming and its effects on phytoplankton community composition, the dynamics of spring bloom events and interactions within the plankton community are still difficult to extract and predictions on future developments (Greve et al. 2004, Wiltshire & Manly 2004) as well as modelling has begun just recently (Wirtz & Wiltshire 2005).

In this study, meteorological and hydrological as well as additional morphological and physiological parameters of important phytoplankton species were combined with the regular Helgoland Roads series counts and data on zooplankton groups to put the results into a broader ecological context. In addition to finding correlations between two variables, new multivariate approaches were used to find patterns that might help to explain the observed succession.

Material und Methods

Plankton samples

Sampling for the Helgoland Roads Long-Term Series has been carried out work-daily since 1962. Surface water samples are taken at 54° 11.3' N / 007° 54' E known as the "Kabeltonne" location. For the Helgoland Foodweb Spring Bloom Campaign in 2004, zooplankton samples were taken on Tuesdays and Thursdays using a 150 µm net with an opening of 0.023 m² for vertical hauls of mesozooplankton and a 500 µm CalCOFI net for macrozooplankton. The water body sampled using the mesozooplankton-net was determined with the help of a HYDRO-BIOS Flowmeter (Model 438, HYDRO-BIOS Apparatebau GmbH, Kiel, Germany) using the following equation:

Volume (L) = $n \times 0.3 \times m^2$ (opening of the net) $\times 1000$

With n = Δ rotation speed of the flowmeter

0.3 = factor for calculating the distance of the haul

m^2 = area of the opening

Samples were fixed with 4% Borax-buffered Formaldehyde. In the laboratory, they were washed on a 40 μm sieve with filtered seawater and then transferred to a Bogorov counting chamber. Details on the procedure can be found in Laakmann (2004).

Physical and chemical parameters

The temperature was measured on site immediately after taking the sample. For salinity analysis, a GDT Autosol salinometer (GAT Gamma Analysentechnik GmbH, Bremerhaven, Germany) was used. The nutrients NH_4^+ , NO_2^- , NO_3^- , SiO_4^{2-} and PO_4^{3-} were measured photometrically using the methods described by Grasshoff & Johannsen (1974) and Grasshoff et al. (1999).

Phytoplankton cell counts and biovolume measurements

The surface water sub-samples for phytoplankton were fixed with Lugol's solution and, depending on the overall phytoplankton concentration; either 25 or 50 ml of the sample were filled into Utermöhl settling chambers and allowed to settle for 12 or 24 hours respectively. After settling, samples were counted under an inverted microscope (Table 2.1). The measurements for cell sizes were taken from the same sample. To calculate the cell volume, the formulas given in Hillebrand et al (1999) were used.

Table 2.1: Procedure for phytoplankton counts.

Magnification	Counts
50x	- all species > 60 μm - whole chamber - count dominant species in a max of 4 tracks and a minimum of 50 individuals
100x	- all species between 30 μm and < 60 μm - whole chamber - count dominant species in a max of 4 tracks and a minimum of 50 individuals

Table 2.1 continued

200x	<ul style="list-style-type: none"> - all species between 12,5 µm and < 30µm - count all species in tracks - count a min. of 50 individuals of the dominant species. - max. of 4 tracks even if no species reaches 50 individuals
400x	<ul style="list-style-type: none"> - all species below 12,5 µm - count all species in tracks - count a min. of 50 individuals of the dominant species - max. 2 tracks even if no species reaches 50 individuals

Photosynthetic quantum yield

To determine the effective quantum yield of photochemical energy conversion the PAM (Pulse Amplitude Modulation) method of fluorescence measurement was applied. Since an energy conversion of 100% is impossible, a small fraction of the light energy absorbed by a cell has to be released again usually as chlorophyll a fluorescence originating in Photosystem II. These fluorescence emissions are highest when the yield of photochemistry and heat dissipation, the two other pathways of de-excitation, are lowest. Therefore, fluorescence yields reflect directly the efficiency of a plant's photosynthetic performance. To determine the quantum yield of Photosystem II the first initial fluorescence (F_0) is induced by low levels of actinic light after a period of dark adaptation. When the emission reaches a stable level, a strong pulse of light (Saturation Pulse) is applied. This leads to a quick reduction of the electron transport chain between the two photosystems, forcing the cells to emit all energy as fluorescence. This maximum fluorescence value (F_m) is recorded and the effective quantum yield calculated from $F = \frac{(F_m - F_0)}{F_m}$ for dark adapted and

$$F' = \frac{(F'_m - F'_0)}{F'_m} \quad \text{for previously light exposed samples (Genty et al. 1989).}$$

Fluorescence-based measurements of photosynthetic efficiency are non-invasive and allow the assessment of the photosynthetic electron transport in real time since the relationship between quantum yield and electron transport rates is linear over a wide range of quantum flux densities (Schreiber et al. 1995, Heinz Walz GmbH 1999).

One of the factors influencing photosynthetic efficiency is nutrient concentration. Under nutrient-replete conditions, the maximum quantum yield or energy efficiency for microalgae is between 0.6 and 0.7 (Falkowski & Kolber 1995, D. Hanelt, pers. communication). It was hypothesized by Falkowski & Kolber (1995) that nutrient

limitation in general affects plastid protein synthesis leading to a reduction in functional PSII units. A common mechanism of damage to the reaction centers by an excess of light harvesting over energy demand under such conditions was proposed by Geider et al. (1993). Nitrogen limitation for example interferes with the efficiency of electron transportation from the antenna complexes to the reaction centers (Kolber et al. 1988, Falkowski et al. 1989) and reduces RUBISCO as well as the PSII reaction center core protein D1 (Geider et al 1993). Losses of photochemical quenching were also reported for iron limitations (Greene et al. 1991, Greene et al. 1992) while the effects of phosphorus depletions are less pronounced (Geider et al. 1993). However, those effects are reversible and responses shown in increases of quantum yield are reported to range within minutes to hours (Geider et al. 1993, Lippemeier et al. 1999) Other factors influencing the quantum yield are reactions to physical stress e.g. temperature fluctuations (El-Sabaawi & Harrison 2006, Robison & Warner 2006) which also affect structure and functionality of PS II reaction centers.

The overall community quantum yield was measured using a Xenon-PAM (Heinz Walz GmbH, Effeltrich, Germany). It uses a Xe-flash light emitting a broad spectrum of light that can be adjusted to the desired wavelength by optical filters, in this case a SP 695 (short-pass filter, Schott) to avoid interfering longer wavelengths. Samples were dark-adapted for five minutes, then transferred into a 10x10 mm quartz cuvette with a volume of 3 ml and the fluorescence yield measured. To measure the photosynthetic efficiency of single cells, a MicroPAM, a PAMControl unit attached to a microscope, was used (Heinz Walz GmbH, Effeltrich, Germany). In non-limited phytoplankton the F -values amount to 0.6 to 0.7 [arbitrary units] (Kromkamp and Peene 1999)

Meteorological and hydrological parameters

The weather data was provided by the DWD (Deutscher Wetterdienst, German Meteorological Service) operating a survey station at Helgoland. Data on currents was taken from the BSH (Bundesamt für Seeschifffahrt und Hydrographie) reports.

Statistical analysis

To test for significant relationships, correlation matrices were run in STATISTICA 7 (StatSoft Inc. Tulsa, Oklahoma, USA). For overall relationships of biotic and abiotic parameters, RDAs (Redundancy Analysis, Brodgar, Highland Statistics Ltd.,

Newburgh, UK) were used, an analysis based on Principal Component Analysis (PCA) but allowing for calculating directly the effects of independent variables on dependent ones. Before the analyses, the datasets were examined for outliers and square root or logarithmic transformations were applied when necessary. The physical and chemical parameters used as independent factors in the analysis were tested for autocorrelations calculating the variance inflation factors. High VIF values indicate autocorrelation of variables and therefore redundant information (Zuur et al. 2007). Independent factors with values higher than 10 were removed and the VIF calculation repeated until all values met the criterion. In a first run of the RDAs, all factors were included in the analyses. Then partial RDAs and partial correlations with water temperature as covariable were performed to filter out seasonal effects. To obtain insights on the importance and mutual influences of variables, classification trees (PS-Explore, VGSPS mbH, Neustadt/Wied, Germany) were applied to the data set. The purpose of this type of analysis is to determine a set of *if-then* logical (split) conditions that permit accurate prediction or classification of cases (Statsoft Inc, 2006). In contrast to the PCA and RDA, there is no differentiation between independent and dependent variables. Since the sign of a correlation does not affect the results of the analyses, they were performed only once.

Results

Meteorological, oceanographical and chemical parameters

Air and water temperatures started to rise from mean values at about 3 °C (air) and 4 °C (water) at the mid of March, with the highest values being 11.9 °C (air) and 10.8 °C (water) at the end of the observation in May. As shown by correlations, classification trees and a PCA of abiotic parameters, the air temperature was mostly influenced by the date ($p = 0.000$), relative humidity and hours of sunshine (Fig. 2.3 & 2.4). The water temperature (Fig. 2.5) followed closely but with less fluctuation. The relative humidity varied between 59 % and 98 %, being mostly influenced by cloud cover and negatively correlated to the air pressure and sunshine hours.

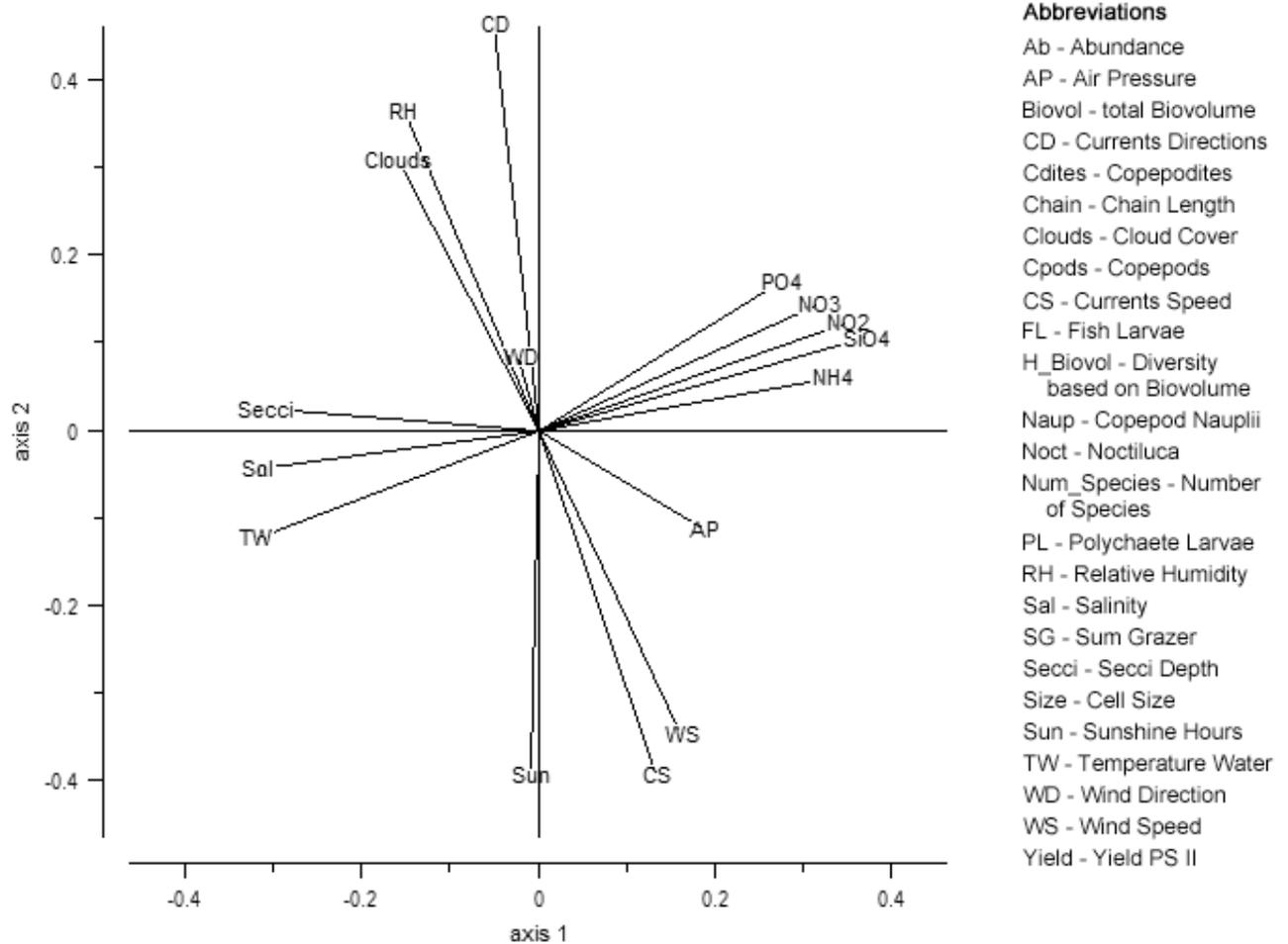


Fig. 2.3: Principal Component Analysis of the measured abiotic parameters. The longer a line, the better it is represented by an axis. Correlations are indicated by the angles between variables. Small angles show positive correlations, 90° are neutral relations and angles above 90° indicate negative relationships.

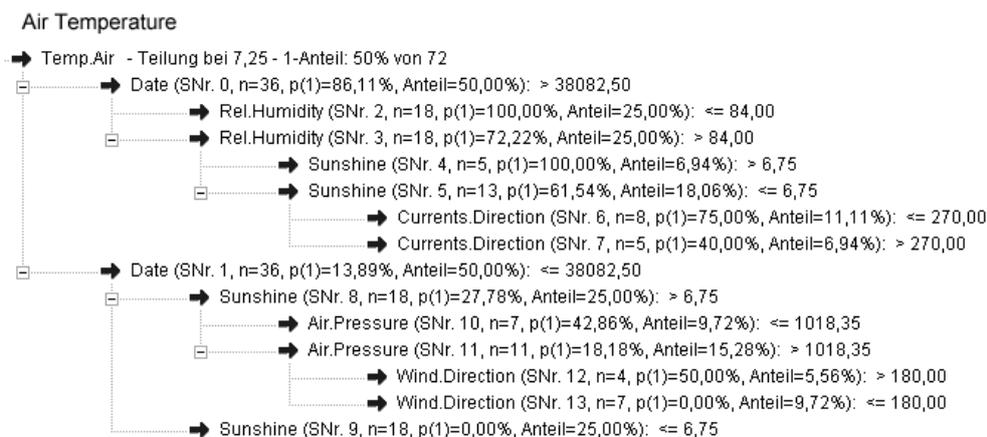


Fig. 2.4: Classification tree for air temperature. SNr: segment number, n: number of cases in a segment, p(1): probability that cases of this segment have an abundance > 0, Anteil: percentage of the whole data set, Fehlwertklasse: variable with missing values.

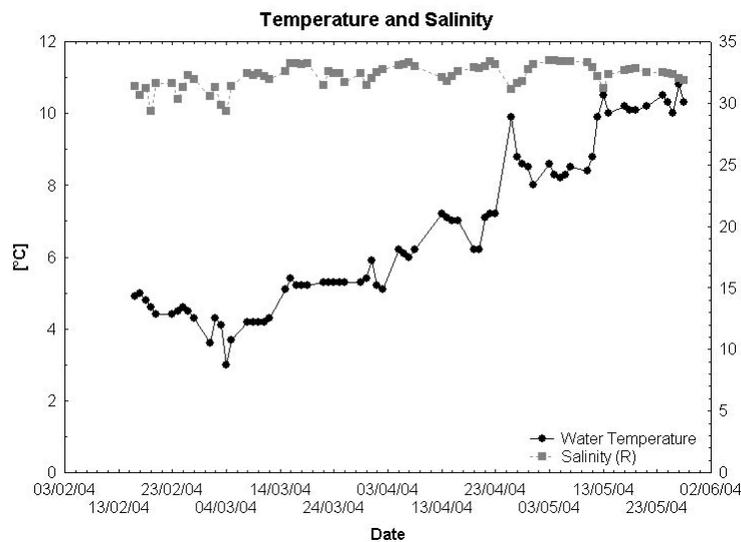


Fig. 2.5: Development of water temperature and salinity at Helgoland Roads 2004.
<http://www.pangaea.de>

For air pressure, values between 987.2 hPa and 1034.5 hPa were observed with most cases between 1015 hPa and 1035 hPa, indicating a majority of high pressure conditions. According to the correlation trees (Fig. 2.6), relative humidity, sunshine hours, air temperature and air pressure were most influential on the wind speed and wind directions. Throughout the observations, wind speed varied mostly between 15.5 km h^{-1} and 44 km h^{-1} (3 Bft and 6 Bft), coming from all directions (Fig. 2.7).

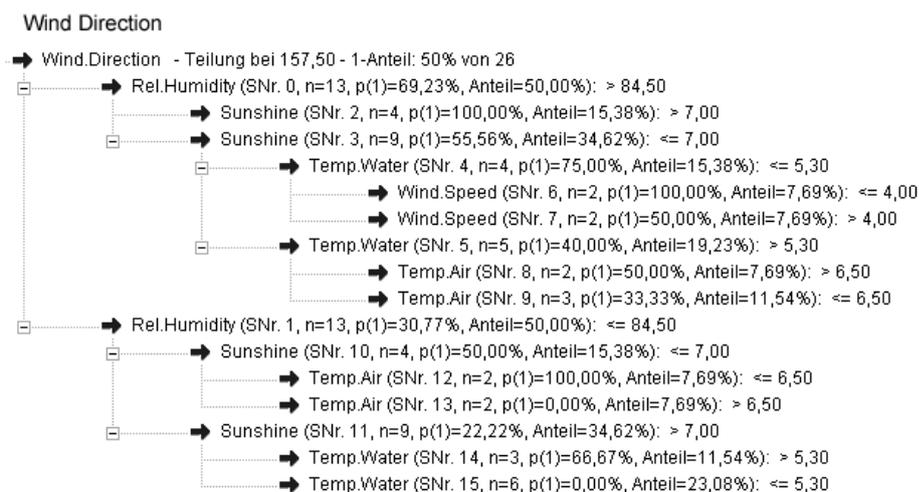


Fig. 2.6: Classification trees for wind direction and wind speed. SNr: segment number, n: number of cases in a segment, p(1): probability that cases of this segment have an abundance > 0, Anteil: percentage of the whole data set, Fehlwertklasse: variable with missing values.

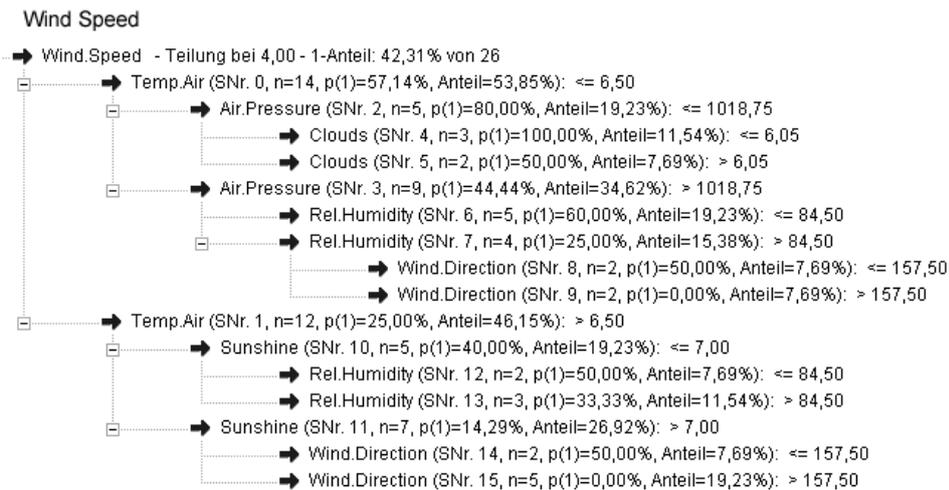
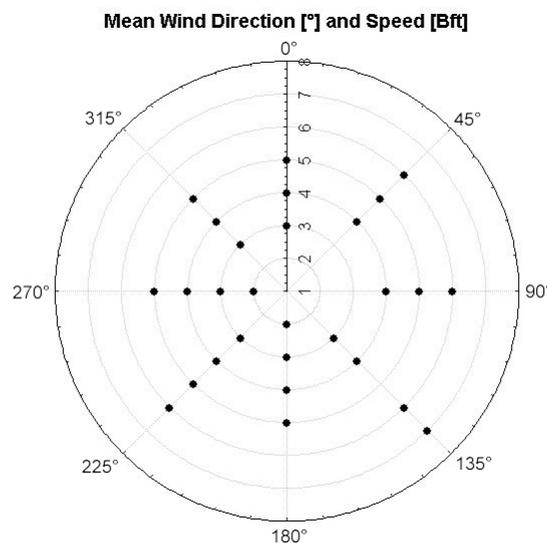


Fig. 2.6: continued

Fig. 2.7: Mean wind direction and speed. <http://www.dwd.de>

The combination of wind direction and speed directly influenced currents in the area. Over the course of the study, no eastern currents could be observed and of all other directions, western currents dominated the situation (Fig. 2.8). The speed of currents was between 0.043 km h^{-1} and 0.72 km h^{-1} (0.8 knts and 1.3 knts). For the further interpretations of the relationships between wind, currents, biotic and abiotic parameters the reversed nomenclature of directions has to be kept in mind. Wind directions are defined by the direction the wind is coming from, currents by the directions they are flowing to. A numerically negative correlation between the directions therefore actually indicates a positive relationship.

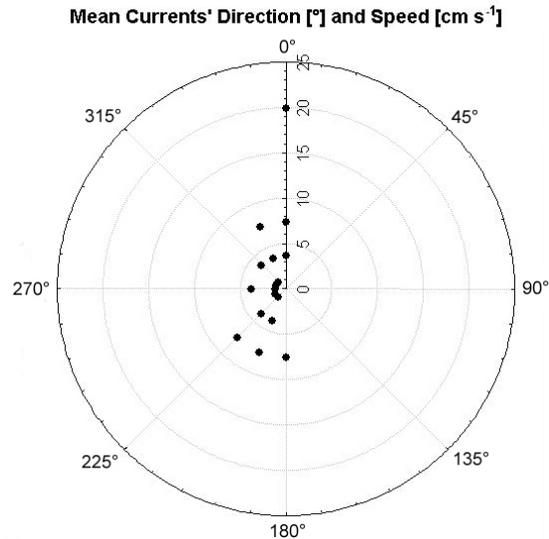


Fig. 2.8: Mean currents, direction and speed. <http://www.bsh.de>

Apart from a few freshwater input events, the salinity varied between 31.8 and 33.5 (Fig. 2.5). With the lowest values measured during February and the first week of March, it was also positively correlated with time ($p = 0.005$).

All measured major nutrients, NH_4^+ , NO_2^- , NO_3^- , SiO_4^{2-} and PO_4^{3-} declined over the course of the observations (Fig. 2.9). Regarding physical parameters, all nutrient concentrations were influenced by air pressure as well as speed and directions of wind and currents (Fig. 2.10). They were also significantly negatively correlated with general biological parameters as for example the number of algal species ($0.000 \leq p \leq 0.042$) and the total algal biovolume (for nitrate $p = 0.021$ and silicate $p = 0.024$).

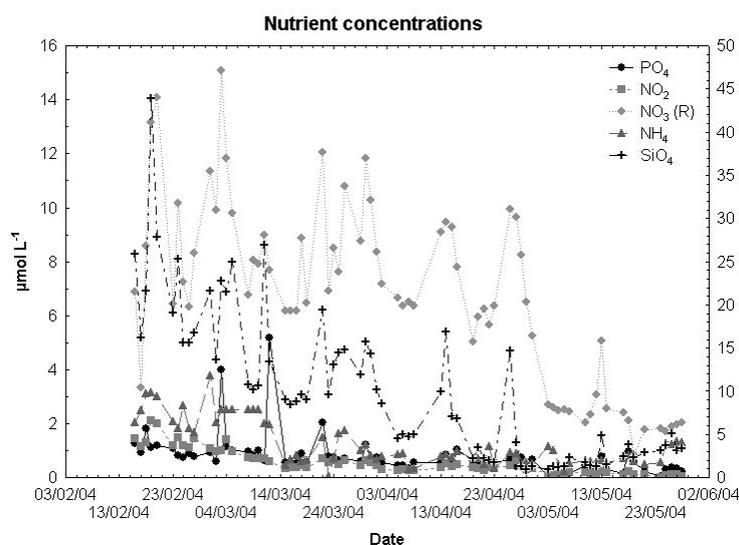
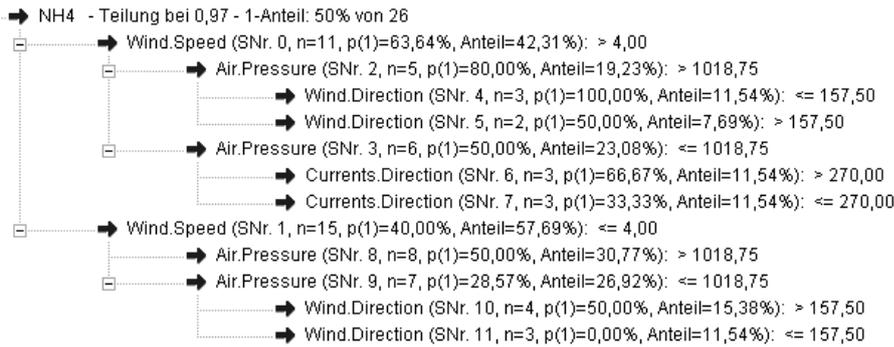
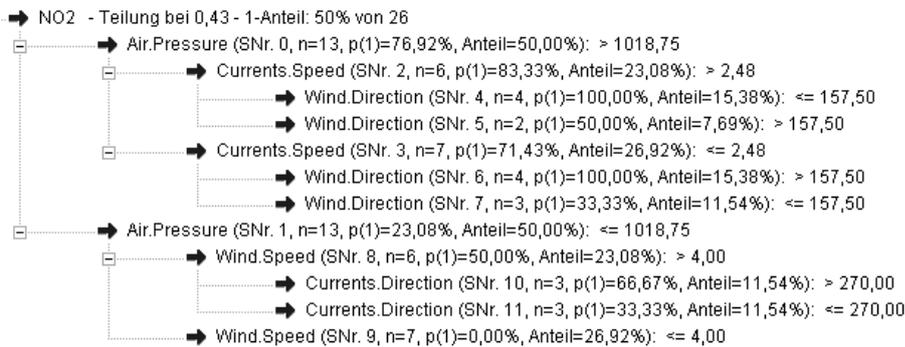


Fig. 2.9: Nutrient concentrations. (R): concentrations are noted on the right y - axis <http://www.pangaea.de>

Ammonium Concentration



Nitrite Concentration



Nitrate Concentration

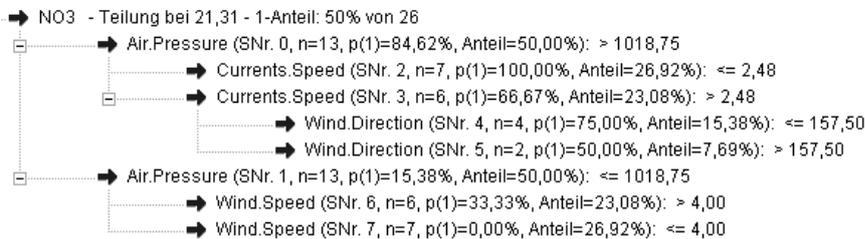


Fig. 2.10: Classification trees for nutrient concentrations. SNr: segment number, n: number of cases in a segment, p(1): probability that cases of this segment have an abundance > 0, Anteil: percentage of the whole data set, Fehlwertklasse: variable with missing values.

This was confirmed by the full RDA analysis (Fig. 2.12) In the partial correlations and the partial RDA (Fig 2.12) that removed seasonal effects by using water temperature as covariable, most nutrient concentrations became positively correlated with total algal biovolume and the number of phytoplankton species.

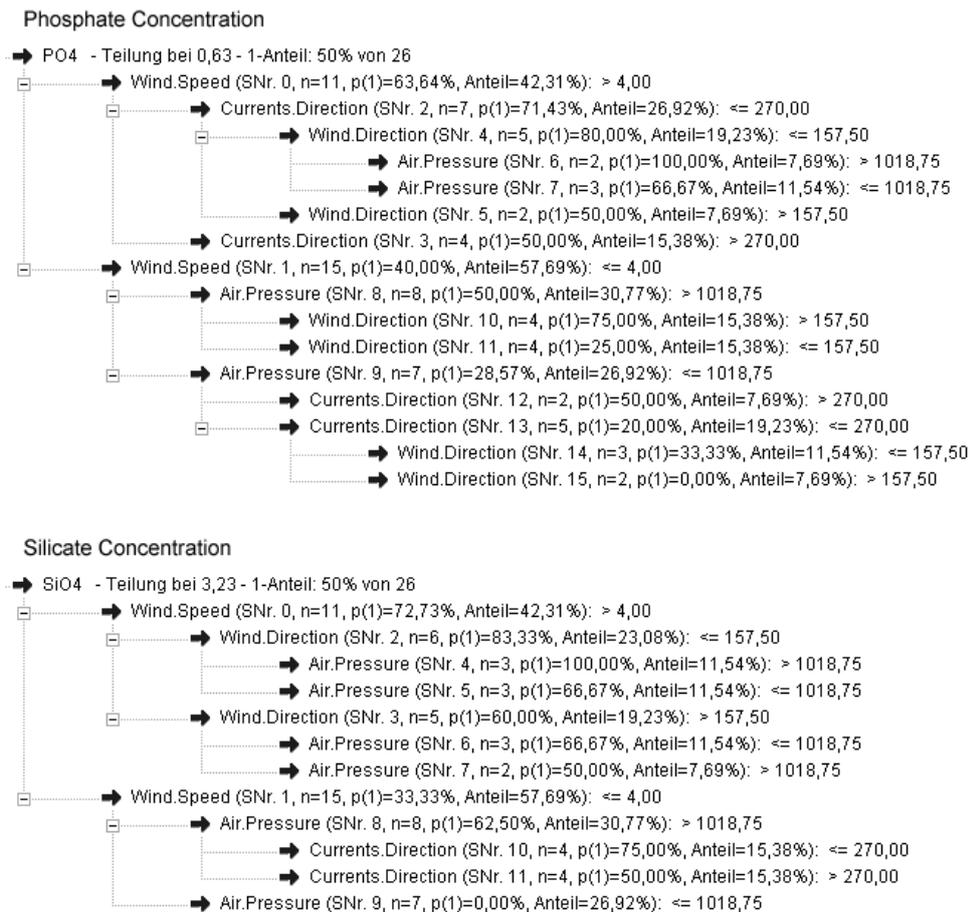


Fig. 2.10: continued

Zooplankton

Copepod nauplii, copepodites and adults were observed over the whole course of the study with numbers peaking in mid April (Fig. 2.11). Nauplii showed no significant correlations at all with any of the measured parameters or plankton community data like e.g. diversity or algal abundance. The general plankton community (Fig. 2.12) RDA analysis indicated negative correlations with wind and currents' speed as well as a positive relationship with salinity and the direction of wind and currents. The phytoplankton community parameters total biovolume and number of species were put at neutral angles while algal diversity (based on biovolume) showed a slightly positive correlation in the partial RDA. The classification tree (Fig. 2.13) identified wind speed, currents' directions as well as algal diversity and number of species as most influential parameters but correlations were only weak ($0.197 \leq p \leq 0.976$). According to the correlation tree (Fig. 2.13), the abundance of copepodites was mostly influenced by nauplii abundance, algal diversity and the direction of currents,

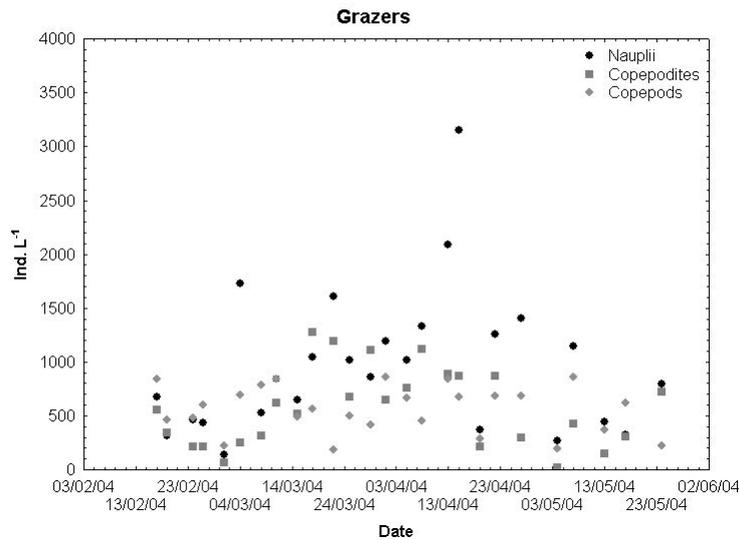


Fig. 2.11: Abundances of copepod nauplii, copepodites and copepods.

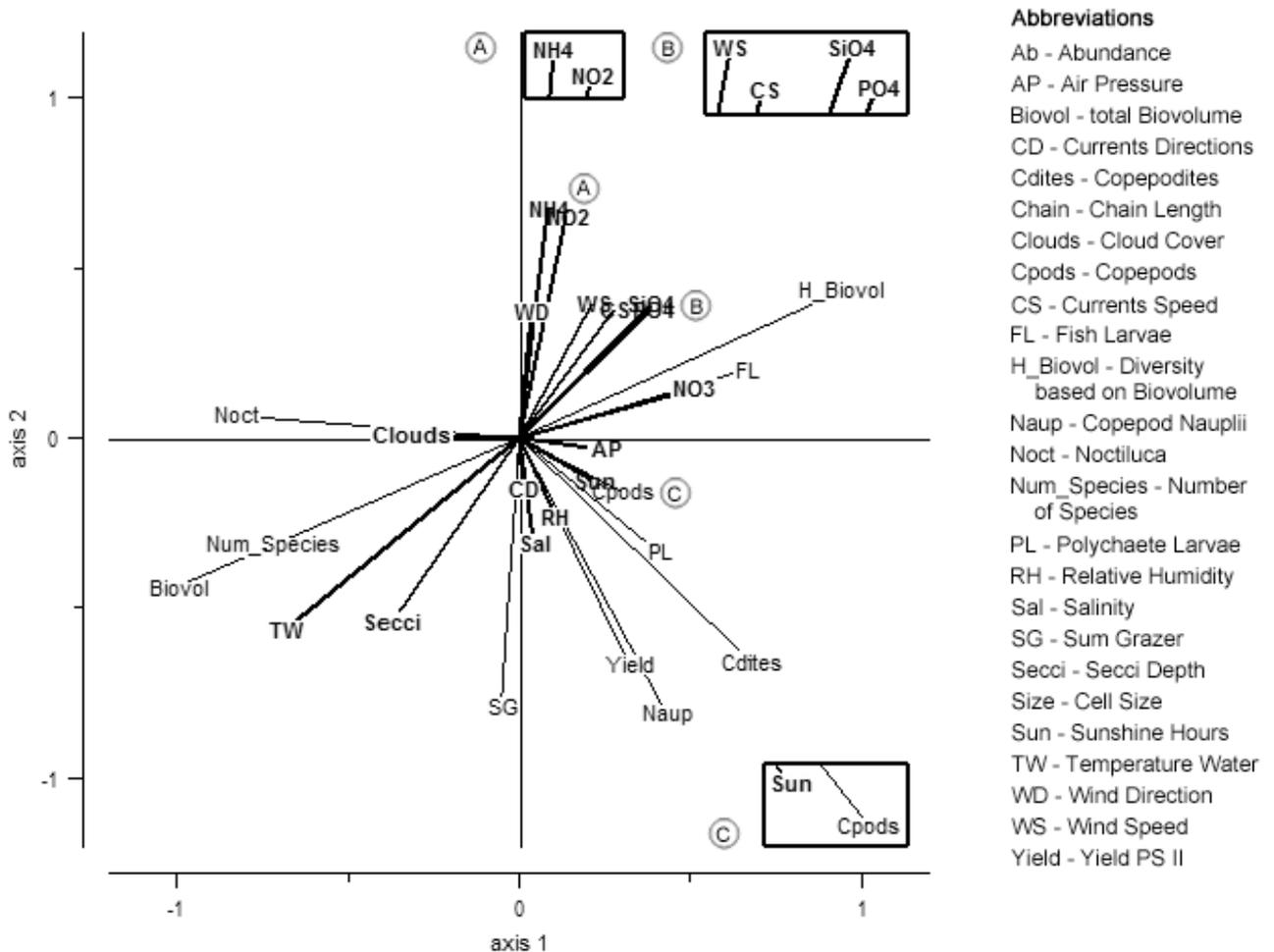


Fig. 2.12: RDA triplots of the plankton community data. Full RDA (upper) and partial RDA (lower plot). Thick lines and bold labels represent independent variables, thin lines dependent variables. The longer a line, the better it is represented by an axis. Correlations are indicated by the angles between variables. Small angles show positive correlations, 90° are neutral relations and angles above 90° indicate negative relationships.

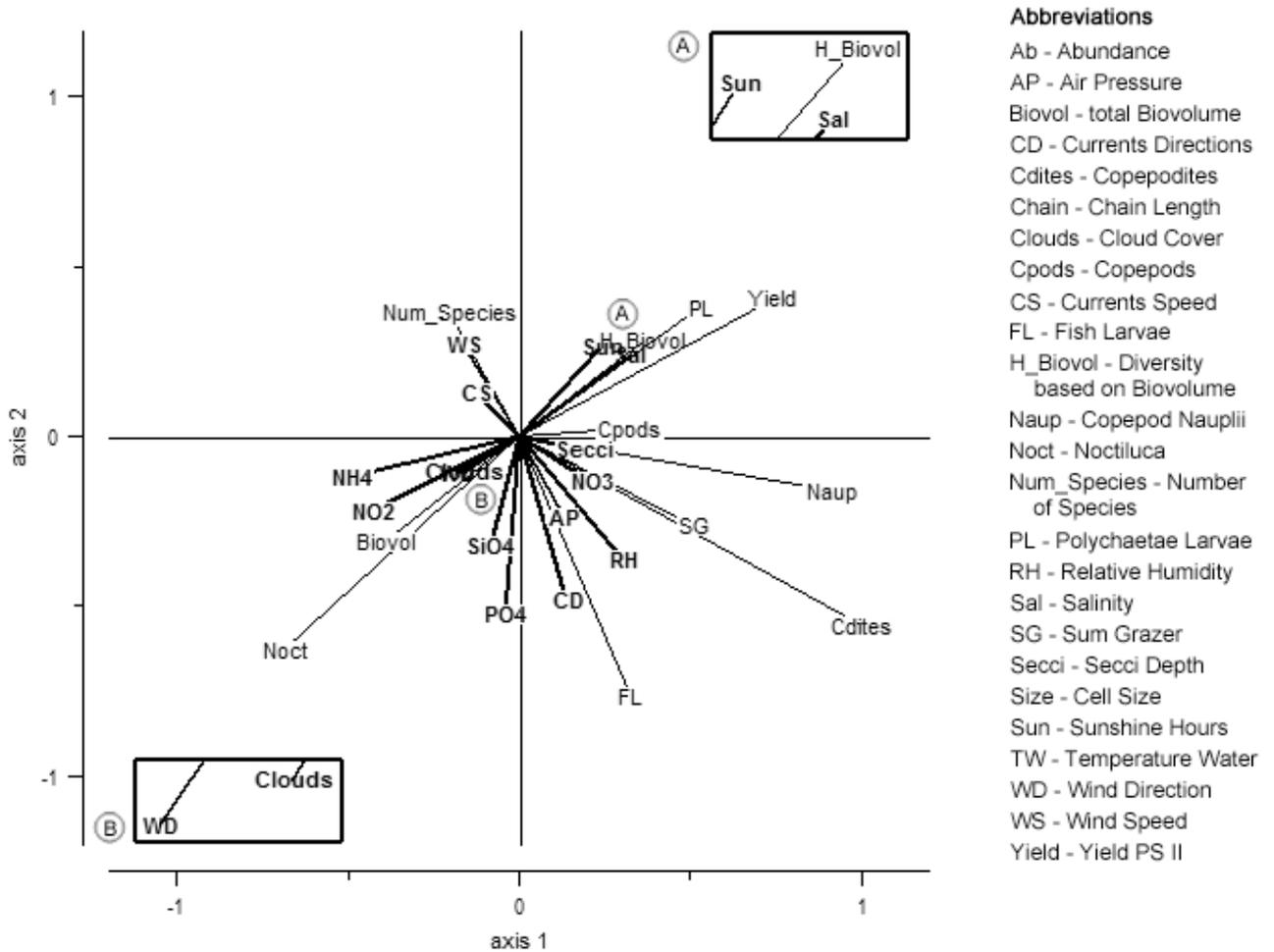


Fig. 2.12 continued

copepodite abundance and the number of algal species was a negative one. Phytoplankton diversity was slightly positively correlated and currents directions, fish larvae and nauplii showed a clearly positive relationship

In the classification tree analysis (Fig. 2.13), the abundance of copepods was highly influenced by phytoplankton diversity and abundance of fish larvae as well as copepod nauplii and the direction of currents. The correlation matrix showed the relationships with diversity, nauplii and currents directions to be positive while fish larvae were negatively correlated. Only the correlation with nauplii was found to be significant with $p = 0.034$. The partial RDA (Fig. 2.12) also detected strong positive correlations with algal diversity, nauplii and currents directions but placed fish larvae on a positive angle, too.

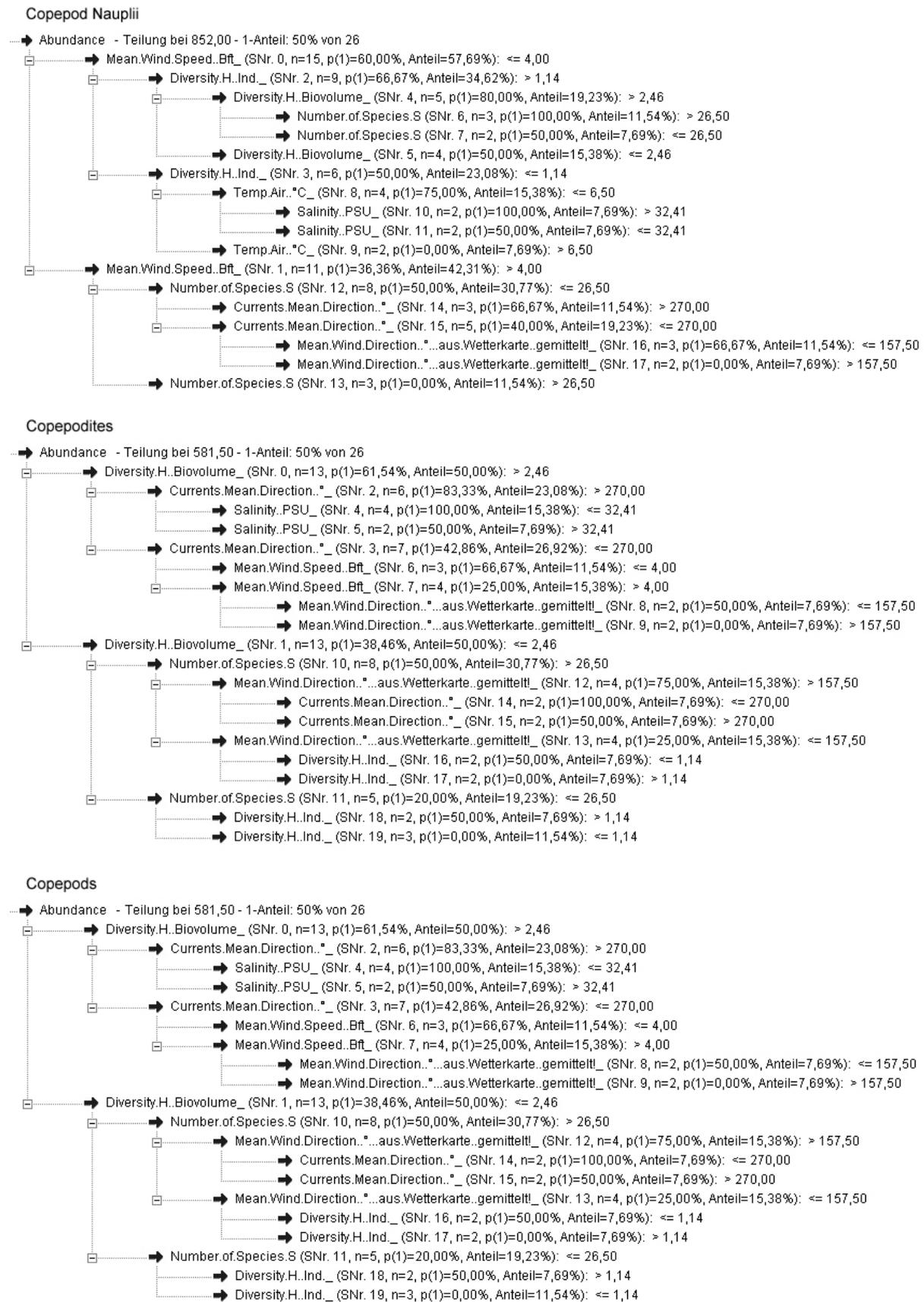


Fig. 2.13: Classification trees copepod life stages. SNr: segment number, n: number of cases in a segment, p(1): probability that cases of this segment have an abundance > 0, Anteil: percentage of the whole data set, Fehlwertklasse: variable with missing values.

The abundance of *N. scintillans* (Fig. 2.14) was found to be mainly influenced by wind speed and, for more than 4 Bft, by the number of algal species and their diversity. For lower wind speeds, the directions of the currents were most influential and, depending on their values, either wind directions or the phytoplankton's number of species and diversity (Fig. 2.15). Of these relationships, the number of phytoplankton species, currents directions and *N. scintillans* abundances were positively correlated. The relationships with algal diversity, wind speed and wind directions were negative ones. Only the correlation with phytoplankton diversity was found to be significant ($p = 0.008$). In addition to those parameters, the correlation matrix identified total algal biovolume as a significantly positive relationship ($p = 0.001$). The partial RDA (Fig. 2.12) generally confirmed these patterns.

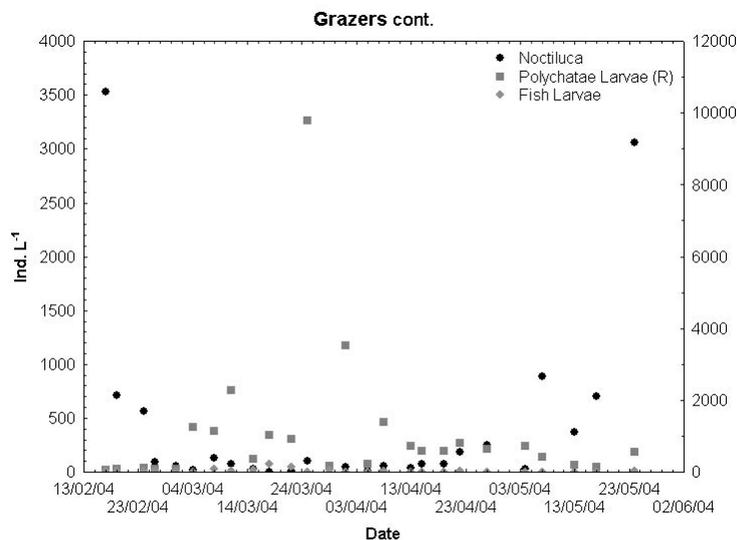


Fig. 2.14: Abundances of *N. scintillans scintillans*, polychaete and fish larvae.

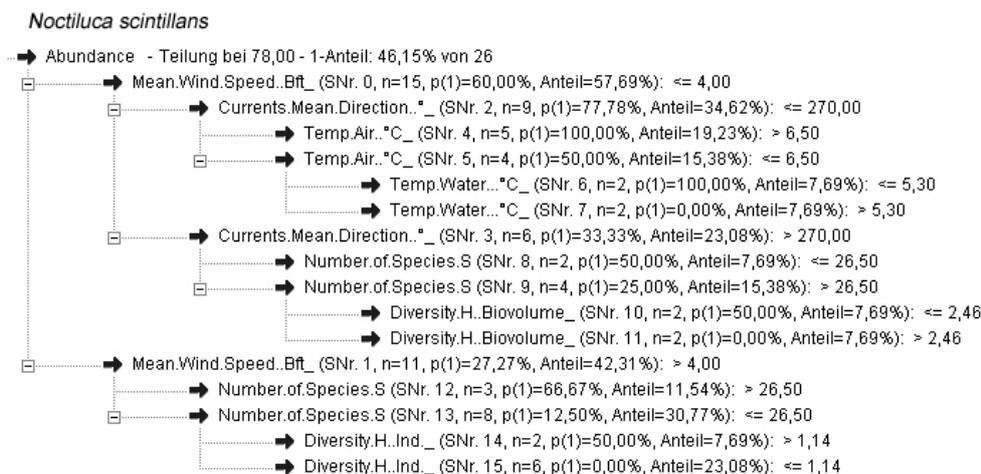


Fig. 2.15: Classification tree for *N. scintillans*. SNr: segment number, n: number of cases in a segment, p(1): probability that cases of this segment have an abundance > 0, Anteil: percentage of the whole data set, Fehlwertklasse: variable with missing values.

The abundance of polychaete larvae (Fig. 2.14) was most influenced by phytoplankton diversity, number of species as well as the directions of winds and currents (Fig. 2.16). The partial RDA (Fig. 2.12) also found a strong positive link with diversity while the other factors were placed at neutral angles. Only wind speed was found to be negatively correlated. While the correlation matrix generally found the same positive or negative relationships, none were classified as being significant.

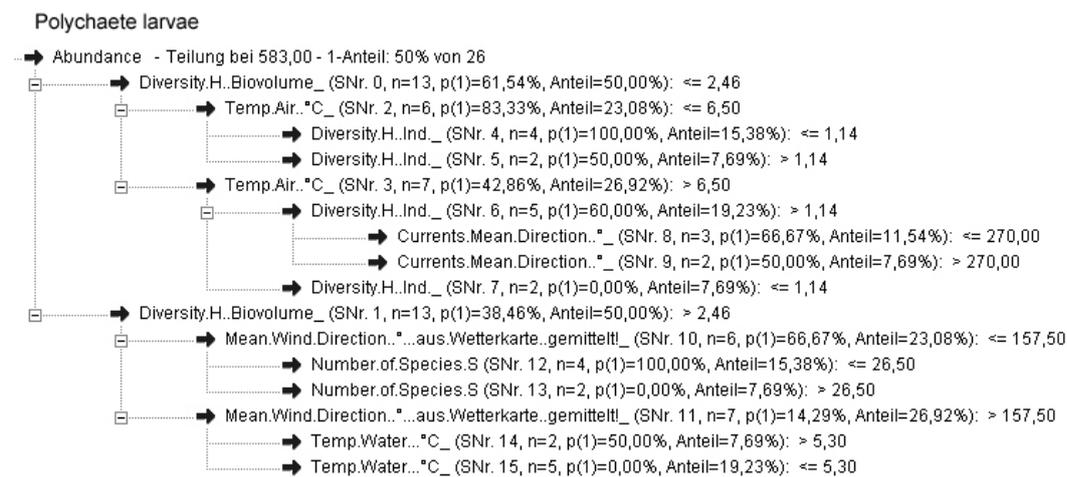


Fig. 2.16: Classification tree for polychaete larvae. SNr: segment number, n: number of cases in a segment, p(1): probability that cases of this segment have an abundance > 0, Anteil: percentage of the whole data set, Fehlwertklasse: variable with missing values.

According to the classification tree, the abundance of fish larvae (Fig. 2.14) depended mostly on the water temperature. For temperatures below 5.3 °C, a threshold reached at the end of March, the direction of the currents and the abundance of copepod nauplii had the most influence. For temperatures above 5.3 °C, the diversity of the phytoplankton community and then the current's direction explained best the abundance of fish larvae (Fig. 2.17). RDAs (Fig. 2.12) and correlations showed that the relationship with temperature was an overall negative one (significant with $p = 0.021$). Algal diversity and the abundance of the larvae were positively related in the correlation matrix and the full RDA while the partial RDA indicated a slightly negative angle. Although the correlation with the overall phytoplankton abundance was not significant, a significantly negative correlation was observed with the number of algal species ($p = 0.014$). Both the full and the partial RDA confirmed this negative relationship. The RDAs also indicated a positive correlation with the abundance of copepods as well as a positive relationship with

copepodites. The link with copepodites was found to be significant ($p = 0.008$) in the correlation analysis.

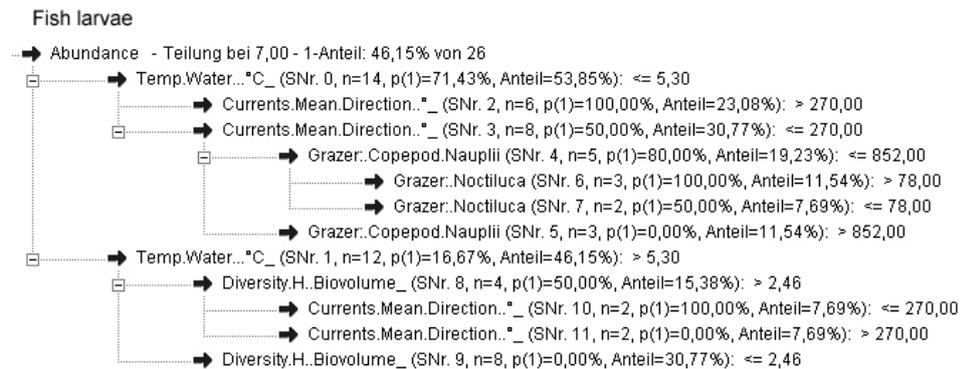


Fig. 2.17: Classification tree for fish larvae. SNr: segment number, n: number of cases in a segment, p(1): probability that cases of this segment have an abundance > 0, Anteil: percentage of the whole data set, Fehlwertklasse: variable with missing values.

General phytoplankton community parameters

The classification tree (Fig. 2. 18) found the number of algal species to be highly influenced by the water temperature, a significantly positive interaction as found by correlation analysis ($p = 0.0000$) and the RDA (Fig. 2.12). Below a threshold of 5.3 °C, the abundance of polychaete larvae and cloud cover were chosen to explain the number of species. Above 5.3 °C, the speed of the currents fitted best. However, none of these factors was found to be significant in correlations. In the RDA triplot, polychaete larvae were put at a neutral angle while cloud cover was classified as positive influence and the current's speed as a negative one. The correlation matrix also found significantly negative relationships with all measured nutrients ($0.0025 \leq p \leq 0.0421$) and the number of fish larvae ($p = 0.0258$). The RDA also classified all of those correlations as negative.

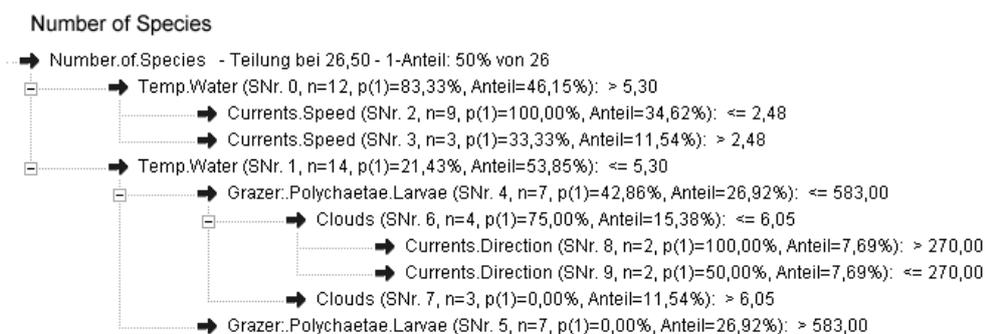


Fig. 2.18: Classification tree for the number of phytoplankton species. SNr: segment number, n: number of cases in a segment, p(1): probability that cases of this segment have an abundance > 0, Anteil: percentage of the whole data set, Fehlwertklasse: variable with missing values.

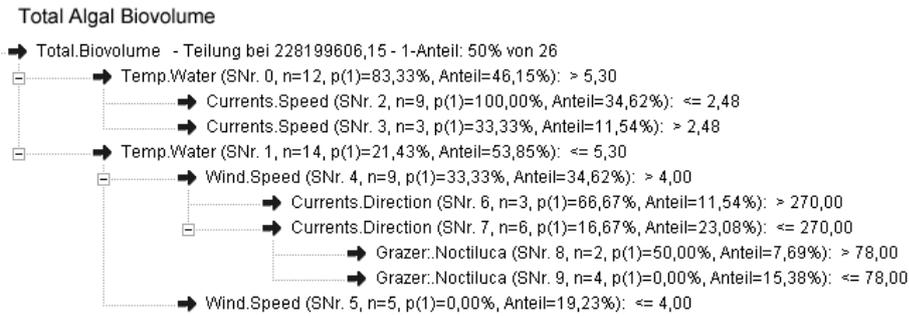


Fig. 2.19: Classification tree for total algal biovolume. SNr: segment number, n: number of cases in a segment, p(1): probability that cases of this segment have an abundance > 0, Anteil: percentage of the whole data set, Fehlwertklasse: variable with missing values.

The total algal biovolume was also mainly influenced by the water temperature, a significantly positive relationship ($p = 0.0000$). The classification tree (Fig. 2.19) also identified the speed of winds and currents as important factors but those influences were not significant. The RDA (Fig. 2.12) also showed a strong positive relationship between the total biovolume and the water temperature. Wind speed and currents speed were evaluated as having a slightly negative correlation. All nutrients showed a negative correlation with total algal biovolume but only the relationship with nitrate was identified as being significant by the correlation matrix ($p = 0.021$). The RDA also classified all nutrients as having negative correlations with phytoplankton biovolume. With the exception of the abundance of *N. scintillans* cells, copepod nauplii and the sum of grazer abundance, the RDA identified all grazer types as having a negative relationship with the total algal biovolume which was also confirmed by the correlations. But with the exception of *N. scintillans* ($p = 0.001$), none of them were significant.

The diversity (Fig. 2.20) calculated on the basis of biovolumes was mostly influenced by grazers (Fig. 2.21). The most important abiotic factors were nitrate concentrations and the speed of currents. In the correlation matrix, the diversity showed a significantly

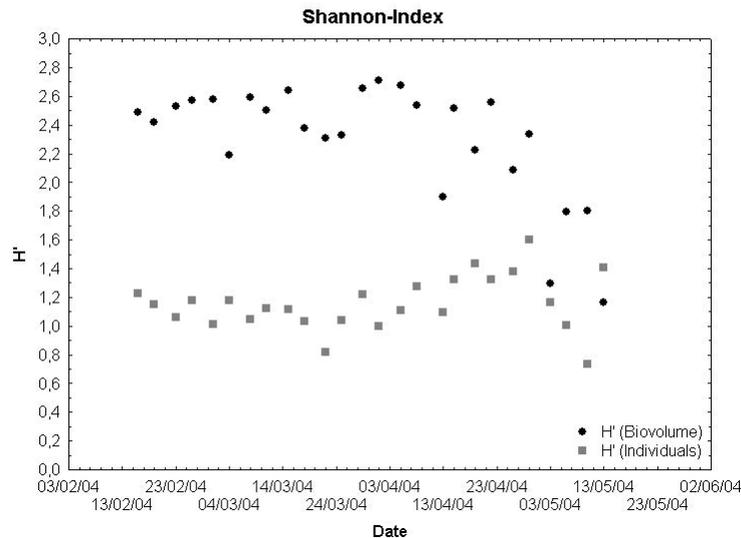


Fig. 2.20: Phytoplankton diversity calculated on the basis of biovolume and number of individuals.

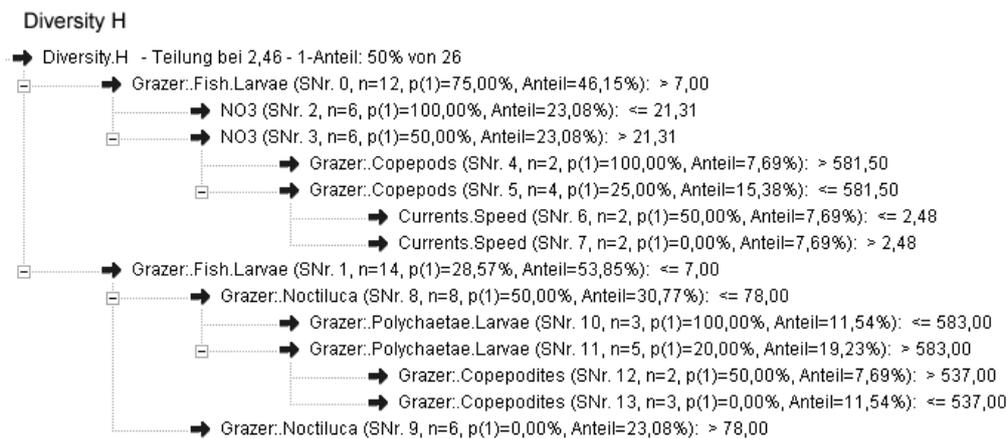


Fig. 2. 21: Classification tree for phytoplankton diversity. SNr: segment number, n: number of cases in a segment, p(1): probability that cases of this segment have an abundance > 0, Anteil: percentage of the whole data set, Fehlwertklasse: variable with missing values.

negative correlation with water temperature ($p = 0.000$) and positive ones with nitrate concentrations ($p = 0.032$) and the abundance of *N. scintillans* ($p = 0.008$). The abundances of fish and polychaete larvae were also positively correlated but not on significant levels. The influence of temperature and nitrate was confirmed by the RDA (Fig. 2.12) which also identified strong positive correlations with the number of fish larvae. Slightly weaker positive correlations were shown with the other nutrients and polychaete larvae. The abundances of all copepod groups were shown as slightly positive to neutral influences while *N. scintillans* was classified as being negatively correlated.

General photosynthetic efficiency (Fig. 2.22) as analysed by the classification tree (Fig. 2.23) depended mostly on salinity. For values above 32.4, grazers became most important while below algal diversity and total biovolume had the highest influence. In the correlation matrix, quantum yield showed a significantly positive correlation with salinity ($p = 0.0129$) but was significantly negatively correlated with most nutrients ($0.0020 \leq p \leq 0.0177$). Grazers had no significant impact as well as the slightly negative correlation with total algal biovolume and the positive one with diversity. The RDA confirmed the relation between quantum yield and salinity and also identified a negative relationship with nutrient concentrations. With the exception of *N. scintillans*, grazers were classified as having a positive influence. The partial RDA also found the negative impact of total algal biovolume and the positive correlation with diversity while the full RDA put both factors on neutral angles.

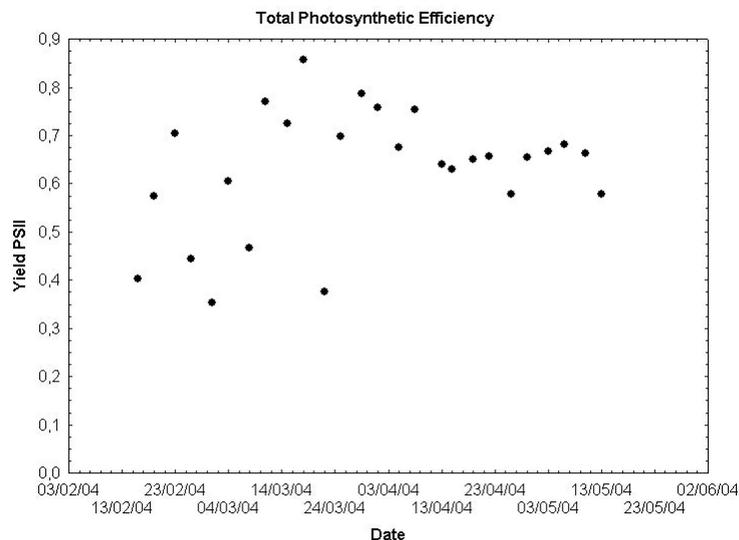


Fig. 2.22: Total photosynthetic efficiency of the phytoplankton community measured by a Xe-PAM.

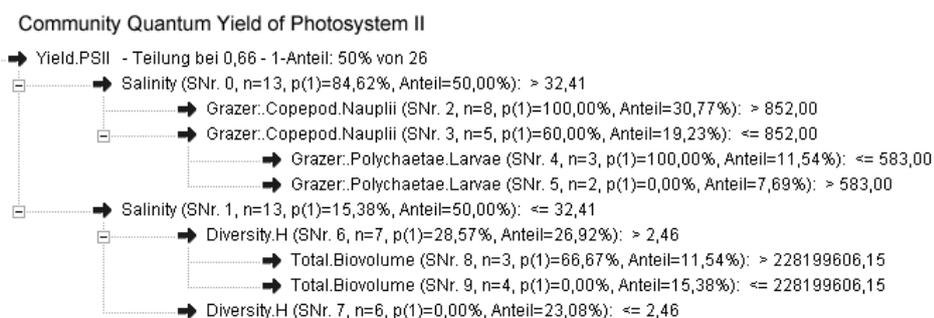


Fig. 2. 23: Classification tree for the community quantum yield. SNr: segment number, n: number of cases in a segment, p(1): probability that cases of this segment have an abundance > 0, Anteil: percentage of the whole data set, Fehlwertklasse: variable with missing values.

Phytoplankton species

Guinardia delicatula

Cells of this species were observed from the beginning of April on. Numbers rose very quickly peaking in the middle of May with a maximum of 283740 cells L⁻¹ (Fig. 2.24). According to the classification tree (Fig. 2.25), water temperature had the highest influence on the abundance. For water temperatures below 6 °C, nitrite concentrations controlled abundances while for higher temperatures, nitrate concentrations and weather conditions were of importance. The correlation matrix classified water temperature as a significantly positive correlation ($p = 0.000$) while nitrite and nitrate were significantly negatively correlated with abundances ($p = 0.000$). Phosphate, ammonium and silicate were also negatively correlated ($0.000 \leq p \leq 0.004$). In the partial correlation with temperature as covariable nitrite and ammonium became positive relationships. Other significant correlations found by the full correlation matrix were positive relationships with cloud cover ($p = 0.002$) and salinity ($p = 0.042$) while a negative correlation with air pressure was observed ($p = 0.018$). Of the grazer groups, copepod nauplii, *N. scintillans*, the larvae of fish and polychaete as well as the total grazer abundance showed positive correlations while copepodites and copepods were negatively correlated. None of those correlations were significant. The RDA (Fig. 2.26) confirmed the strong positive relationship of *G. delicatula*'s abundance with water temperature and also found the positive correlations with cloud cover as well as the negative one with air pressure. It also placed all nutrients at angles indicating negative correlations. Of the grazers, Noctiluca and the total grazer abundance were classified as being positively correlated while all other grazers were found to have a negative influence. The partial RDA found the same patterns.

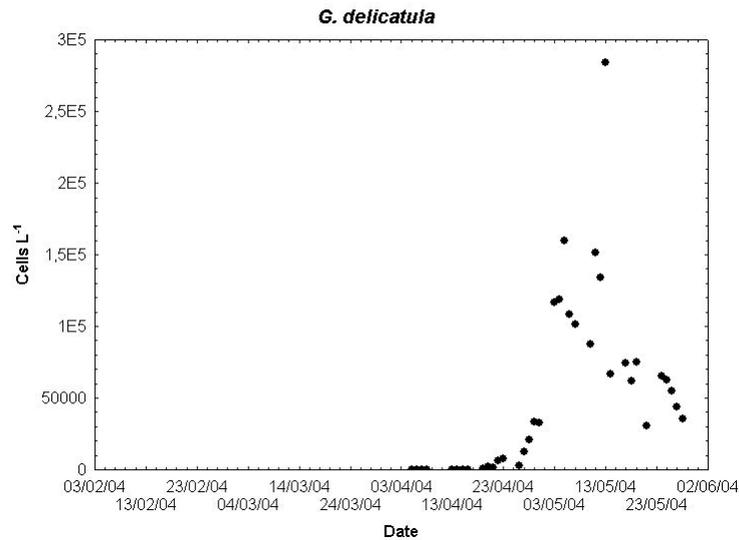


Fig. 2.24: Abundance of *Guinardia delicatula*. <http://www.pangaea.de>

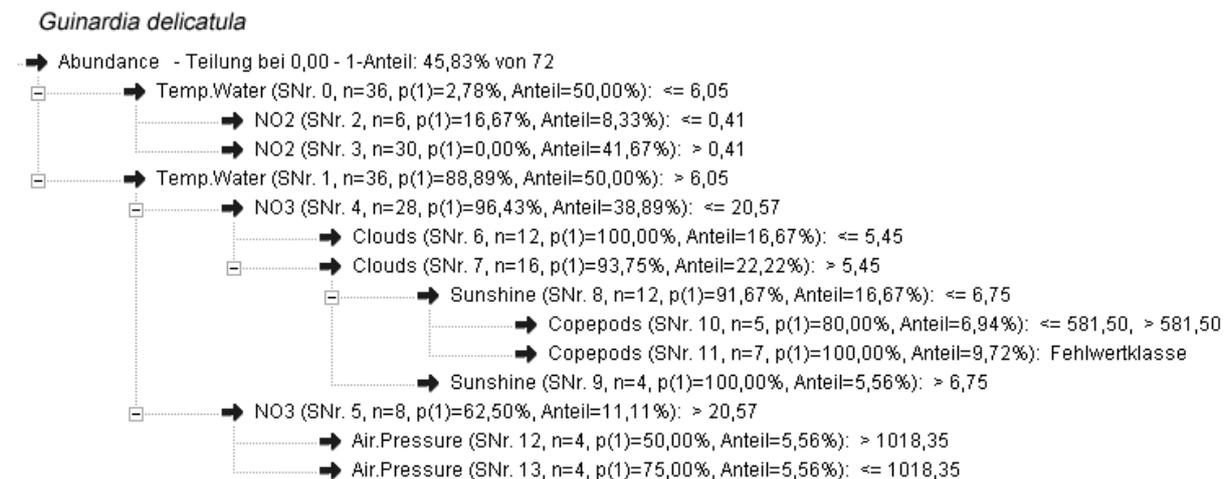
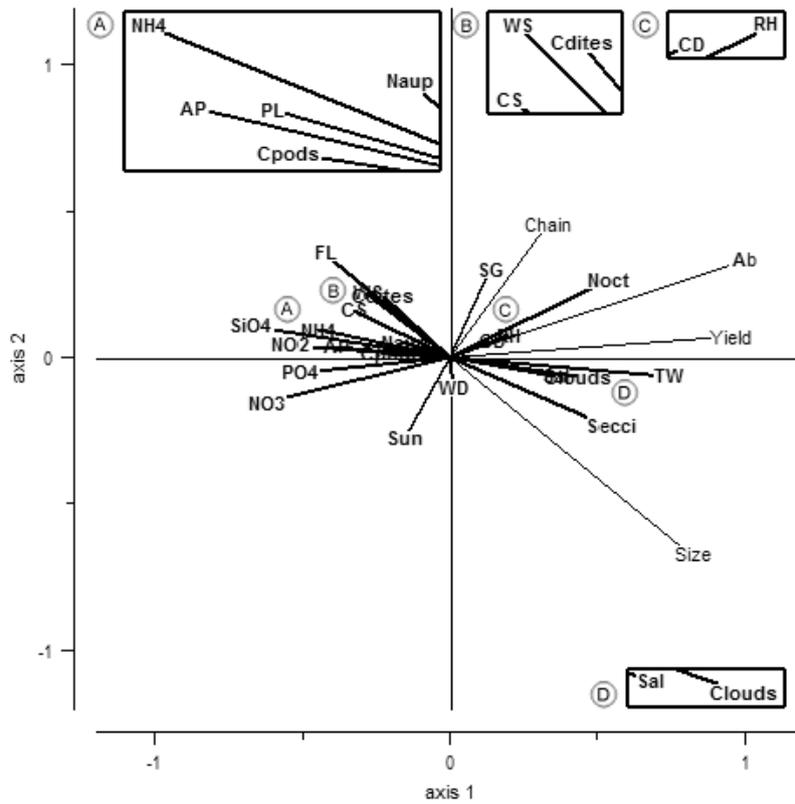


Fig. 2.25: Classification tree for the abundance of *G. delicatula*. SNr: segment number, n: number of cases in a segment, p(1): probability that cases of this segment have an abundance > 0, Anteil: percentage of the whole data set, Fehlwertklasse: variable with missing values.

The size of cells first increased together with the abundance but reached its maximum of $16775 \mu\text{m}^3$ by the end of April and then declined again to medium ranges between $10122 \mu\text{m}^3$ and $13784 \mu\text{m}^3$. Apart from abundance ($p = 0.021$) significant positive correlations were found with water temperature ($p = 0.011$), cloud cover ($p = 0.02$), copepods ($p = 0.004$) and the total grazer abundance ($p = 0.014$). Negative correlations were observed with air pressure ($p = 0.043$), the speed of currents (0.037), the abundance of copepod nauplii ($p = 0.000$), Noctiluca ($p = 0.000$) and the larvae of fishes and polychaete ($p = 0.000$). All nutrient concentrations also



Abbreviations

- Ab - Abundance
- AP - Air Pressure
- CD - Currents Directions
- Cdites - Copepodites
- Chain - Chain Length
- Clouds - Cloud Cover
- Cpods - Copepods
- CS - Currents Speed
- FL - Fish Larvae
- Naup - Copepod Nauplii
- Noct - Noctiluca
- PL - Polychaete Larvae
- RH - Relative Humidity
- Sal - Salinity
- SG - Sum Grazer
- Secci - Secci Depth
- Size - Cell Size
- Sun - Sunshine Hours
- TW - Temperature Water
- WD - Wind Direction
- WS - Wind Speed
- Yield - Yield PS II

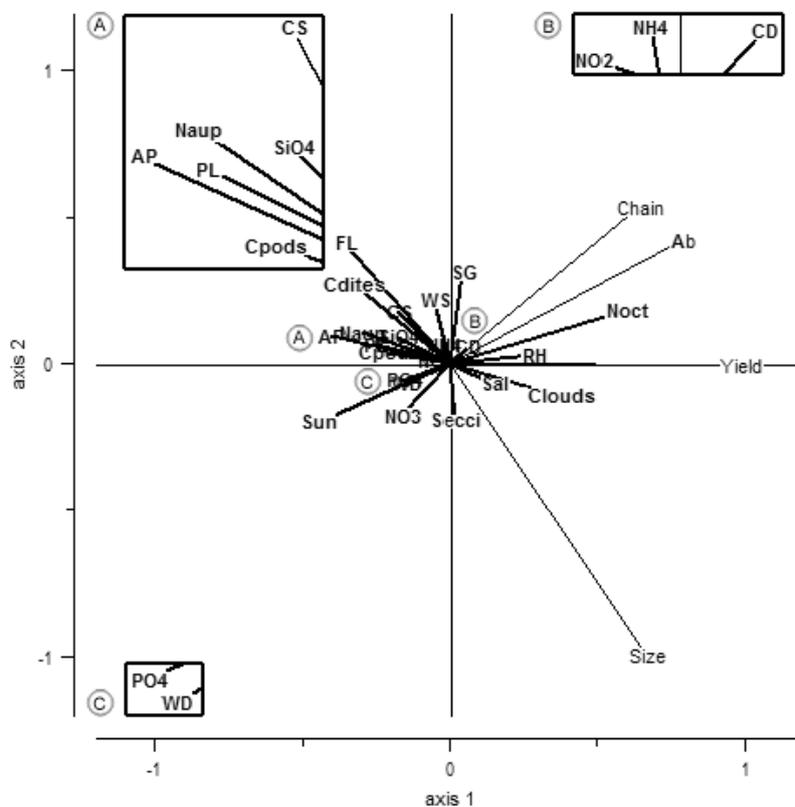


Fig. 2.26: RDA triplots for *G. delicatula*. Full RDA (upper) and partial RDA (lower plot). Thick lines and bold labels represent independent variables, thin lines dependent variables. The longer a line, the better it is represented by an axis. Correlations are indicated by the angles between variables. Small angles show positive correlations, 90° are neutral relations and angles above 90° indicate negative relationships.

showed negative correlations but only the correlation with silicate was found to be significant ($p = 0.007$). In the second runs with temperature as a covariable, the same overall patterns were found. Only nitrate concentrations were now positively correlated but without significance and from the grazer groups, copepodites now showed a weak positive correlation. The full RDA (Fig. 2. 26) confirmed the positive correlation with water temperature and cloud cover but placed copepods and total grazer abundance at negative angles. Further agreements with the results of the correlation matrix were found for air pressure, the speed of currents, nutrient concentrations, nauplii and larvae. The partial RDA (Fig. 2.26) also generally detected the same patterns as the partial correlations.

The mean chain length showed a similar pattern as cell size, increasing alongside the abundance and cell size with the largest colonies being observed at the beginning of May with a mean colony size of 14 cells per chain and then declining again. Significantly positive correlations were observed with temperature ($p = 0.000$) and salinity ($p = 0.006$) as well as the abundances of copepod nauplii ($p = 0.000$), *N. scintillans* ($p = 0.000$) and the larvae of fish and polychaetes ($p = 0.000$). Negative correlations were found with nutrient concentrations ($p = 0.000$), copepods ($p = 0.000$) and total grazer abundance ($p = 0.001$). The partial correlation found the same patterns. Both RDAs (Fig. 2.26) found the same patterns as the correlation matrices with the exceptions of putting total grazer abundance on a positive angle while fish larvae were classified as neutral and nauplii as well as polychaete larvae as negative influence.

Photosynthetic efficiency was at medium levels of about 0.4. Significantly positive correlations with temperature ($p = 0.000$), salinity ($p = 0.002$), the direction of currents ($p = 0.036$) and chain length ($p = 0.00$) were observed. All nutrients were negatively correlated ($0.000 \leq p \leq 0.001$). Further significantly negative correlations were found with wind speed ($p = 0.006$), air pressure ($p = 0.018$) and the speed of currents ($p = 0.008$). The partial correlations found the same relationships with the exceptions of nitrate showing a positive correlation and abundance ($p = 0.002$) now being negatively correlated with quantum yield. Again both RDAs (Fig. 2.26) generally confirmed the patterns found by the correlation matrices.

Odontella aurita

Individuals of this species were found over the whole time of this study, starting to appear as early as 16th of February (Fig. 2.27). But abundances remained rather low with only three minor peaks reaching maxima between 540 cells L⁻¹ and 1020 cells L⁻¹ until the middle of April. Between April 5th and April 8th, *O. aurita* was found only once in the sample with an abundance of 40 cells L⁻¹. From April 13th, numbers grew from 8660 individuals L⁻¹ to 10300 cells L⁻¹ within two days. Afterwards, the abundance declined again as quickly with the last detection of this species during this study in the sample of May 7th with 40 cells L⁻¹.

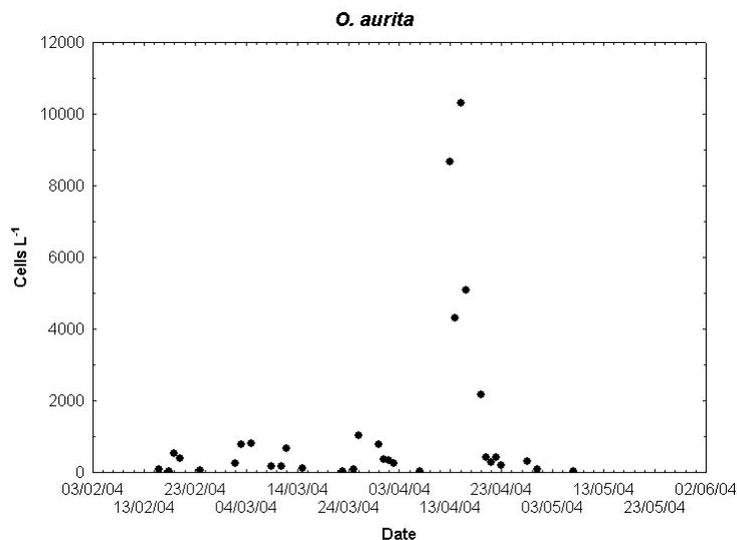


Fig. 2.27: Abundance of *Odontella aurita*. <http://www.pangaea.de>

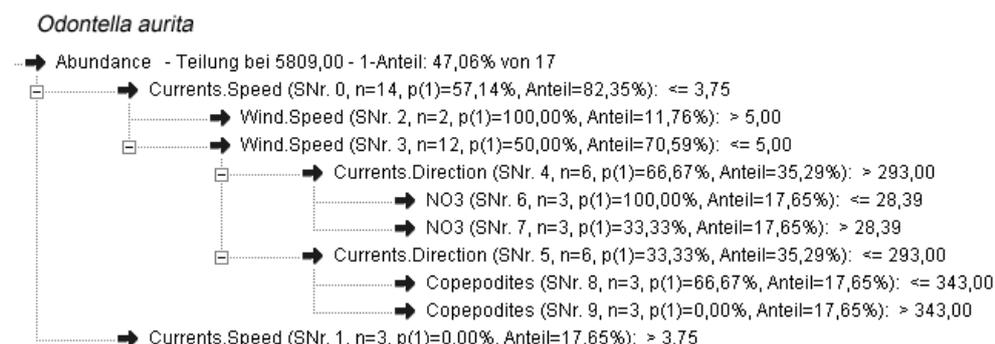
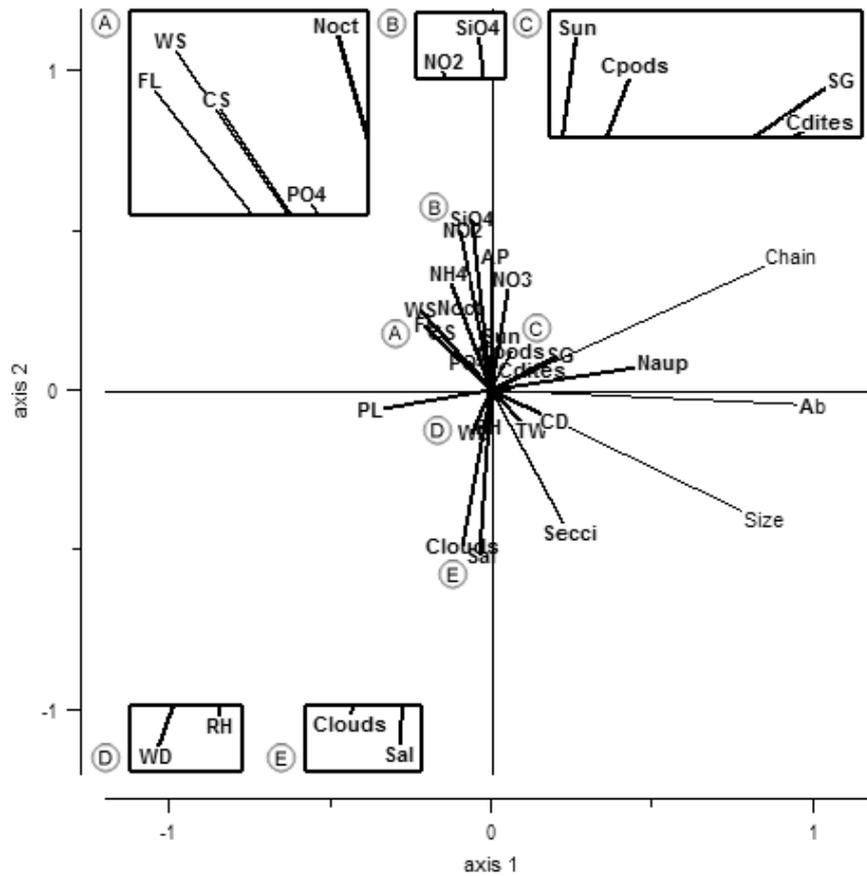


Fig. 2.28: Classification tree for the abundance of *O. aurita*. SNr: segment number, n: number of cases in a segment, p(1): probability that cases of this segment have an abundance > 0, Anteil: percentage of the whole data set, Fehlwertklasse: variable with missing values.



Abbreviations

- Ab - Abundance
- AP - Air Pressure
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- Cdites - Copepodites
- Chain - Chain Length
- Clouds - Cloud Cover
- Cpods - Copepods
- CS - Currents Speed
- FL - Fish Larvae
- Naup - Copepod Nauplii
- Noct - Noctiluca
- PL - Polychaete Larvae
- RH - Relative Humidity
- Sal - Salinity
- SG - Sum Grazer
- Secci - Secci Depth
- Size - Cell Size
- Sun - Sunshine Hours
- TW - Temperature Water
- WD - Wind Direction
- WS - Wind Speed
- Yield - Yield PS II

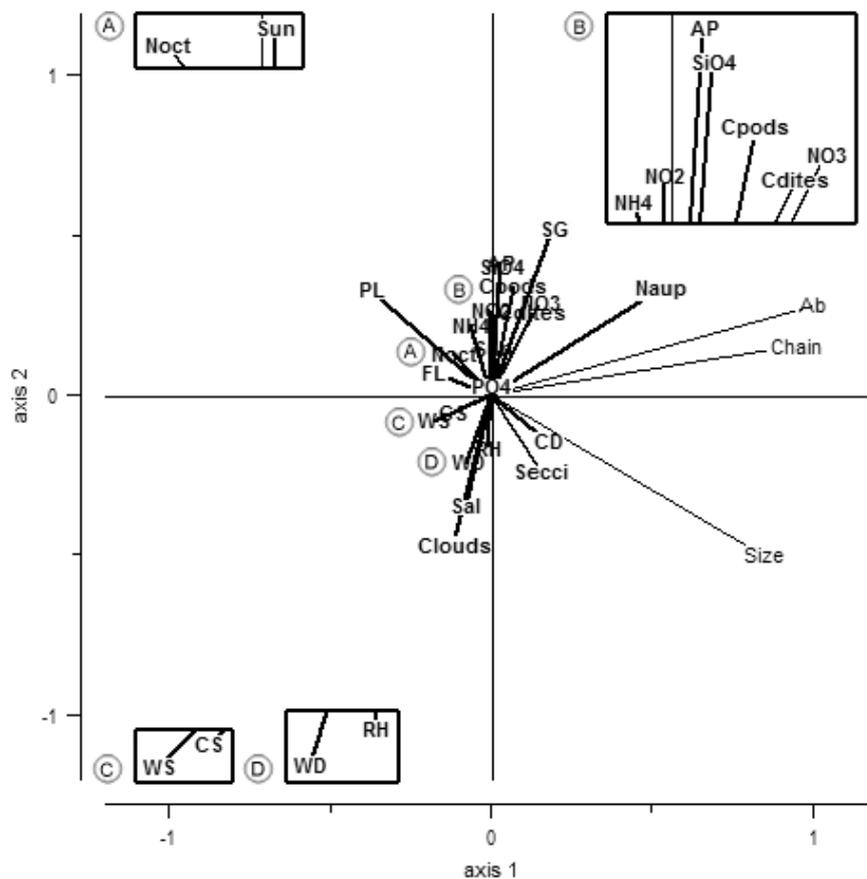


Fig. 2.29: RDA triplots for *O. aurita*. Full RDA (upper) and partial RDA (lower plot). Thick lines and bold labels represent independent variables, thin lines dependent variables. The longer a line, the better it is represented by an axis. Correlations are indicated by the angles between variables. Small angles show positive correlations, 90° are neutral relations and angles above 90° indicate negative relationships.

Because of this, correlations with physical and chemical parameters were only weak. This was also reflected in the partial RDAs and correlations where using temperature as covariable did not change the results. The classification analysis (Fig. 2.28) identified speed of currents, wind speed and currents direction as the most important factors for the distribution of this species alongside with nitrate concentrations for west-north-west and northern currents and the abundance of copepodites for currents between west and south. With the exceptions of nitrate and the direction of currents, classified as positive influence by the partial RDA, the RDAs (Fig. 2.29) placed all these parameters on angles indicating neutral or negative relationships. But only the negative relationship with wind speed was found to be significant by the correlation matrix ($p = 0.029$). The RDA classified copepod nauplii, copepodites, copepods and the total amount of grazers as having a positive relationship with the abundance of *O. aurita*. *N. scintillans*, fish and polychaete larvae were identified as being negatively correlated to cell numbers. For copepodites and the total sum of grazers these relations were found to be significant ($p = 0.037$ and $p = 0.001$).

The mean cell size rose and fell with the abundance with higher cell numbers coinciding with larger sizes ($p = 0.000$). Apart from this relationship, significantly positive correlations with the number of copepodites ($p = 0.005$), copepods ($p = 0.003$) and the overall sum of grazers ($p = 0.0000$) were observed as well as significantly negative ones with the abundances of nauplii ($p = 0.002$), Noctiluca ($p = 0.001$), polychaete larvae ($p = 0.008$) and fish larvae ($p = 0.001$). This was generally confirmed by the RDA (Fig. 2.29) with the exception of nauplii being classified as positive influence. The RDA also showed positive influences of the water temperature and the direction of the currents as well as a negative relationship with wind speed and direction.

According to the correlation analysis, the mean chain length showed significantly negative correlations with abundance ($p = 0.000$), cell size (0.000) as well as the abundances of copepodites, copepods and total grazer abundance ($0.0000 \leq p \leq 0.001$). Nauplii, *N. scintillans*, polychaete and fish larvae, were positively correlated ($0.000 \leq p \leq 0.011$). The RDA triplot (Fig. 2.29) however classified the algae's abundance and cell size as positive correlations as well as the correlation with copepodites, copepods and the sum of all grazer's abundances. *N. scintillans*, polychaete and fish larvae were put on negative angles. Only the correlation with the abundance of nauplii showed the same sign as in the correlation matrix.

Odontella aurita was found only once in the samples used to assess photosynthetic efficiency showing a mean quantum yield of 0.48. Therefore no further analysis was possible.

Paralia sulcata

Cells of *P. sulcata* were found at each sampling day over the whole course of this study (Fig. 2.30). The abundance was highest from February to the end of March with

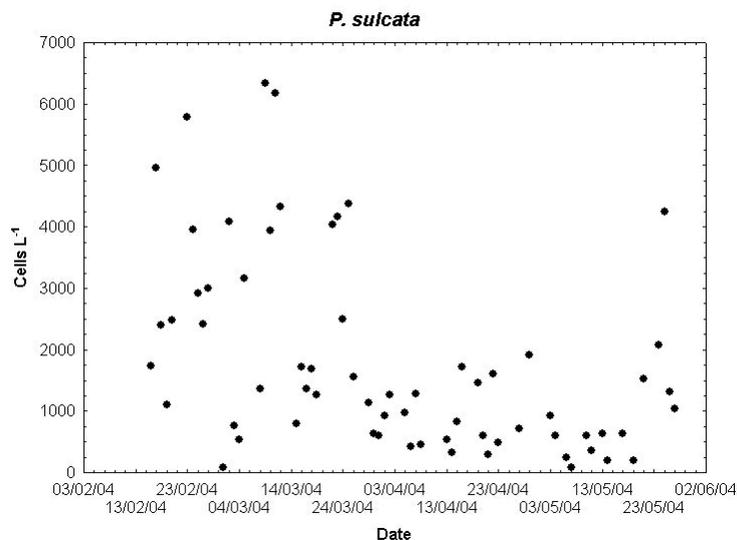


Fig. 2.30: Abundance of *Paralia sulcata*. <http://www.pangaea.de>

a maximum of 6340 cells L⁻¹. During April and May, cell numbers varied between 80 and 1920 cells L⁻¹. A second smaller increase in abundance was observed at the end of this study with numbers reaching a peak of 4240 cells L⁻¹ at the beginning of June but declined again afterwards. The classification tree (Fig. 2.31) found the water temperature as the main influence on cell numbers. For *P. sulcata*, a threshold value of 5.4 °C was identified. This temperature was reached in the middle of March. Below 5.4 °C, nitrite and nitrate concentrations were of importance, followed by the speed of currents. Above the threshold temperature, current's speed came first to explain the abundance, followed by ammonium and phosphate concentrations. In the correlation matrix, the relationship with water temperature was a significantly negative one ($p = 0.001$) and the abundance was positively correlated with the concentrations of ammonium ($p = 0.000$), nitrite ($p = 0.003$), phosphate ($p = 0.012$) and silicate ($p = 0.00$). Another significantly positive relationship was found with wind speed ($p = 0.005$) and currents speed ($p = 0.044$). In the partial correlation analysis, these

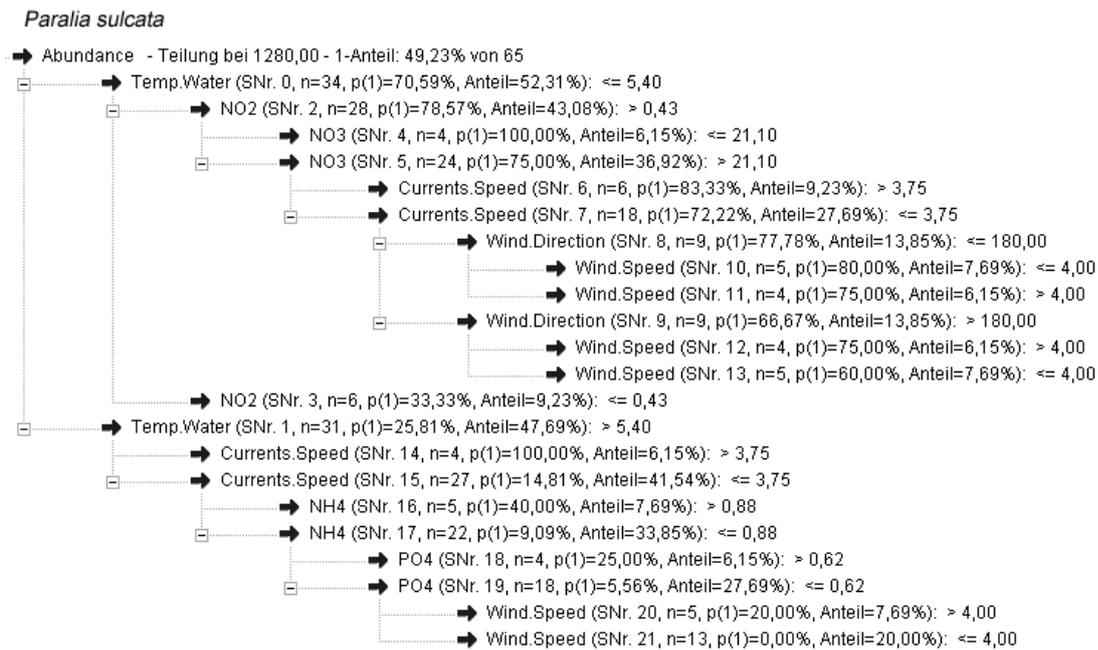


Fig. 2.31: Classification tree for the abundance of *P. sulcata*. SNr: segment number, n: number of cases in a segment, p(1): probability that cases of this segment have an abundance > 0, Anteil: percentage of the whole data set, Fehlwertklasse: variable with missing values.

patterns remained the same. However only the correlations with ammonium and wind speed were still found to be significant ($p = 0.000$ and $p = 0.005$). No significant correlations were observed regarding the numbers of grazers. The RDA (Fig. 2. 32) confirmed the negative link of cell numbers with water temperature as well as the positive relationships with nutrients and wind speed. The partial analysis also found these correlations. Regarding the impact of grazers, the RDA classified all groups as positive relations with the exception of nauplii showing a slightly negative link.

Cell size varied between $313 \mu\text{m}^3$ and $3196 \mu\text{m}^3$ with most values being found between $1263 \mu\text{m}^3$ and $2922 \mu\text{m}^3$. According to the partial correlations, none of the meteorological or oceanographical variables measured had a significant effect on cell sizes. The same was found for most nutrients with only nitrite showing a significantly negative correlation ($p = 0.026$). Cell sizes also had a significantly negative relationship with chain length ($p = 0.000$) and with photosynthetic efficiency ($p = 0.000$) although quantum yield was only measured at four days and therefore the significance may be an artefact. Abundance had no significant influence. Of all grazer groups only copepodites, copepods and total grazer abundance showed significantly positive correlations ($p = 0.000$) while nauplii, *N. scintillans*, fish and polychaete larvae were significantly negatively correlated ($p = 0.000$). This was partially

confirmed by the RDAs (Fig. 2.32) which classified copepodites, copepods and fish larvae as positive influence. *N. scintillans* and total grazer abundance were put at neutral angles while polychaete larvae and nauplii were found to be negatively correlated with cell size. As in the correlation matrix, photosynthetic efficiency and chain length showed a strong negative correlation with cell sizes while the abundance was placed at neutral or slightly positive angles. Most physical and chemical parameters were placed at angles indicating neutral or almost neutral relationships.

The mean chain length varied between two and 33 cells per chain and showed significantly positive correlations with water temperature ($p = 0.02$), quantum yield ($p = 0.000$ which as described above may be an artefact due to limited data) as well as the abundances of copepod nauplii ($p = 0.000$), *N. scintillans* ($p = 0.000$), polychaete larvae ($p = 0.000$) and fish larvae ($p = 0.000$). Significantly negative correlations were found with cell size ($p = 0.000$), the abundances of copepodites ($p = 0.000$), copepods ($p = 0.000$) and total grazer abundance ($p = 0.000$). The partial correlations confirmed no significant links to any physical and chemical parameters. Both RDAs generally (Fig. 2. 32) also confirmed the patterns found by the correlations.

The photosynthetic efficiency was measured on four days with mean values between 0.68 at the beginning of the observations and 0.42 at the end, showing a continuous decline.

Rhizosolenia pungens

As in the case of *P. sulcata*, cells of *R. pungens* were present at all sampling days throughout the study (Fig. 2.33). Abundances were low until the beginning of April with maxima of about 300 cells L⁻¹. During the first week of April, cell numbers began to rise with the highest growth rates at the end of April, resulting in a maximum concentration of 13021 cells L⁻¹ on April 28th. Afterwards, cell numbers declined again at the same rate to the initial range of abundances. The number of cells showed significantly positive correlations with the water temperature ($p = 0.005$) while significantly negative relationships were observed with silicate ($p = 0.000$) and ammonium ($p = 0.014$) concentrations.

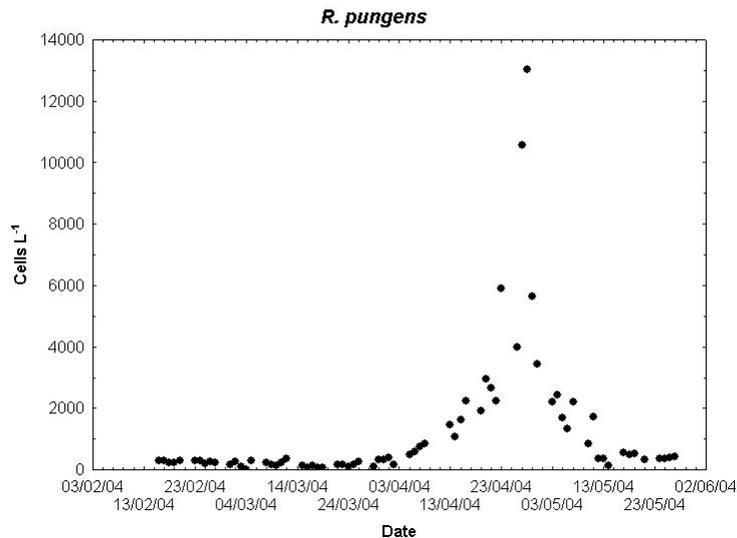


Fig. 2.33: Abundance of *Rhizosolenia pungens*. <http://www.pangaea.de>

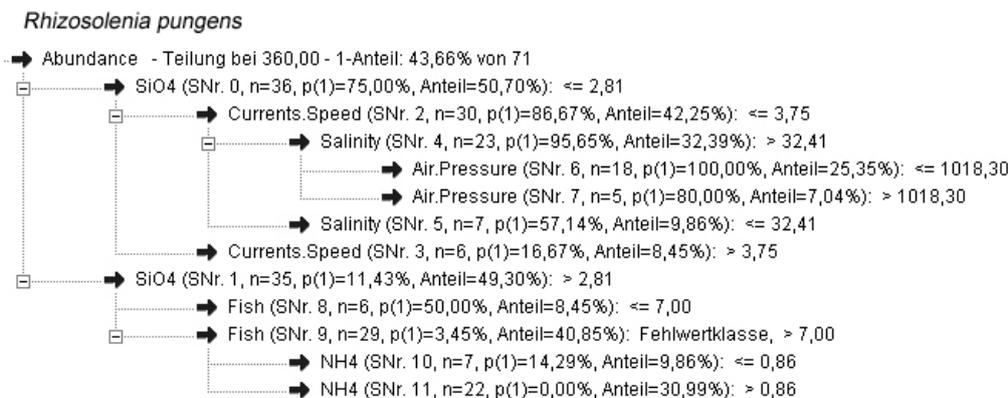
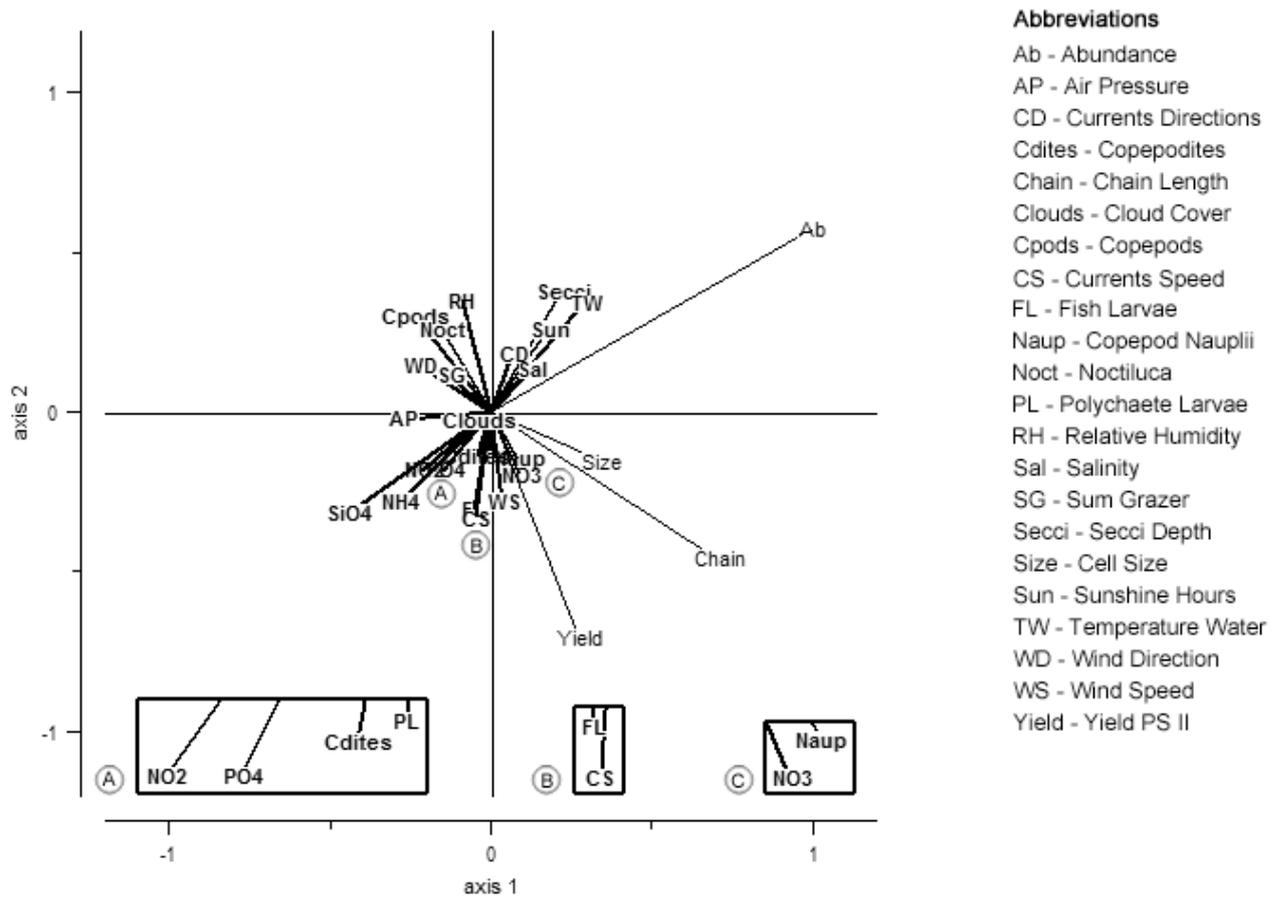


Fig. 2.34: Classification tree for the abundance of *R. pungens*. SNr: segment number, n: number of cases in a segment, p(1): probability that cases of this segment have an abundance > 0, Anteil: percentage of the whole data set, Fehlwertklasse: variable with missing values.

The classification tree (Fig. 2.34) identified silicate as the most important factor. For a concentration above 2.8 $\mu\text{mol L}^{-1}$, the number of fish larvae and ammonium concentrations were of importance. For silicate concentrations below the threshold of 2.8 $\mu\text{mol L}^{-1}$, currents' speed and salinity had an influence on the abundance of *R. pungens*. These results were confirmed by the first run of the RDA (Fig. 2.35) analysis which also classified water temperature to have a strong positive link with the abundance and placed all nutrient concentrations at angles indicating negative relations. Most grazers were classified as neutral or negative correlations. Partial correlations showed that neither currents nor wind parameters had significant influences on the abundances of *R. pungens*. Salinity was negatively correlated with abundances and the negative correlations of silicate ($p = 0.02$) and ammonium were also found by the partial analysis.



- Abbreviations**
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 - AP - Air Pressure
 - CD - Currents Directions
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 - Chain - Chain Length
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 - Cpod - Copepod
 - CS - Currents Speed
 - FL - Fish Larvae
 - Naup - Copepod Nauplius
 - Noct - Noctiluca
 - PL - Polychaete Larvae
 - RH - Relative Humidity
 - Sal - Salinity
 - SG - Sum Grazer
 - Secci - Secci Depth
 - Size - Cell Size
 - Sun - Sunshine Hours
 - TW - Temperature Water
 - WD - Wind Direction
 - WS - Wind Speed
 - Yield - Yield PS II

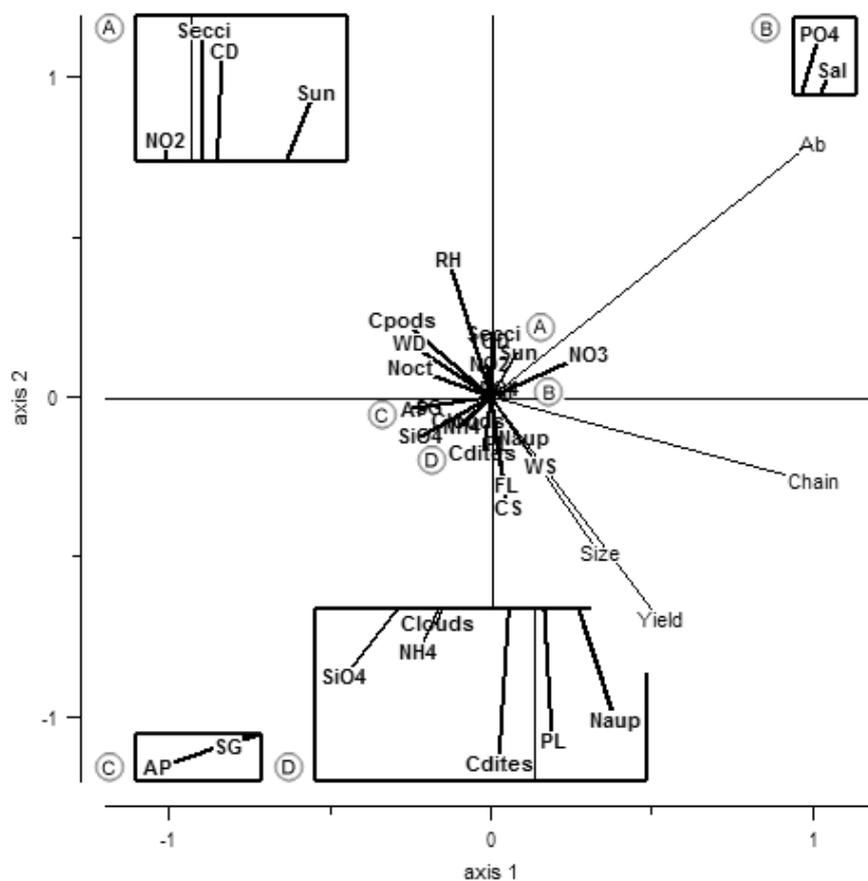


Fig. 2.35: RDA triplots for *R. pungens*. Full RDA (upper) and partial RDA (lower plot). Thick lines and bold labels represent independent variables, thin lines dependent variables. The longer a line, the better it is represented by an axis. Correlations are indicated by the angles between variables. Small angles show positive correlations, 90° are neutral relations and angles above 90° indicate negative relationships.

However, the other nutrient patterns changed. Nitrite and nitrate were now positively correlated with the abundance of *R. pungens* although only in the case of nitrate this relationship was significant ($p = 0.004$). The partial RDA generally confirmed these results although most nutrients were not represented very well by the triplot. According to the partial correlations, nauplii were positively, copepodites and copepods negatively correlated with the abundance of *R. pungens*, although none of the copepod life stages had a significant influence. A neutral correlation was found for *N. scintillans* while polychaete larvae and juvenile fish had a positive relationship with the diatom's abundance. The partial RDA put nauplii at a neutral and the other grazer groups at negative angles.

Cell size was highly variable with values between $468 \mu\text{m}^3$ and $75230 \mu\text{m}^3$. Apart from photosynthetic quantum yield, a positive correlation with $p = 0.002$, significant correlations were only observed with all grazer groups. Nauplii, *N. scintillans*, polychaete and fish larvae were positively correlated ($p = 0.000$) while for copepodites, copepods and the sum of grazer abundances the relationship was found to be negative ($p = 0.000$). Both RDAs (Fig. 2.35) generally confirmed those correlations.

Over the whole course of the observations, *R. pungens* cells were found to be solitary. If chain formation occurred, only two cells were attached to each other.

The photosynthetic quantum yield was measured on five occasions. With the exception of the last observation having a value of 0.4, all other results were between 0.58 and 0.62.

Skeletonema costatum

Cells of *S. costatum* were found between February 20th and April 29th with another singular appearance at May 27th (Fig. 2.36). Until the second half of April, abundances varied between 2490 cells L^{-1} and 9129 cells L^{-1} with a first peak of 29508 cells L^{-1} at March 11th. From April 15th to April 29th, cell numbers then rose to a final abundance of 139419 cells L^{-1} . For the following weeks, *S. costatum* was absent from the samples until May 27th, when 2213 cells L^{-1} were observed. While the classification tree (Fig. 2.37) identified the speed of currents and winds as most important influences on abundance, the only significant correlations were a positive link with water temperature ($p = 0.018$) and a negative relationship with silicate concentrations ($p = 0.044$).

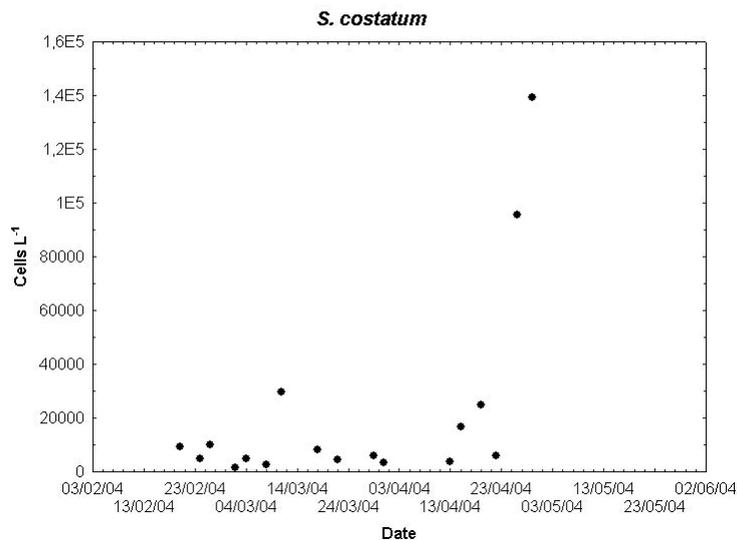


Fig. 2.36: Abundance of *Skeletonema costatum*. <http://www.pangaea.de>

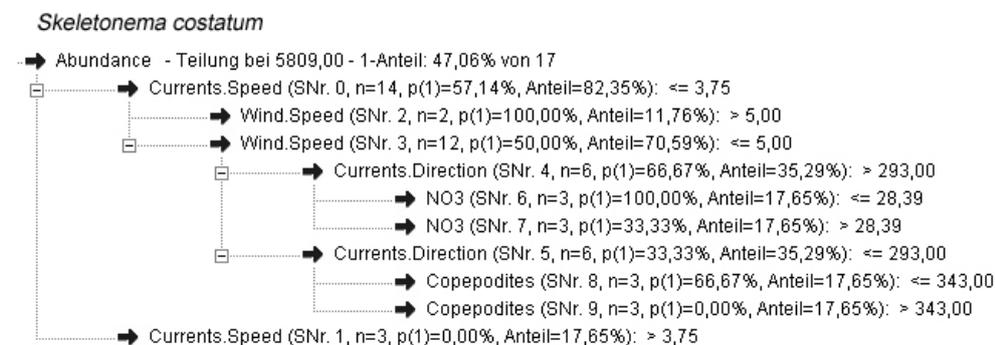


Fig. 2.37: Classification tree for the abundance of *S. costatum*. SNr: segment number, n: number of cases in a segment, p(1): probability that cases of this segment have an abundance > 0, Anteil: percentage of the whole data set, Fehlwertklasse: variable with missing values.

The RDA (Fig. 2.38) also classified temperature as having an important positive correlation with the abundance as well as confirming the negative relationship for silicate. Speeds of winds and currents were put at negative angles. In the partial correlation with temperature as a covariable, speeds of currents had a negative relationship with abundance while wind speed had a positive influence. The partial RDA also identified wind speed=0,00 as a positive correlation while it put the speed of currents at a neutral angle. Salinity, phosphate, ammonium and silicate were negatively correlated with abundances while nitrite and nitrate showed a positive relationship. However, only the correlation with nitrate was significant ($p = 0.002$). The partial RDA also found these correlations, placing salinity, phosphate, ammonium and silicate on negative angles while nitrite and nitrate were classified as positive influence. Of the grazer groups, copepod nauplii, copepodites, Noctiluca and the larvae of polychaetes and fish were found to be of negative influence

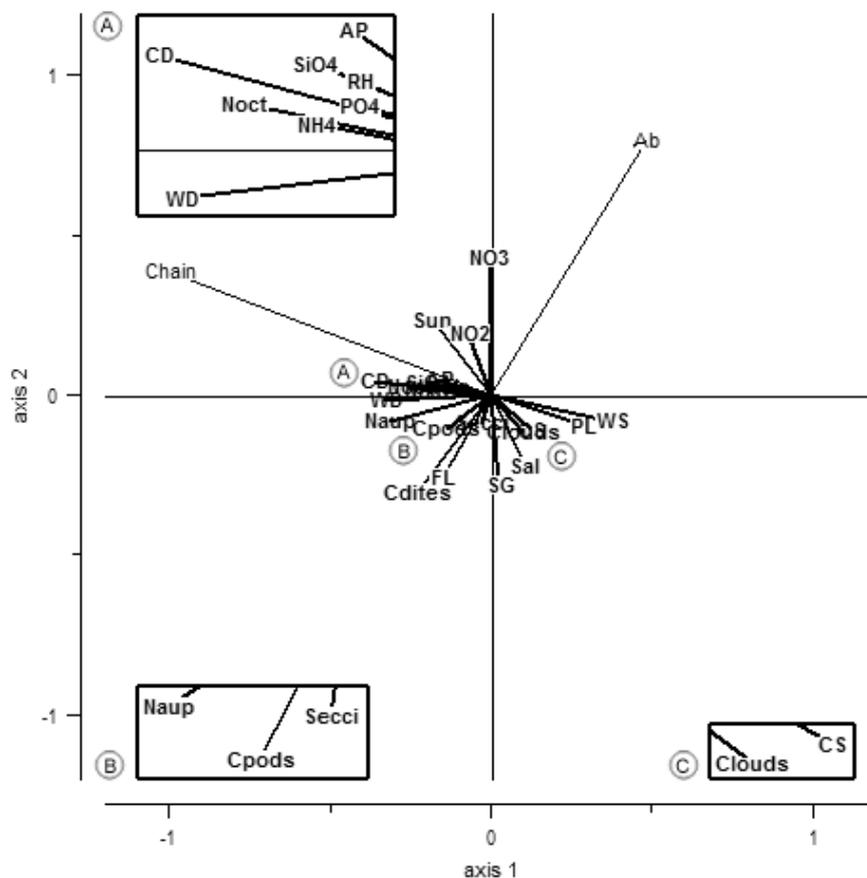
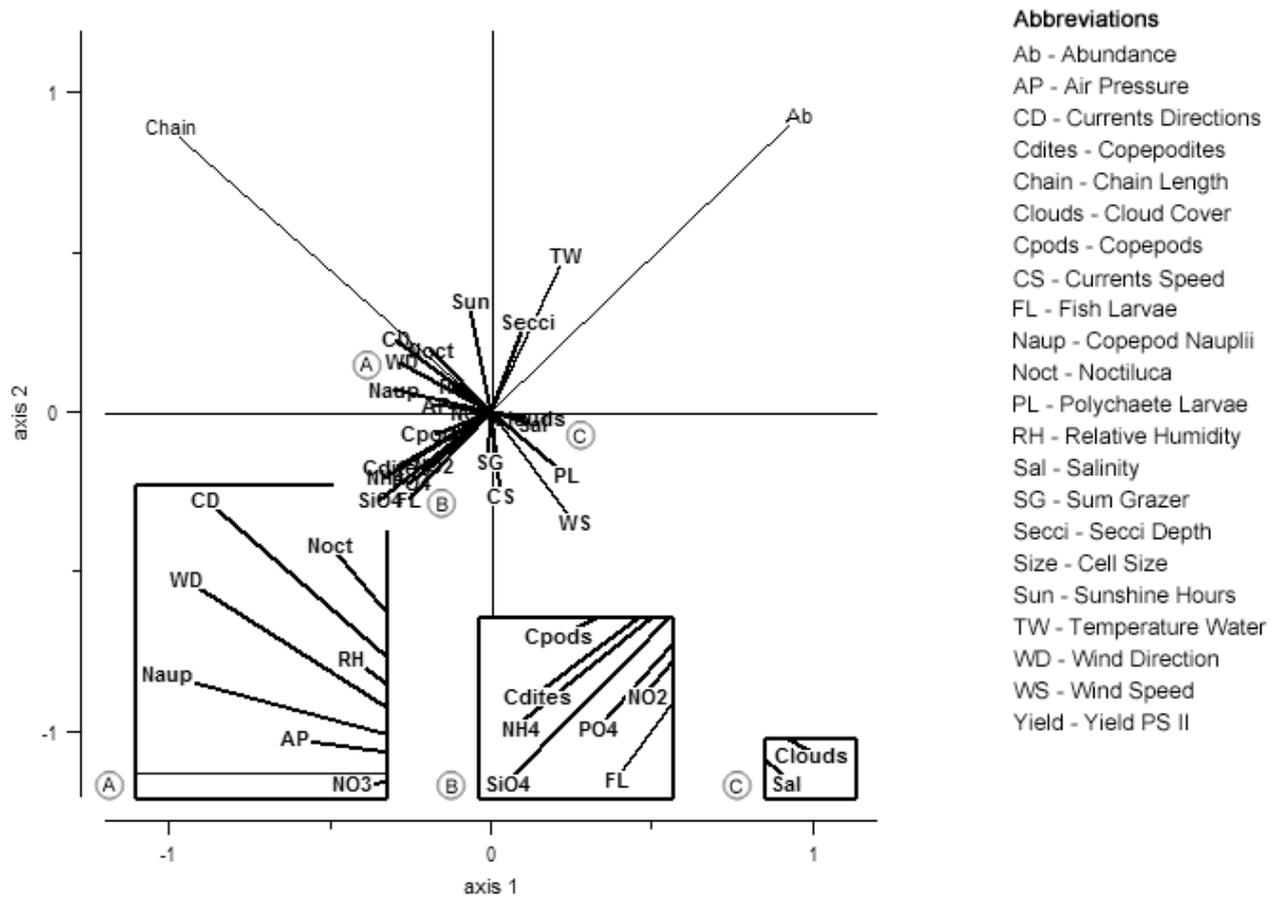


Fig. 2.38: RDA triplots for *S. costatum*. Full RDA (upper) and partial RDA (lower plot). Thick lines and bold labels represent independent variables, thin lines dependent variables. The longer a line, the better it is represented by an axis. Correlations are indicated by the angles between variables. Small angles show positive correlations, 90° are neutral relations and angles above 90° indicate negative relationships.

while adult copepods and the total sum of grazers were positively correlated. With the exception of polychaete larvae, the partial RDA placed all grazers at negative angles.

Since cells of this species are rather small, cell size did not vary enough to allow for the detection of significant differences with the microscope used.

Chain length varied between 4.8 and 10.2 cells and showed significant correlations with all grazer groups. Negatively correlated were copepodites, copepods and the sum of all grazers ($p = 0.000$) while nauplii, *N. scintillans* and the larvae of fish as well as polychaetes showed a positive correlation ($p = 0.000$). Except for polychaete larvae, the RDAs (Fig. 2.38) however classified all grazer groups as being positively correlated with chain length.

In the samples for the measurement of photosynthetic efficiency *S. costatum* was found only once with a yield of 0.46.

Thalassionema nitzschioides

This species was found between February 16th and April 29th (Fig. 2.39). Cell numbers varied mostly between 2320 cells L⁻¹ and 7800 cells L⁻¹ with four peaks reaching 13920 cells L⁻¹ (March 11th), 13380 cells L⁻¹ (April 1st), 36140 cells L⁻¹ (April 15th) and a maximum of 41800 cells L⁻¹ at April 29th. According to the classification tree (Fig. 2.40), the abundance was most influenced by currents and wind speed as well as the direction of currents, nitrate concentrations and the abundance of copepodites. The correlation matrix however only found significant correlations with all grazer groups. Copepodites, copepods and the sum of grazers were positively correlated ($0.000 \leq p \leq 0.001$) while nauplii, Noctiluca, polychaete larvae and fish larvae showed a negative correlation ($0.000 \leq p \leq 0.002$). The RDA (Fig. 2.41) placed winds and currents on neutral or negative angles and identified salinity and water temperature as strongest positive correlations with abundance. Nitrate concentrations were also classified as negative. Regarding grazers, positive correlations were found for the sum of grazer abundances and nauplii. Polychaete larvae showed a weak neutral link and all other grazer groups were put at angles indicating negative relationships. The partial RDA placed wind speed, salinity and nitrate at positive angles while all other nutrients as well as other parameters of winds and currents were classified as having negative relationships with the

abundance. The partial correlation matrix again only found significant correlations with the grazer groups.

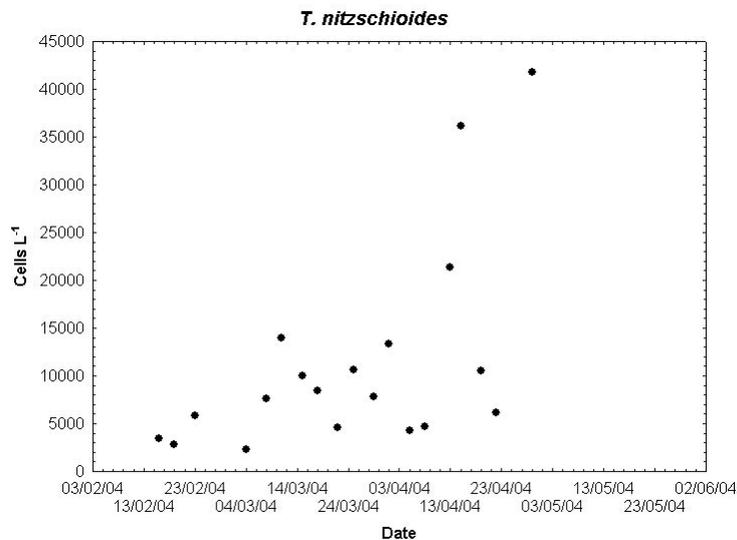


Fig. 2.39: Abundance of *Thalassionema nitzschioides*. <http://www.pangaea.de>

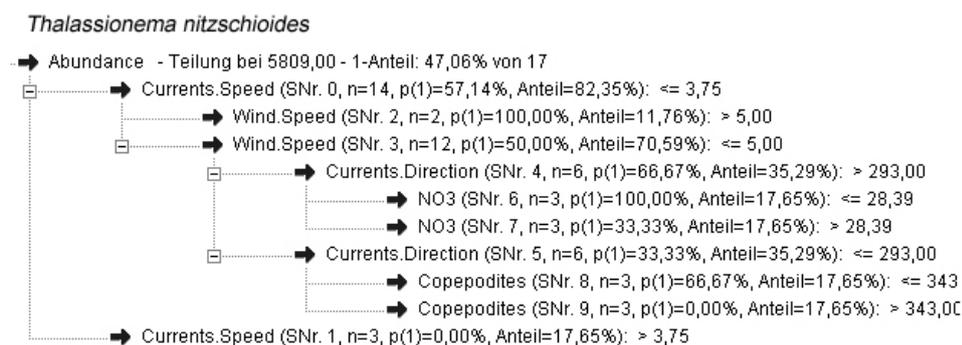
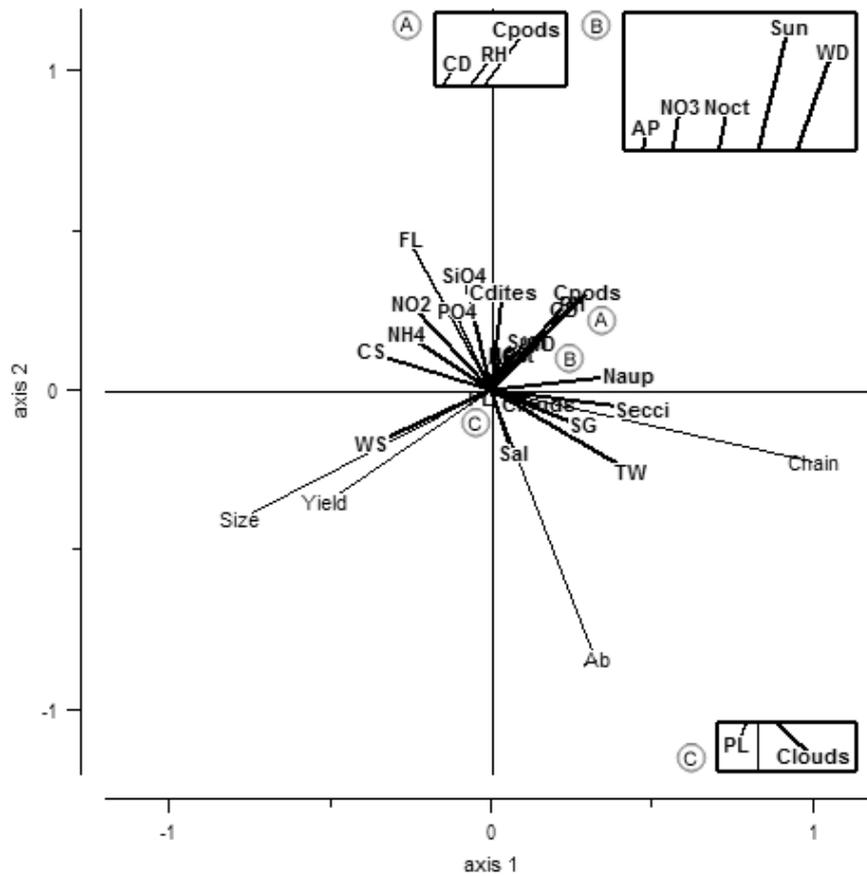


Fig. 2.40: Classification tree for the abundance of *T. nitzschioides*. SNr: segment number, n: number of cases in a segment, p(1): probability that cases of this segment have an abundance > 0, Anteil: percentage of the whole data set, Fehlwertklasse: variable with missing values.

The numbers of copepodites ($p = 0.001$), copepods ($p = 0.001$) and the sum of grazers ($p = 0.000$) were positively correlated with the abundance of *T. nitzschioides* while nauplii ($p = 0.001$), *N. scintillans* ($p = 0.001$), polychaete larvae ($p = 0.002$) and fish larvae ($p = 0.000$) had a negative relationship. The partial RDA also classified the sum of grazers as positive correlation as well as putting polychaete larvae and fish larvae at negative angles. For the other grazer groups, correlations were reversed. Cell size varied between $294 \mu\text{m}^3$ and $790 \mu\text{m}^3$ and showed a general decreasing trend over time. It was negatively correlated with salinity and showed positive correlations with all nutrients. However, none of these correlations were found to be significant.



Abbreviations

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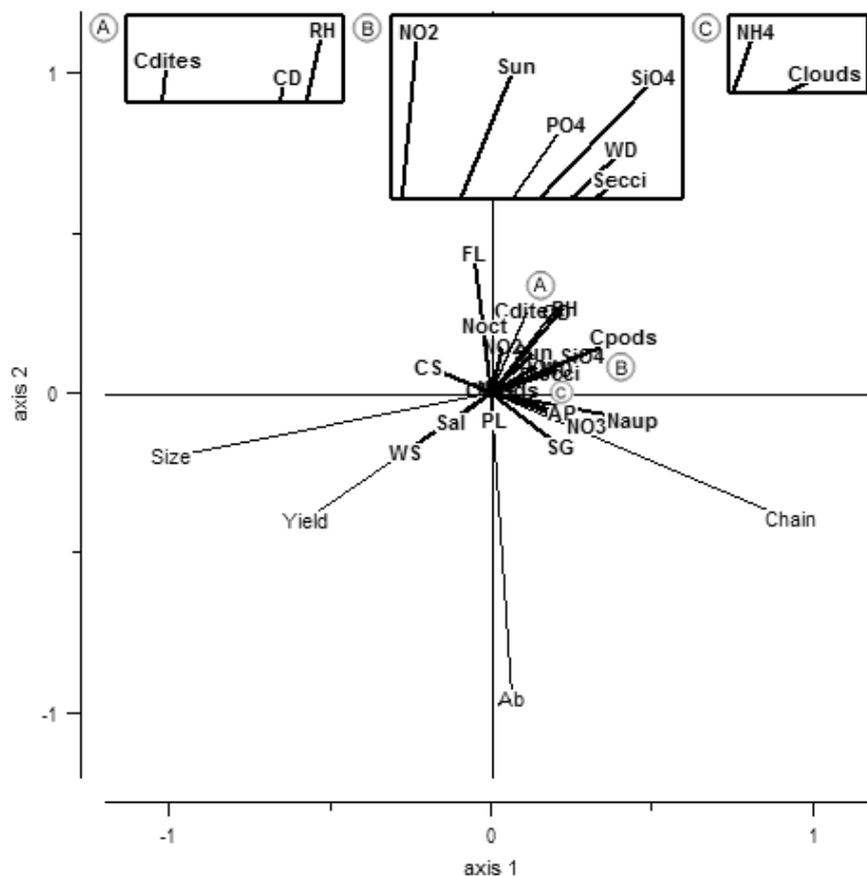


Fig. 2.41: RDA triplots for *T. nitzschioides*. Full RDA (upper) and partial RDA (lower plot). Thick lines and bold labels represent independent variables, thin lines dependent variables. The longer a line, the better it is represented by an axis. Correlations are indicated by the angles between variables. Small angles show positive correlations, 90° are neutral relations and angles above 90° indicate negative relationships.

The RDA (Fig. 2.41) placed ammonium and nitrate at angles indicating slightly positive correlations while salinity, phosphate and silicate were classified as neutral. Only nitrate was found to have clear negative angle but was not very well represented on the triplot. The partial RDA classified all nutrients as negative correlations. Regarding the relationship with grazers, the full and partial correlation analyses found significantly positive correlations with copepodites ($p = 0.0000$) and copepods ($p = 0.0000$) as well as with the sum of grazer abundances ($p = 0.000$). Significant negative correlations were found for nauplii, Noctiluca and larvae of fish and polychaetes ($p = 0.000$). Apart from polychaete larvae and in the case of the full RDA also fish larvae, grazers were put on angles indicating negative correlations by the RDA analyses.

Colonies were found to have mean chain lengths between 2.6 and 22.5 cells per aggregate with the majority of observations being below 10 cells per chain. Significant negative correlations were observed with abundance and cell size ($p = 0.000$). Colony size was positively correlated to wind speed and directions but had a negative relationship with the speed of currents. Salinity showed a positive correlation while all other nutrients had negative relationships. However, none of these correlations were found to be significant. In contrast to cell size and abundance, the significant correlations of colony size had an exactly reversed pattern being positively correlated with nauplii, Noctiluca, polychaete and fish larvae (all $p = 0.000$) while showing a negative relationship with copepodites, copepods and the sum of grazer abundance ($p = 0.000$). The RDAs (Fig. 2.41) classified abundance and cell size as positive correlations. Salinity was also evaluated as positive relationship. Apart from nitrate, the full RDA placed nutrients at angles indicating negative correlations while the partial RDA also identified silicate and phosphate as positive influences. The RDAs placed nauplii, copepods and the sum of grazers at positive, polychaete larvae at a neutral and all other grazers at negative angles.

Photosynthetic efficiency was assessed on nine occasions. The mean quantum yield ranged from 0.45 to 0.63. A significantly negative correlation was observed with sunshine hours ($p = 0.049$) while relative humidity ($p = 0.026$) and cloud cover ($p = 0.050$) were positively correlated with quantum yield. Of the nutrients silicate concentrations ($p = 0.035$), ammonium, nitrite and nitrate were negatively correlated with photosynthetic efficiency while a positive relationship with phosphate was found. Regarding the other algal parameters measured, quantum yield showed a

significantly positive correlation with chain length ($p = 0.000$) as well as significantly negative relationships with its abundance ($p = 0.047$) and cell sizes ($p = 0.003$). In relation to grazers, photosynthetic efficiency had a significantly positive correlation with nauplii ($p = 0.001$), *N. scintillans* ($p = 0.001$), polychaete larvae ($p = 0.003$) and the number of fish larvae ($p = 0.001$) as well as significantly negative ones with the sum of grazers ($p = 0.041$). Copepodites and copepods were also negatively correlated to photosynthetic efficiency. The RDAs (Fig. 2.41) confirmed the negative correlation with sunshine hours but also put relative humidity at a negative angle. Cloud cover was not represented very well in the triplot but showed a slightly negative tendency. The full RDA classified ammonium and nitrite as positive and all other nutrients as being negatively correlated with quantum yield. The partial RDA put all nutrients at negative angles. Regarding abundance, cell size and chain length, the RDAs found relationships opposite to the correlation matrix with the yield having the closest positive correlation with cell size but being negatively correlated to chain length. Apart from polychaete larvae, both RDAs classified all grazer groups as negative correlations.

Discussion

In this study, we examined the development of a spring bloom event, the factors behind the appearance of important species at the Helgoland roads and their interactions. These factors were not only limited to nutrient concentrations and interactions with grazers but also included meteorological and oceanographical parameters. Because of the complexity of the interactions, several uni- and multivariate techniques were applied to identify important influences for each of the species and to gain insight the in common importance or species specificity of the parameters measured.

Using several statistical approaches proved to be useful in pattern detection and explanation although in some cases contrasting results were produced. However, especially the use of classification trees and RDA gave good overviews on the relation of all parameters and their relative importance impossible to detect by correlations alone.

Regarding parameters influencing the whole phytoplankton community, temperature was the main driving factor behind the overall number of algal species during this spring bloom with highly significantly positive results while the biodiversity was

negatively correlated with temperature. This is of particular interest with regard of the warming trend in the North Sea. According to the Helgoland Roads Long-term Series, the mean water temperature has risen by 1.13 °C since 1962 (Wiltshire & Manly 2004) with first consequences visible like delayed spring blooms (Wiltshire & Manly 2004). The consequences of shifts in phenology could affect the whole food web as higher trophic levels depend on matching life cycles (Edwards & Richardson 2004).

One of this warm water species becoming more abundant in recent years is *Guinardia delicatula* (Wiltshire et al. 2005). In this study, temperature also had one of the highest positively correlated influences on all the diatom's parameters measured. In a recent study by Mieruch et al. (2006) on new bloom type classification criteria, *G. delicatula* was assigned to type C, species with typically narrow peaks of high abundances. This was also observed in this study where *G. delicatula* showed fast growth rates and a high peak in the middle of May with declining abundances afterwards. This pattern has also been described for other ecosystems, e.g. Bahía Blanca, Argentina (Gayoso 1999) and the western Irish Sea (Gowen et al. 1999). These peaks should be even more pronounced when calculated on biovolume basis since the mean cell size was also significantly positively correlated with water temperature. The high influence of currents and wind may be explained by the tendency of *G. delicatula* to form long chains and thereby developing a large surface area exposed to these factors. Regarding nutrients, *G. delicatula* was positively correlated with nitrite and ammonium but showed a negative correlation with nitrate in the partial analyses. In the original data the concentrations of all nutrients were already declining when *G. delicatula* became abundant. Already depleted nutrients could explain the medium photosynthetic efficiency measured since the photosynthetic quantum yield is a very sensitive indicator for nutrient stress as for example reported by Lippemeier et al. (1999) who found a recovery time of 20 minutes in *Thalassiosira weissflogii* cells after the previously silicate-limited cells were exposed to a pulse of silicate addition. Since long chains are susceptible to breaking in turbulent water, the significantly positive correlation of quantum yields with colony size as well as the negative one with wind speed also show the importance of mechanical stress for the photosynthetic performance.

Odontella aurita was present during the whole sampling campaign but with relatively low abundances and, after a period of absence from the samples, a peak in the middle of April. In Mieruch et al. (2006), *O. aurita* is classified as a type A species. In this category, species show the highest abundances the faster they start reproducing from scratch. Other studies suggest low winter temperatures as triggers for *O. aurita* blooms (Martens 2001). Since the winter 2003/04 was rather warm with water temperatures between 7 °C and 2.6 °C (mean temperature: 5 °C) from December 21st 2003 to March 21st 2004, 2004 may just have been not an *O. aurita* year. Apart from the positive correlation of the cell size with the abundance of this species, the positive correlation with grazers, except fish larvae, could be explained by a stronger grazing pressure by copepods on smaller cells, leaving only the upper size range for observation. The increase of smaller cells in the sample with the abundance of fish larvae could also be a top-down effect, in this case predation of fish on copepods. Chain lengths followed a reversed pattern of correlations i.e. larger cells were found in smaller colonies. This may also be connected to the grazing behaviour of zooplankton. As shown by Schnack (1983), small zooplankton like copepods in some cases of algae with large cell sizes and long chains prefer shorter colonies. Since they are preyed upon by fish larvae, the observed patterns in chain length can again be explained by top-down control.

Paralia sulcata was also found over the whole course of this study with the highest abundances during the first weeks. This may be explained by the high demands of silicate since this species has heavily silicified valves. Because *P. sulcata* is a benthic species as well, it fits that the abundance in the plankton community was also significantly influenced by turbulence connected parameters like currents and wind speed. This is confirmed by McQuoid & Nordberg (2003) who found higher abundances in well mixed, nutrient rich water columns than in stratified, nutrient depleted situations, especially with low phosphorus concentrations. In our study, nutrient concentration was of general importance for the abundance and a pronounced link with phosphorus concentrations was found for temperatures above 5.4 °C. The cell size varied over the course of time but was positively correlated with all grazers which may also be caused by a stronger predation on smaller cells. In contrast to the report of McQuoid & Nordberg (2003), no link between cell size, water temperature and phosphorus concentrations could be found. Chain length was

negatively correlated to copepodites, copepods and the total grazer abundance. It is known that copepods can position diatom chains to eat the cells one by one so that only the cells size is important for food selection, not the length of the chain (Paffenhöfer et al. 1982). It is therefore possible that in the case of *P. sulcata*, long chains with small cells were preferred.

Rhizosolenia pungens was another species present over the whole time of the observations. The abundance of this species showed a sharp peak in the end of April and was significantly positively correlated with temperature and negatively with ammonium and silicate concentrations. The strong influence of silicate may be explained by the highly variable cell size with up to $75230 \mu\text{m}^3$. Wind directions being negatively correlated to cell size could also be explained by the high demand for silicate as the numerically lower first half of directions corresponds to wind coming from the mainland driving nutrient rich coastal waters into the German Bight. The same applies to the positive correlation with the directions of currents since the nomenclature is directly reversed from wind directions.

The abundance of *Skeletonema costatum* showed two peaks, one in the first half of March and the second one at the end of April. In Mieruch et al. (2006), *S. costatum* is classified as a type B bloomer. Species in this category can have varying low abundances and small growth rates at the beginning of a bloom and have several peaks in cell numbers. The results of this study match the pattern described by Mieruch et al. The strongest positive influence found on the abundance was again the water temperature while copepodites, copepods and fish larvae were negatively correlated. Since the cells of *S. costatum* are rather small, they may be very well suited as food source for higher trophic levels and therefore be heavily grazed upon by copepods and other heterotrophs.

Thalassionema nitzschioides was found between February and the end of April with four peaks in its abundance between March 11th to April 29th. Again, this matches the classification of Mieruch et al. (2006) who classified this species as type B, alongside *S. costatum*. The significantly positive correlations with all grazer groups may be a time effect of the abundances since the increasing cell size was also being positively correlated with the number of grazers, pointing again to a potential selection effect by higher trophic levels. Interestingly, colony size showed an exactly reversed pattern

and was negatively correlated to the grazers' abundances. This may have the same cause as in the case of *P. sulcata* described above with grazers likely selecting cell sizes first. A preference for colonies with smaller cells can be explained with the chain formation of *T. nitzschioides* forming zigzag bands and not simple straight chains. Therefore, not only a cell's diameter but also its length may be a factor in food selection.

The significantly negative correlations of photosynthetic efficiency with sunshine hours and the positive ones with cloud cover and relative humidity point to an adaptation to low light conditions. This preference was also shown by e.g. Furuya et al. (1996) where *T. nitzschioides* was found in depths between 40 m and 120 m.

In this study, the different species showed in some cases common reactions to biotic and abiotic parameters, e.g. the importance of water temperature for abundances and the high influence of grazers on cell sizes and chain lengths, but also many different responses that point to adaptations and niches. The patterns found for each species matches in most cases known blooming behaviour. The importance of including data on large scale hydrodynamics and meteorology in addition to the more commonly used parameters such as temperature and nutrient concentration was clearly shown by the classification trees that revealed the in some cases strong impacts on diatom species. Although this study describes only a single bloom event, we therefore conclude that large scale patterns should be included in community studies to further explain the observed successions. But as every scientist knows, correlation doesn't mean causation and therefore more detailed research on the blooming conditions of each species and the mechanisms behind succession is needed, especially disentangling time, temperature and nutrient effects. Hence, we consider this study as a basis for future research on these subjects.

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Morphological Adaptations in Diatoms: Influence of Grazer Presence and Turbulence

The aim of this study was to investigate if cyclomorphosis, as an induced developmental response to predation, is an existing phenomenon in the predator-prey relationship between the copepod *Acartia tonsa* and the diatoms *Thalassiosira rotula* and *Thalassionema nitzschioides*. Additionally, the influence of turbulence and, in the case of *T. rotula*, bacteria and different culture media was also determined. The experiments were performed on two strains of *T. rotula* and one strain of *T. nitzschioides*. To insure an equal level of grazer exposure and as recent research shows the importance of infochemicals, indirect grazer presence by *Acartia* culture water was used. Turbulence was provided by shaking tables. Results show that the copepod water led to smaller chain lengths but this effect is without statistical significance. Stirring, however, had a significant influence on growth rates and colony size.

Introduction

Aquatic organisms are subject to strong selection pressures whereby predation is a significant mortality factor. This has favoured the evolution of many elaborate defence adaptations (Brönmark & Hansson 2000). Many species have evolved defence strategies like toxicity, swarming, diel vertical migration or changes in morphology and life history (Larsson & Dodson 1993). Regarding morphological changes, defence mechanisms can either be fixed or induced. The fixed morphological defences occur irrespectively of the presence or absence of the predator. The induced responses occur when the predator (or an associated excretion product) is present (Lass & Spaak 2003a, 2003b, Verschoor et al. 2004) and are reduced when the predator is no longer present. This indicates that there may be a cost to the production of morphological predator defences (Dodson 1989) that could also be an important factor for the succession in algal communities.

The role of chemical signals as inducers of defences is becoming more and more recognized, especially in the aquatic environment. These chemicals influence the ecology, the evolution and the behaviour of plankton. Two kinds of chemical signals exist to warn prey of the proximity of predators: substances released directly by the predator (kairomone) and substances released by an injured prey (alarm substances) (Brönmark & Hansson 2000). Wiltshire & Lampert (1999) found that one of the excretory products of the zooplankter *Daphnia* Müller, urea, can act as kairomone as it can induce colony formation in *Scenedesmus* Meyen.

A factor influencing the quality of the chemical signal could be the presence of bacteria. The role of the prevalent bacteria in symbiosis is well known. For example, Ringelberg & Van Gool (1998) reported that fish-associated chemicals enhance phototactic downward swimming in *Daphnia*. In their experiment, they treated perch with the antibiotic ampicillin and by this, the enhancement significantly decreased. Therefore, they concluded that it is not fish itself, but bacteria associated with fish that produce this kairomone. Cyclomorphosis, primarily regarded as temporal cyclic morphological changes of planktonic organisms (Black & Slobodkin 1987), can also be seen as a direct response to chemicals released by predators. Cyclomorphosis as a developmental response induced by predation is a rare phenomenon (Dodson 1989), and the study presented here refers some new aspects of this in the diatom-copepod relationship regarding chain length, length of the threads between cells and mean biovolume.

Predator defence mechanisms as a reason for different morphologies in diatoms is however confounded by the fact that even in culture the morphology of algae can be extremely variable. It can also be influenced by temperature, turbulence, nutrients, salinity and light. (Grover 1989, Lüring & van Donk 1999, Trainor 1992, Trainor 1993, Arin et al. 2002, Meave del Castillo et al. 2003). Abiotic factors like for example temperature and turbulence also have an important influence on how kairomones are produced, perceived and reacted to. Lass & Spaak (2003) have shown that *Daphnia galeata* exhibits higher growth rates when exposed to fish kairomones produced at higher temperature compared to kairomones from lower temperatures.

Turbulence for example is important for the dispersion of chemicals and nutrients as well as keeping diatom cells in suspension. As Huisman et al. (2002) demonstrated, intermediate levels of turbulence can keep a diatom population in the euphotic zone even if the individual cells themselves are sinking. On the other hand, it can lead to disruption of diatom colonies by mechanical forces or may interfere with the vertical migration of species like *Rhizosolenia* spp. that are positively buoyant and known for active density regulation (Moore & Villareal 1996). All of these possible ways of influence by turbulence on the phytoplankton, may affect the development of the algae and changes of the community structure. (Arin et al. 2002).

Additionally, it has been demonstrated that reactions of algae to abiotic stress like nutrient limitation or UVB radiation may also serve as defence mechanisms against grazers (van Donk & Hessen 1993, van Donk & Hessen 1995).

Bacteria are not only important in the expression of potential kairomones. They are also found in symbiosis with microalgae, facilitating for example the release of nutrients in mucopolysaccharide sheaths around algae. This is also reported for benthic communities where for example Garcia et al. (2004) showed the ability of bacteria inhabiting mucopolysaccharide layers of corals to degrade proteins and lipids as well as reducing nitrates to nitrites. It is also well known that although bacteria compete with algae for nutrients, diatoms don't grow well under axenic conditions as they need symbiotic bacteria for the remineralisation of nutrients (Grossart 1999). For example, bacteria are extremely important in the remineralisation of Si and without them siliceous algae would have problems attaining Si for their frustule. (Bidle et al. 2003). Although the remineralisation of Si by bacteria is influenced by many factors, e.g. temperature, species of bacteria and diatoms involved and the effectiveness of

the enzymes used, bacteria can determine whether a system is limited by nitrogen or silicate (Bidle & Azam 2001, Bidle 2002)

Thus the factors governing cyclomorphosis, are likely to be complex. In the literature papers investigating predator prey reactions related to other morphological inducers are rare (Wiltshire & Lampert 1999) Generally, predator prey reactions are considered separately from other morph inducing factors, for example Lampert et al. (1994, 1994) investigating responses of *Scenedesmus* spp. or *Daphnia* spp. to predator released chemicals while Yurista (2000) studied the induction of cyclomorphosis in *Daphnia lumholtzi* by temperature.

Most of the studies examining these complex interactions are carried out under freshwater conditions. We aim here to investigate the relative importance of different factors on morphological adaptation- i.e. turbulence, presence of bacteria and potential predator prey interrelationships in a marine environment.

Two strains of the diatom *Thalassiosira rotula* Meunier and one strain of the diatom *Thalassionema nitzschioides* (Grunow) Mereschkowsky and one of their predators *Acartia tonsa* Dana, a calanoid copepod, were used because of their ubiquitous distribution and their importance for the aquatic food web. At Helgoland, where this study was conducted, all three are regarded as key species of the pelagic food web.

Material and methods

Diatom cultures

Stock cultures were grown from algae previously isolated from the North Sea at Helgoland Roads. The diatoms were kept in continuous cultures at 15° C and a light-dark cycle of 12:12 hours. Two strains of *Thalassiosira rotula*, one isolated in April, the other one in August and one strain of *Thalassionema nitzschioides* were used.

Copepod cultures

Copepods were obtained from *Acartia tonsa* stock cultures reared from individuals isolated from the North Sea. In the cultures, the copepods were fed *Rhodomonas* spec., a common cryptomonad in the waters around Helgoland.

Experimental setup

The impact of copepods and turbulence on the diatoms was investigated with regard to changes in cell abundances, growth rate, biovolume, chain length (number of cells per chain) and, in the case of *T. rotula*, length of threads between cells in a chain.

In the *T. rotula* experiment, two strains and two different types of culture media were used in order to determine a possible effect on the tested parameters. Additionally, the bacterial abundance was analysed. In order to estimate how the different treatments might have influenced the physiological condition of the algae and to monitor possible costs of morphological changes, the photosynthetic activity of the algae was determined with the pulse-amplitude-modulation measuring principle (PAM) for *T. nitzschioides* cultures and the chlorophyll *a* concentrations using High Performance Liquid Chromatography (HPLC).

The two media, *f/2* and Drebes, were chosen for this study because of their common use for culturing algae (e.g. Karwath et al. 2000, Mock & Gradinger 2000, Pistocchi et al. 2000, Glud et al 2002, Olli & Anderson 2002, Ciglenecki et al 2003) and a wide range of experiments from studies on growth rates under various nutrient conditions (Somers 1972, Lopez-Rodas et al. 1992), food quality assessments for maricultures (Daume et al. 2003), cell division mechanisms (Cande & McDonald 1985), studies addressing microbiological interactions (Suttle et al. 1990, Grossart 1999, Kaczmarska et al. 2001) to all kind of ecological questions, e.g. studies on the effects of pollution (Pistocchi et al 2000). Although both media supply algae not only with nutrients but also with vitamins and minerals, there are differences in their composition that called for a check for media effects on the results. While the concentrations of nitrogen and phosphate are the same for both media, the Drebes medium contains about four times as much silicate. Also, the concentrations of all vitamins are much higher in the Drebes medium while *f/2* contains a much more varied mix of metals. Both media were prepared using filtered and autoclaved seawater from Helgoland Roads. The water was filtered four times over two 10µm and two 3 µm stainless steel candle-filters (Wolftechnik Filtersysteme, Weil der Stadt, Germany). The detailed composition of the medium components is given in tables 3.1 and 3.2. After adding all stock solutions except the vitamins, the seawater was autoclaved and allowed to cool down before the vitamins were added.

Table 3.1: f/2-medium modified after Guillard and Ryther (1962)

Substance	Concentration of stock solution	Amounts of stock solution added to 1 l of filtered seawater
NaNO ₃	75 g L ⁻¹	1.0 ml
Na ₂ HPO ₄	5 g L ⁻¹	1.0 ml
Na ₂ SiF ₆	5 g L ⁻¹ (obtain pH 3,0 with HCl (1N))	2.0 ml
HCl	0.04 mol l ⁻¹	0.1 ml
Metals		
Na ₂ EDTA x 2H ₂ O	5 g L ⁻¹	1.0 ml
ZnSO ₄ x H ₂ O	15 mg L ⁻¹	1.0 ml
CuSO ₄ x 5 H ₂ O	10 mg L ⁻¹	1.0 ml
CoSO ₄ x 7H ₂ O	12 mg L ⁻¹	1.0 ml
MnSO ₄ x H ₂ O	0.2 g L ⁻¹	1.0 ml
FeCl ₃ x 6H ₂ O	0.5 g L ⁻¹	1.0 ml
Na ₂ MoO ₄ x 2H ₂ O	6.5 mg L ⁻¹	1.0 ml
Vitamins		
Vitamin B ₁₂	1 mg L ⁻¹	1.0 ml
Biotin	1 mg L ⁻¹	1.0 ml
Thiaminiumdichlorid	20 mg L ⁻¹	1.0 ml

To obtain the copepod water, one litre of medium with 400 adult female copepods was incubated for 24 hours in darkness at 15 °C. To prepare for the *T. rotula* experiments, the copepods were fed with 15 ml of a *T. rotula* suspension and 50 ml of a *Rhodomonas* suspension. This yielded a total carbon content of 274 µg C L⁻¹ during the incubation with Drebes medium and 357 µg C L⁻¹ for the f/2 medium. For the *T. nitzschioides* experiment, the copepods were fed 200 ml of a *T. nitzschioides* suspension corresponding to a total carbon content of 250 µg C L⁻¹. After the incubation period, the copepod-algae suspension was first passed through a net to remove the copepods and then filtered through 50 µm and 0.2 µm pore size cellulose-nitrate-filters to remove the algae and obtain sterile media.

Table 3.2: Drebes-medium modified after von Stosch and Drebes (1964)

Substance	Concentration of stock solution	Amounts of stock solution added to 1 l of filtered seawater
MnCl ₂ x 4H ₂ O	0.2 g L ⁻¹	0.1 ml
NaNO ₃	42 g L ⁻¹	0.1 ml
Na ₂ HPO ₄ x H ₂ O	5.94 g L ⁻¹	0.1 ml
FeSO ₄ x 7 H ₂ O	0.278 g L ⁻¹	1.0 ml
Metals		
Na ₂ EDTA x 2H ₂ O	3.27 g L ⁻¹	1.0 ml
Na ₂ SiO ₃ x 9H ₂ O	8.36 g L ⁻¹	5.0 ml
Vitamins		
Vitamin B ₁₂	1 g L ⁻¹	50 µl
Biotin	0.1 g L ⁻¹	5 µl
Thiaminiumdichlorid	0.2 g L ⁻¹	25 µl

Turbulence was generated by a rotating shaking table (IKA KS 501 digital, IKA-Werke GmbH & CO. KG, Staufen, Germany) set at 12 rpm (rounds min⁻¹).

The general experimental setup was identical for both the *T. rotula* and the *T. nitzschioides* experiments. To test for the effects of different media, turbulence and alarm substances or kairomones, the following setup for all experiments was chosen: Each treatment was replicated four times. To start the *T. rotula* experiments, 300 ml Erlenmeyer flasks were filled with 200 ml of solution consisting of 70 ml algal inoculum and 130 ml f/2 or Drebes medium for the turbulence and the control treatment. For the treatments containing copepod water, 70 ml algal inoculum, 20 ml copepod water and 110 ml of f/2 or Drebes medium were mixed together. For the experiments with *T. nitzschioides*, 300 ml Erlenmeyer flasks were filled with 9 ml algal inoculum and 191 ml f/2 or Drebes medium for the control and the turbulence treatment. For the copepod treatments, 9 ml algal inoculum, 20 ml copepod water and 171 ml of medium were mixed. Since the copepod water was obtained by adding copepods to the full media, additional nutrient input by copepod excretion was considered insignificant.

The flasks were incubated at 15 °C with a light intensity of 6-12 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (*T. rotula*) and 12-14 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ (*T. nitzschioides*) respectively and a light-dark cycle of 12 hours.

Samples were taken directly after inoculation and then on every second day for eight days. Before sampling, the solutions were carefully shaken. In the case of *T. rotula*, the first sample of 13 ml was preserved using Lugol's solution for later cell counts, biovolume determination and morphological analyses. A second sample of 10 ml was taken for measurements with High Performance Liquid Chromatography (HPLC) and Pulse amplitude modulated (PAM) fluorometer. Additionally, on day t_0 , one sample of 15 ml was taken and on t_8 15mls of each replicate of each treatment batch were taken. These samples were fixed with 0.86 ml of formaldehyde (35 %) to a final concentration of a 2 % solution for determination of the bacterial abundances. During the experiment with *T. nitzschioides*, 10 ml were taken and preserved with Lugol's solution. A second sample of 13 ml was taken for pigment composition and fitness analyses, measured as photosynthetic efficiency. Bacteria densities were not determined in this experiment. The volumes of the second samples in the *Thalassiosira rotula* and the *Thalassionema nitzschioides* experiment differed, because a larger subsample for *T. nitzschioides* was required to avoid underestimation of the chlorophyll *a* concentration by the HPLC (N. Aberle-Malzahn, pers. communication)

All samples were taken under sterile conditions.

Analytical methods

Algae counts and biovolume calculation

Three replicates from each treatment of the fixed samples were counted under an inverted ZEISS microscope (Carl Zeiss AG, Oberkochen, Germany) at 100 \times magnification. The number of cells per chain was determined for ~400 aggregates (Lund et al., 1958). In the paper of Lund et al. (1958), the authors achieve an accuracy of 10 % (expressed as percentage of count) for a count of 400 organisms using a 0.95 confidence coefficient assuming the distribution to be random. Growth

rates were calculated as $\mu = \frac{\Delta LNn}{\Delta t}$ (Sommer 1998).

Furthermore, the algae were measured in order to estimate the biovolume using the formulas published by Hillebrand et al. (1999). In the case of *T. rotula*, the lengths of

the threads between the cells in a chain were also determined. The measurements were made at 200× magnification using an ocular micrometer.

Hillebrand et al. (1999) reported that for a measurement of 20 cells the standard error in three of four diatom species is $\leq 5\%$ of the mean. Therefore, the size of the cells of 20 chains and, in the case of *T. rotula*, the lengths of up to 20 threads between the cells in a chain were measured. To verify the results, a second method was applied. In this case the number of cells per chain was divided by the chain length [μm] in up to 20 chains. For *T. nitzschioides*, the size of the cells in up to 20 chains was also estimated.

Only one cell was measured in every chain, because the variation within a filament is generally lower than that between cells of different filaments (Sicko-Goat et al. 1977).

Bacteria counts

The determination of the bacterial abundances was done only for *T. rotula*. In three replicates of each treatment, the number of cells in the formaldehyde preserved samples was analysed using the nucleic acid stain 'SYBR Green I' (Sigma Inc., St. Louis, MO, USA) (Noble and Fuhrman, 1998).

Chlorophyll a content

The chlorophyll *a* content of the samples was determined by using high-performance liquid chromatography (Waters Corporation, Milford, MASS, USA). This method is widely accepted to be a very accurate method for the quantification of pigment concentrations in aquatic systems (Wiltshire et al., 1998). In order to determine the chlorophyll *a* concentrations in the experimental units, 5 ml of the algal solutions were filtered through a 0.2 μm glass micro fibre filter (GF/C, Whatman) during the *T. rotula* experiment and 10 ml during the *T. nitzschioides* experiment. Afterwards, the filters were folded, put into a plastic tube (Falcon tubes, 15 ml) and 2 ml of acetone (100 %) were added. Subsequently, the tubes were kept frozen at $-80\text{ }^{\circ}\text{C}$ for at least 48 h. Prior to further processing, a small spatula of quartz sand (Merck) was added to the tubes and the filters were crushed with a pestle. Afterwards, the extracts were sonified in an ice-filled ultrasonic bath (Bandelin Sonorex Super RK 103 H, BANDELIN electronic GmbH & Co. KG, Berlin, Germany) for 90 minutes and filtered through 0.2 μm pore size cellulose filters (Schleicher & Schuell). The extracts were filled into 1.5 ml glass screw flasks and injected into the HPLC system (Waters

Alliance 2695 Separation Module). The pigments were identified and quantified using a photodiode array detector (Waters 996). 60 μl of each extract was packed in between two 20 μl water 'plugs' and injected in duplicate straight into the system via a cooled autosampler (Wiltshire, 2000). The flow rate used for all investigations was 1 $\text{ml}\cdot\text{min}^{-1}$. A reversed phase 5C₁₈ column of 25 cm length was used and kept at 15 °C in a column oven. The gradient required 4 solvents, of which the solvent used as "A" consisted of a solution of 800 ml methanol + 200 ml water + 1.5 g tetra butyl amine acetate + 7.7 g ammonium acetate, while a 900 ml methanol + 100 ml acetone mix was used as solvent "B". Solvent "C" consisted of 500 ml methanol + 385 ml propanol and solvent "D" was made of 100 ml methanol + 900 ml H₂O solution. In order to determine the actual amount of chlorophyll *a* in the samples, a four point calibration was made (r^2 ranging between 0.99 and 1) before every series of measurements. For this purpose, a commercial chlorophyll *a* standard (Sigma) was diluted to concentrations of 0.2, 0.5, 1.0 and 4.0 $\mu\text{g ml}^{-1}$ and the actual amount of chlorophyll *a* in the standards was checked spectrophotometrically.

The chlorophyll *a* detection limit of the instrument was 0.008 $\mu\text{g ml}^{-1}$.

Determination of photosynthetic quantum yield

For *T. nitzschioides*, photosynthetic efficiency was monitored using PAM fluorometry. The quantum yield during photosynthesis is a very sensitive indicator of a plant's physiological state and stress level. To determine the effective quantum yield of photochemical energy conversion, the PAM (Pulse Amplitude Modulation) method of fluorescence measurement was applied using a Xenon-PAM (Heinz Walz GmbH, Effeltrich, Germany). To acquire the quantum yield of Photosystem II, the first initial fluorescence (F_0) is induced by low levels of actinic light after a period of dark adaptation. When the emission reaches a stable level, a strong pulse of light (Saturation Pulse) is applied. This leads to a quick reduction of the electron transport chain between the two photosystems, forcing the cells to emit all energy as fluorescence. This maximum fluorescence value (F_m) is recorded and the effective quantum yield calculated from

$$F = \frac{(F_m - F_0)}{F_m} \text{ for dark adapted and } F' = \frac{(F'_m - F'_0)}{F'_m} \text{ for previously light exposed}$$

samples (Heinz Walz GmbH 1999) In non-limited phytoplankton the F -values amount to 0.6 to 0.7 [arbitrary units] (Kromkamp and Peene 1999). Prior to the

measurement, the samples were dark-adapted for five minutes. The measurements were repeated whenever the signal was too intense (dilution of sample) or the signal was too low (increase of gain).

Statistical analysis was performed using ANOVA and Tukey's HSD post-hoc test with $p = 0.05$ as level of significance to detect any significant differences between the treatments.

Results

Differences between media – f/2 medium

All treatments of the *T. rotula* April strain showed an initial cell number of approximately $219 \cdot 10^3$ cells per litre. Until day eight, the abundances of the non-stirred control (0) and the copepod-water treatments (C) showed an exponential increase to $2 \cdot 10^7$ cells per litre, while the stirred (S) and the stirred copepod-water (SC) cultures increased only to $5.5 \cdot 10^6$.

These differences were confirmed to be significant from day four onwards ($0.0046 \leq p \leq 0.027$). Growth rates (Fig. 3.1) were highest between day two and six, differing significantly from the rates at the beginning and the end of the experiment ($0.0046 \leq p \leq 0.0287$). Between day two and four, stirred treatments grew at a significantly lower rate than their non-stirred equivalents ($0.0046 \leq p \leq 0.0207$). During the following two days of the experiment, significant differences between treatments were observed between cultures treated with copepod-water and the ones without grazer water ($p = 0.0283$).

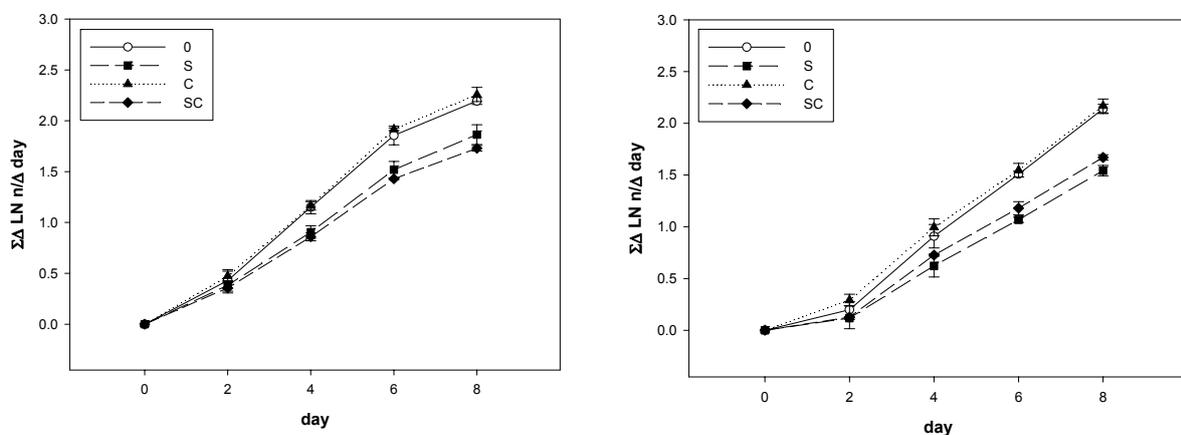


Fig. 3.1: Mean growth rates. Left: *T. rotula* April, right: *T. rotula* August. ○ control, ■ stirred, ▲ copepod water, ◆ stirred copepod water. Error bars show standard deviations.

Cell dimensions varied between 22.3 μm and 31.7 μm in diameter and 9.9 μm and 16.4 μm in height. This was without any correlation to treatments or day of sampling. The number of chains per litre showed significant differences between stirred and non-stirred treatments from day two onwards ($0.0005 \leq p \leq 0.0364$), the highest numbers being observed in the copepod-water treatment followed by the control. Colony size varied over the course of the experiment, growing slowly towards the end (Fig. 3.2). At the end of the experiment, stirred treatments had the highest number of cells per chain and stirred copepod-water cultures the lowest. But no significant differences between the treatments could be observed. The distance between two cells in a chain varied during the experiment but showed no significant reaction to the different treatments.

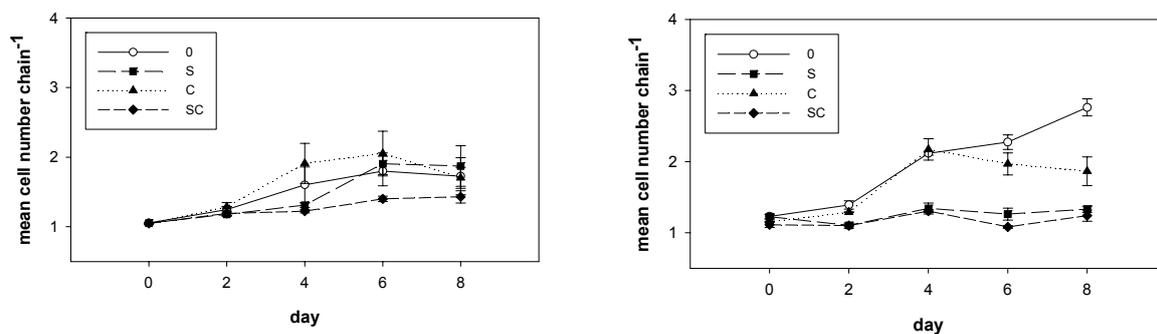


Fig. 3.2: Mean chain length. Left: *T. rotula* April, right: *T. rotula* August. ○ control, ■ stirred, ▲ copepod water, ◆ stirred copepod water. Error bars show standard deviations.

In all August *T. rotula* strain treatments the initial cell abundance amounted to approximately $25 \cdot 10^3$ cells per litre on day 0. These lower cell abundances in the beginning naturally led to lower cell densities by the end of the experiment, when compared to the April strain. Until day eight, the abundances of the control and the copepod-water cultures showed an almost exponential increase to $2 \cdot 10^6$ cells per litre, while the stirred and the stirred copepod-water algae increased only to $0.5 \cdot 10^6$ cells. A Tukey's HSD post-hoc test showed a significant difference between the stirred and the non-stirred treatments from day six onwards ($0.0203 \leq p \leq 0.0459$). Growth rates (Fig. 3.1) did not differ significantly between treatments apart from the day four to day six where the control showed significant differences to both stirred treatments ($0.0215 \leq p \leq 0.0275$).

The cell sizes of the August strain differed by as much over the experimental period as those of the April strain. Again, no correlation could be found with treatments or sample dates.

The number of chains per litre increased only slowly over the course of the experiment and at about the same rate for all treatments. Only on the final sampling day did the copepod-water treatment differ significantly from all other cultures ($0.038 \leq p \leq 0.0476$). Until day four, the mean cell number per chain in the control and in the copepod-water treatment increased. After that, the control values still rose, while the values of the copepod-water declined until day eight. The post-hoc test showed significant differences between the stirred and the non-stirred treatments from day two to day six ($0.0002 \leq p \leq 0.0043$). After day six, the control differed significantly from the copepod-water treatment as well ($0.0019 \leq p \leq 0.0043$). The stirred and the stirred copepod-water diatoms remained more or less in the same size range during the eight days and the statistical test showed no significant differences from day two to day eight. As with the April strain, distances between cells in a chain varied but showed no correlation with the treatments.

The chlorophyll *a* content of the *f/2*-medium cultures was below the detection limit during the first days of the experiment.

The April algae cultures had up to $300 \mu\text{g L}^{-1}$ chlorophyll *a* concentrations. These values were higher albeit more fluctuating compared to the August cultures. On day eight, the copepod-water algae contained the most chlorophyll *a*, followed by the control, the stirred and the stirred copepod-water treatments. The post-hoc test showed significant differences between the copepod-water and the stirred copepod-water treatment only for this day ($p = 0.0163$).

The August strain cultures showed lower chlorophyll *a* amounts (below $100 \mu\text{g L}^{-1}$) until the end of the experiment. Only on day six, were some differences noticeable. Here, the stirred treatment contained the highest amounts of chlorophyll *a*, directly followed by the stirred copepod-water, the control and the copepod-water treatment. The statistical test showed that the stirred treatment differed significantly from the control and the copepod-water treatments ($p = 0.0223$ and $p = 0.0028$), and it showed that the copepod-water regimen differed significantly from the stirred and the stirred copepod-water cultures ($p = 0.0028$ and $p = 0.0171$).

The bacterioplankton abundances in the treatments of the *T. rotula* f/2-medium April and August diatom cultures evinced a similar development (Fig. 3.3). The abundances on day 0 were low and differences were seen on day eight. The stirred treatments had the highest bacterial abundances, followed by the control, the copepod-water and the stirred copepod-water treatments, which had the fewest numbers of cells per millilitre.

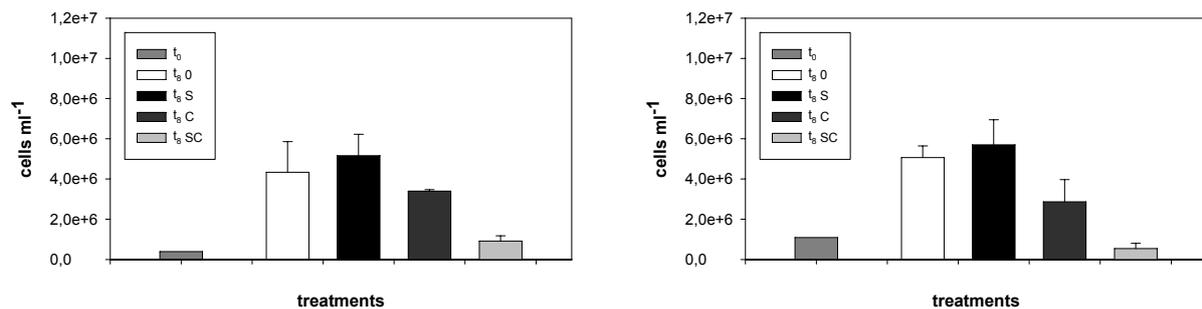


Fig. 3.3: Mean bacterial abundance. Left: *T. rotula* April, right: *T. rotula* August. Error bars show standard deviations.

On day eight, both *T. rotula* strains in the stirred copepod-water treatments evinced significant differences from all other regimens, with a visible tendency to differences between the stirred copepod-water and the copepod-water treatment ($p = 0.11$ in the April strain; $p = 0.12$ in the August strain) as well as between the stirred and the copepod-water treatment ($p = 0.31$ in the April strain; $p = 0.05$ in the August strain). A comparison of both strains led to the result that the stirred copepod-water treatment of the April strain differed significantly from the control and the stirred treatment of the August strain. The stirred copepod-water treatment of the August strain also differed significantly from the control and the stirred treatment of the April strain.

The treatments of the *T. nitzschioides* culture had an initial cell abundance of approximately $244 \cdot 10^3$ cells per litre. The treatments increased exponentially to $2.5 \cdot 10^7$ cells per litre, with no remarkable differences between the treatments until day eight. The post-hoc test did not detect any differences between the treatments during the experiment with the exception of the copepod-water treatment differing significantly from the control and the stirred cultures on day four ($p = 0.0063$ and $p = 0.0068$).

The development of the biovolume was similar to the cells per litre, although the increase in biovolume was somewhat smaller. The algae grew with a constant rate in all treatments until day six (Fig. 3.4). After that, a slight decrease of the growth rate was observed. There were no significant differences between treatments or over the duration of the experiment.

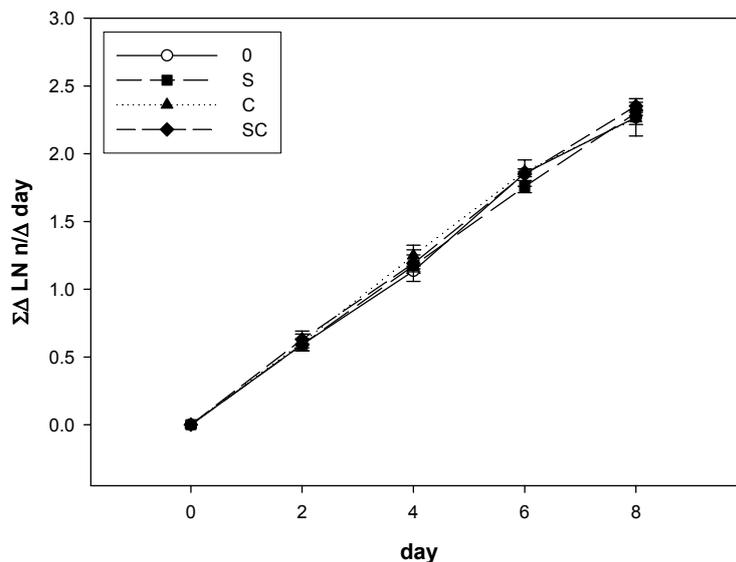


Fig. 3.4: Mean growth rates of *T. nitzschioides*. ○ control, ■ stirred, ▲ copepod water, ◆ stirred copepod water. Error bars show standard deviations.

During the experiment, the size of the apical axis of the *T. nitzschioides* cells varied between 52.2 μm and 54.8 μm and the size of the transapical axis between 5.3 μm and 8.3 μm .

The number of chains per litre of the *T. nitzschioides* cultures increased very slowly until day six over the course of the experiment (Fig. 3.5). Afterwards, the chain numbers increased visibly and the stirred copepod-water treatment had the highest number of chains per litre. The stirred treatment, the copepod-water and the control treatment had the lowest numbers of chains per litre. The statistical test showed that the treatments with copepod-water and the treatments without copepod-water were significantly different on day four ($0.001 \leq p \leq 0.0049$). On day eight, all treatments were significantly different ($0.003 \leq p \leq 0.0174$), with the exception of the control and copepod-water treatments. From day zero until day two, the mean cell number per chain increased in different ways in the different treatments. Subsequently, the cell numbers per chain decreased during the experiment. On day eight, the stirred copepod-water and the stirred treatment showed the strongest decrease. The stirred copepod-water treatment differed significantly from the control ($p = 0.07$) and the

stirred treatment ($p = 0.05$) on day four. On day eight, the stirred and the non-stirred treatments were also significantly different ($0.003 \leq p \leq 0.007$).

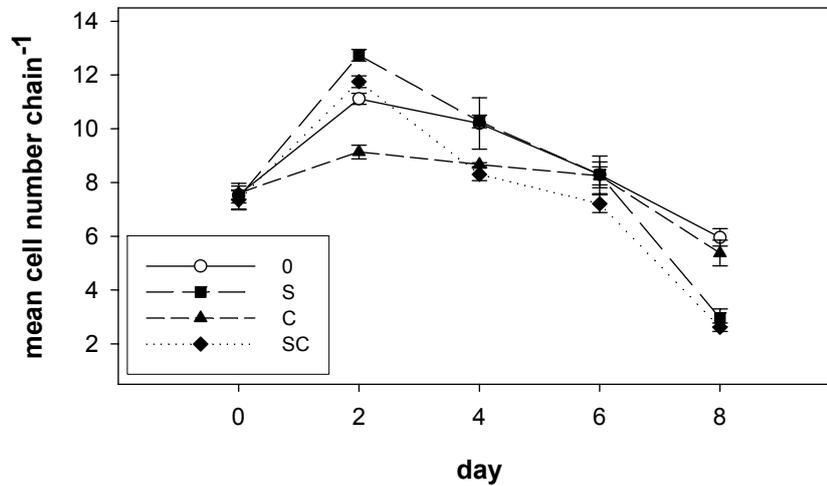


Fig. 3.5: Mean chain length of *T. nitzschioides*. ○ control, ■ stirred, ▲ copepod water, ◆ stirred copepod water. Error bars show standard deviations.

The measured chlorophyll *a* concentrations stayed below 80 µg per litre during the whole *T. nitzschioides* experiment. As in the *T. rotula* experiment, the error bars representing the means of four replicates ± the standard deviation are extremely high in all regimens.

Until day four, the content of the *T. nitzschioides* cultures was below the detection limit. On day eight, the copepod-water treatment showed slightly higher amounts of chlorophyll *a* than the other treatments. Only the copepod-water treatment differed significantly from the stirred treatment ($p = 0.0432$).

The photosynthetic quantum yield (F) of non-limited phytoplankton cells usually ranges between 0.6 - 0.7 [arbitrary units] (Kromkamp and Peene 1999, D. Hanelt, pers. communication). On day 0, the average yield was below 0.6 in all treatments, indicating a reduced physical fitness (Fig. 3.6). Afterwards, the yield increased to values of over 0.6 and then remained constant showing an improved performance. During the following days the yield of all cultures remained at this level, except that the stirred treatment showed a slightly but significantly higher yield on day four ($0.0009 \leq p \leq 0.0076$) and six ($0.0002 \leq p \leq 0.003$).

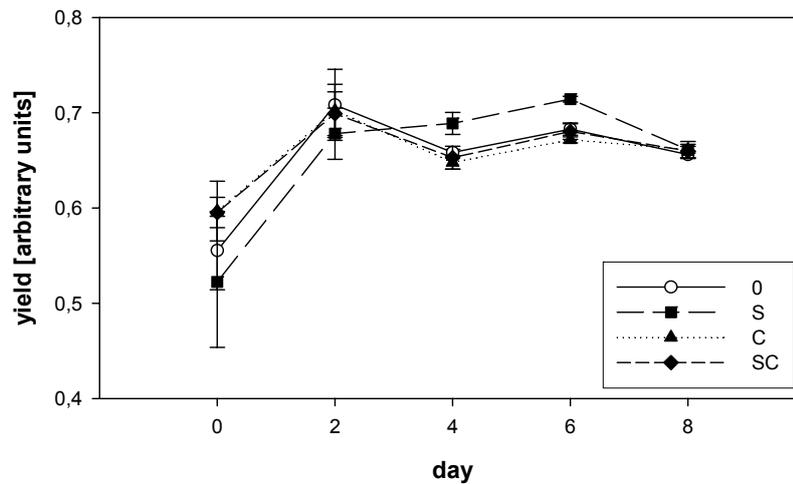


Fig. 3.6: Mean photosynthetic quantum yield of *T. nitzschioides* measured by PAM fluorescence. ○ control, ■ stirred, ▲ copepod water, ◆ stirred copepod water. Error bars show standard deviations.

Differences between media - Drebes-medium

On day zero in the Drebes medium cultures, the number of cells per litre amounted to approximately $12,370 \cdot 10^3$ in each *T. rotula* April strain culture. The cell densities increased to $4 \cdot 10^7$ by day six. Thereafter, the abundances fluctuated only slightly. Only the stirred copepod-water algae had lower abundances during the days of the experiment ($2.5 \cdot 10^6$ on day six) compared to the other treatments. For day six and day eight, this was verified by the statistical analysis, showing the stirred copepod-water treatment as differing significantly from the other regimens ($0.0004 \leq p \leq 0.0006$ and $0.0008 \leq p \leq 0.0013$). All algae showed similar growth rates with the fastest increase from day two to four (Fig. 3.7) although cultures treated with copepod water and stirring showed significantly lower rates after day two ($0.0003 \leq p \leq 0.038$).

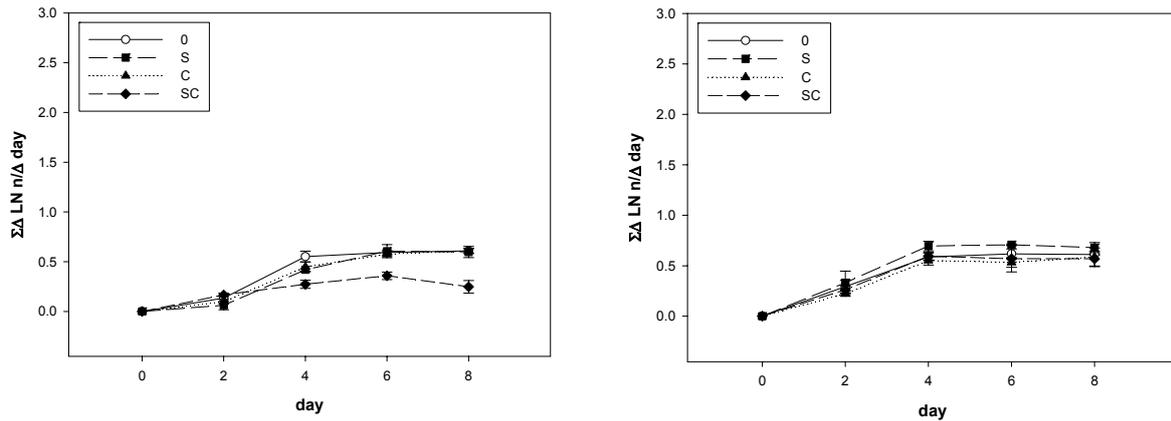


Fig. 3.7: Mean growth rates. Left: *T. rotula* April, right: *T. rotula* August. ○ control, ■ stirred, ▲ copepod water, ◆ stirred copepod water. Error bars show standard deviations.

Cell dimensions varied within the same range as those seen for the $f/2$ experiment.

In all the April strain cultures, the number of chains per litre increased more or less constantly during the eight days of the experiment. Until day six, the treatments fluctuated in slightly different directions and resulted in higher numbers of chains per litre in the stirred, the copepod-water and the control treatment ($3 \cdot 10^7$) on day eight. The post-hoc test confirmed these distinguishable effects, which resulted in significant differences between stirred and non-stirred cultures on day six ($0.004 \leq p \leq 0.0257$) and the stirred copepod-water and all other treatments on day eight ($0.004 \leq p \leq 0.0031$).

The mean cell numbers per chain of the April strain cultures remained almost in the same range over the eight days of the experiment (Fig. 3.8). Merely the stirred algae evinced slightly higher numbers from day four to day six. The stirred copepod-water algae had the lowest cells number per chain from day two onwards. The statistical test showed that the stirred treatment differed significantly from all other treatments on day four ($0.0011 \leq p \leq 0.0179$). On day six, the stirred copepod-water treatment differed significantly from the control and the stirred regimens ($0.0020 \leq p \leq 0.0197$) and on day eight significantly from the control ($p = 0.0148$).

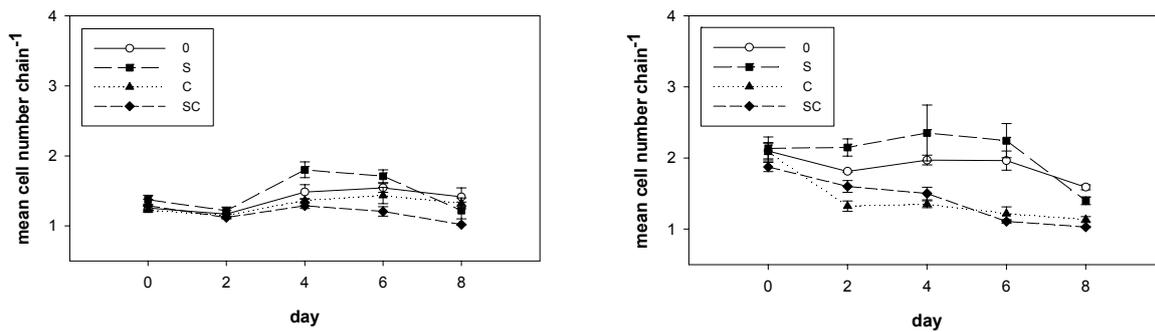


Fig. 3.8: Mean chain Length. Left: *T. rotula* April, right: *T. rotula* August. ○ control, ■ stirred, ▲ copepod water, ◆ stirred copepod water. Error bars show standard deviations.

The initial cell abundance of the August *T. rotula* strain treatments was approximately $11,334 \cdot 10^3$ cells per litre. The cell abundances rose to $3.5 \cdot 10^7$ by day four in all treatments and remained more or less within this range. The only exception was the stirred treatment, which showed slightly higher abundances. These differences could not be confirmed by the statistical test in any of the cases. During day 0 to day four, all treatments of the August strain grew rapidly and ceased growing in day four to day eight (growth rate estimated at between 0.054 and -0.028 cells per litre per 2 days) (Fig. 3.7). No significant differences between the treatments could be detected.

Cell dimensions varied within the same range as for the f/2 experiment.

The numbers of chains per litre in the August strain cultures increased over the course of the experiment and the copepod-water treatments showed higher amounts than the non-copepod-water treatments up to day six. The number of chains per litre of the stirred treatment caught up with the stirred and the control treatments ($3 \cdot 10^7$) by day eight, so that the control then had the lowest chain numbers. On day eight, the statistical analysis backed up these visible differences in the results showing that only the control is significantly different from the other treatments ($0.0020 \leq p \leq 0.0236$).

The mean cell number per chain tended to decrease over experimental time (Fig. 3.8). Especially the stirred copepod-water and the copepod-water algae showed a decrease and the statistical analysis showed significant differences from the other treatments on day six ($0.0004 \leq p \leq 0.0043$) and day eight ($0.0002 \leq p \leq 0.0053$) as well as between the control and the stirred treatments on day two ($0.0003 \leq p \leq 0.0397$) and day eight ($0.0002 \leq p \leq 0.0053$).

The chlorophyll *a* content increased in the April and August strains to $300 \mu\text{g L}^{-1}$ during the experiment, but due to strong variability no consistent tendency with regard to the differences in the treatments was seen. Only on day two and day four, the August strain showed noticeably higher chlorophyll *a* amounts in the stirred treatments, but the statistical results showed no significant differences between all treatments.

The abundance of bacteria in the April strain cultures was low on day 0. (Fig. 3.9) On the last day of the experiment, the stirred copepod-water treatment had the highest bacterial cell abundances per millilitre, followed by the stirred treatment. The copepod-water and the control treatment had nearly the same cell numbers, but there were no significant differences between the treatments on day eight. Bacterial abundances in the August strain cultures were also low on day 0 (Fig. 3.9). Here, the stirred and the copepod-water treatments had almost the same cell amounts, followed (at some distance) by the stirred copepod-water and the control treatments on day eight.

Again, there were no significant differences between the treatments

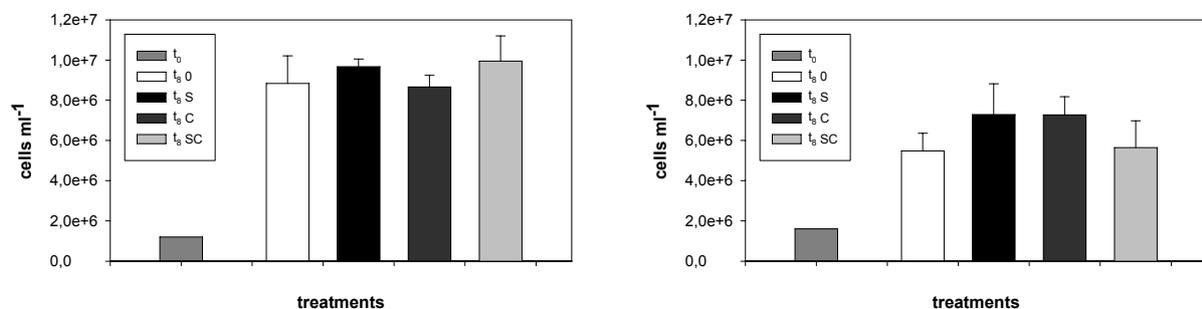


Fig. 3.9: Mean bacterial abundance. Left: *T. rotula* April, right: *T. rotula* August. Error bars show standard deviations.

Discussion

It was our aim here to evaluate the effect of nutrients (two different media), turbulence and predators on the growth rates and the colony size of two common diatom species. We differentiated between summer and winter situations by using different strains of *T. rotula* and another chain forming alga *T. nitzschioides*. As additional abiotic stress, turbulence was chosen since this is a common physical factor with several important aspects for diatoms from mechanical stress on colonies to being kept from sedimentation.

The coevolution of defence mechanisms and counter strategies plays an important role in trophic interactions. In this arms race, many different adaptations like e.g. the production of toxins, swarming, vertical migration etc exist but one of the most common reactions across all trophic levels and species are morphological changes. Chemical signals (kairomones) released by potential grazers are one way of inducing these changes in potential prey organisms (Lampert et al. 1994, Brönmark & Hansson 2000, Lass & Spaak 2003a, Verschoor et al. 2004). This way of signal transmission has been investigated in the present study. Since the development of algae and other organisms may vary in different strains (Krawiec 1982, Karsten 1996), two species of diatoms and within *T. rotula*, two strains were chosen for the experiment.

It has also been ascertained in past publications that morphological changes can be due to other environmental factors such as turbulence and nutrient concentrations. In this context, the authors argued that the availability of nutrients is important for the development of morphological changes (Lampert et al., 1994, Flynn & Martin-Jézéquel, 2000). The chlorophyll *a* content and the photosynthetic activity could provide relevant information with respect to the physiological impact of a morphological change on the algae.

However, most of the studies addressing the relationship between grazer presence and the development of defence mechanisms by prey organisms have been carried out in freshwater systems. To test these results on marine diatoms, our investigations performed on *T. rotula* were carried out with two different media to find out whether differences in the nutritional quality may lead to limitations in the growth and the development of the cultures. It might also be impossible for algae to respond with a morphological change to an environmental impact like the chemicals in the copepod-water or the stirring, when the diatoms are unable to multiply.

Most laboratory studies are conducted using only one medium for culturing algae. With the high number of different media used, we were interested to see how the choice of culture media would influence the results. The two media consist of the same main ingredients (Tables 3.1 and 3.2). The most obvious difference is that there are no metals as zinc, copper, cobalt and molybdenum in the Drebes-medium, which was used in the *Thalassiosira rotula* experiment. Furthermore, the amount of

most of the other ingredients is much lower in the Drebes-medium. The only exceptions are silicate and the vitamin B₁₂, which have much higher amounts than in the f/2 composition. The question arising was, whether the absence or presence of a nutritional component might be the limiting factor for growth and or result in changed morphology especially in the end of the experiment. This is also of interest because it has already been demonstrated by van Donk et al. (1997) that changes in morphological characteristics that are results of nutrient limitations can also impact the life cycle of predators. In the Drebes-medium, the absence of the metals and the lower amounts of most of the other ingredients could lead to the cease of growth during the *T. rotula* experiment as the role of trace metals like iron and zinc as limiting factors has often been demonstrated in the last years, e.g. in general iron fertilisation experiments (e. g. de Baar et al 1995) mesocosms (Lee et al. 2004) or for single plankton species (Wu et al. 2002, Schulz et al. 2004). In the f/2-medium, the lower amount of the vitamin B₁₂ could, for example, be the reason for a reduced growth rate as it has been shown that B₁₂ can be an important factor influencing growth rates (Pommel, 1975) and enzyme activity (Miyamoto et al. 2002).

Generally, in the case of *T. rotula*, the choice of the medium had the strongest influence on the population development. The reaction to the treatments within a medium was very similar in both strains. With the f/2 medium, both strains showed continuous growth after a short initial lag phase with the two unstirred treatments having the highest rates. In the Drebes medium, classic growth curves were observed for both strains with lag phases at the beginning, a log phase and the growth rates reaching a plateau at day four. Again, the unstirred treatments showed higher growth rates during the first days of the experiment. In the case of the April strain, the stirred cultures had a prolonged log phase until day six while the stirred copepod water cultures showed a constant growth rate from day zero to day six and the starting to decline. Regarding the August strain, the graphs for all cultures showed a very similar growth with all treatments reaching the plateau at day four and remaining there until the end of the experiment.

In the f/2-medium experiment, the growth rate decreases only during day six to day eight in the April culture, which has higher cell abundances than the August culture. This could lead to the conclusion that a possible lack of nutrients in the April culture,

caused by the higher cell abundances and consequently a higher consumption of nutrients, starts to limit the growth. In the Drebes-medium experiment, the growth ceased completely during day four to day eight and even started to become negative in the end. This implies that the depletion of nutrients limits growth rates and thus the carrying capacity of the batch culture environment was reached.

In the *Thalassionema nitzschioides* experiments with f/2-medium, the growth rate decreased only during day six to day eight, as found in the April culture of the *T. rotula* experiment with the same medium. This leads to a similar conclusion: A possible lack of nutrients in the culture started to limit the growth, because of the higher cell abundances leading to a higher consumption of nutrients

During the *T. rotula* experiment, all investigations were conducted with two strains of this species. The intention was to get an impression of any possible differences in the development between the spring blooming April strain and the autumn blooming August strain.

Krawiec (1982) investigated the physiological differences of two *T. rotula* clones isolated from two different areas (Baja California and Narragansett Bay, Rhode Islands) regarding their temperature tolerance, their maximum growth rate, their chlorophyll *a* content, and the rates of growth and photosynthesis in response to light intensity and temperature. He found manifest differences between the two clones that corresponded with the hydrographic characteristics of the areas they were isolated from. In addition, he investigated five clones of a local (Narragansett Bay) population isolated in September of 1973 and compared them with the clone isolated in March of 1971. He concluded that there was little physiological variability within this local population and that the differences found in the first experiment were the results of an adaptation of isolated populations to their environment leading to “physiological races” within a species.

The April and August strains show related tendencies, regarding cells per litre, biovolume and growth rate. The proportions of cells per chain show the same tendencies on day four of the f/2-medium April and on day eight of the f/2-medium August culture. The reason for this might be that in both cases the cell densities are approximately the same. Furthermore, the bacterioplankton development was similar for both strains in the f/2-medium with the highest abundance in the S treatment followed by the control, the C and SC treatments at the end of the experiment. These similarities suggest that the two clones, isolated in the same area at different times of

the year, show little physiological variability within this local population, which leads to the same conclusion Krawiec (1982) reached with his results.

In the beginning of the experiments, the aggregate abundance (i.e. the abundance of cells or chains) in some of the treatments was too low to count accurately using the methods of Lund et al. (1958). On day zero and day two, less than 400 cells and chains were counted in the *T. rotula* f/2-medium-August samples and less than 400 chains in the *T. nitzschioides* samples. Interestingly this lack of numbers in aggregates does not seem to have any distinct effect on the error bars.

The intention behind the chlorophyll *a* determinations was to be able to draw conclusions on the development between the different treatments and between the experimental days. Since the chlorophyll *a* concentrations of the samples were below or close to the detection limit. No consistent differences with respect to the treatment dependency were found.

Thus the relevance of chlorophyll *a* as an indicator for algal biomass can only be generally discussed in this study.

As Wiltshire et al. (1998) pointed out, it can be critical to use chlorophyll concentrations as quantitative indicators of algal biomass because of the variability of the chlorophyll content in the cell being a result of varying environmental conditions such as light and nutrients. In this context, a comparison between the results of the biomass and the results of the chlorophyll *a* content analysed with the HPLC-instrument would have been interesting. The effect of the nutrients and their possible limitations at the end of the experiments on the chlorophyll *a* should be investigated in further experiments. In addition to this, it is known that increasing light intensity leads to a decrease in the chlorophyll *a* content (Durbin, 1974). It is possible, that the opposite of the theory happened to the cultures in this study. Especially in the *T. rotula* Drebes-medium cultures, which showed the highest cell abundances (up to 41.000.000 cells/l), shading might have influenced the cultures during the last days.

The photosynthetic quantum yield (F) can be lowered by photoinhibition, water pollution, nutrient limitation and various other natural and anthropogenic stress factors. The only day the yield of the *T. nitzschioides* cultures is lower than in non-limited phytoplankton is day 0. This suggests that the algae were stressed by the initial preparations. The homogenisation of the stock solution (stirring) and the subsequent addition to the experimental media under a well-lit and on a warm clean

bench could be the reasons for the bad initial physiological conditions of the diatoms. However, in the following days, the *T. nitzschioides* algae presented a yield which implied good and non-limited photosynthetic activity. The fact that there is no significant difference between the treatments, underlines the hypothesis that there is no cost of any morphological predator defences induced by the copepod-water. The only exception from this similar development of the treatments is the stirred treatment on day four and day six. The higher and significantly different yields implies that the stirring could have caused a better condition of the algae by facilitating nutrient supply and uptake as well as reducing the effects of shading. However, since on the eight day of the experiment all results again vary only slightly this effect seems not to be a long lasting advantage that could outweigh other factors such as nutrient limitation.

Calanoid copepods are very important in marine ecosystems, because many, like *Acartia tonsa*, feed on phytoplankton and form a direct link between phytoplankton and fish (Mauchline, 1998).

The relation between diatoms and copepods in the food chain and possible responses of the diatoms to the presence of copepods were investigated in this work. Dodson (1989) concluded that most known examples of predator-induced cyclomorphosis were observed in aquatic habitats (including intertidal) and that most cyclomorphic algae are planktonic. The herbivorous zooplankton-algae interface has, in contrast to the well-studied effects of kairomones (substances released directly by the predator) of planktivorous fish on herbivorous zooplankton (*Daphnia*), received relatively little attention (Wiltshire et al. 2003). *Scenedesmus acutus*, a freshwater green algae grows in unicellular form in culture and forms colonies when filtered water of *Daphnia magna* Straus, a potential predator, is added to the culture (Lampert et al. 1994). Wiltshire & Lampert (1999) found that one of the excretory products of the zooplankter *Daphnia*, urea, can induce this colony formation in *Scenedesmus*. A comparable example within the marine system is the ubiquitous phytoplankton genus *Phaeocystis globosa* Scherffel. In this case an increase in colony size is stimulated by dissolved chemicals generated by ambient grazing activities. These chemical signals are not species-specific, because various combinations of grazer species and phytoplankton species resulted in significant colony enlargement in *Phaeocystis globosa* (Tang 2003).

In order to be able to draw any conclusions about whether or not the presence of the copepod-water induces any of the aforementioned variations in the algae used in this work, the quality of the additive copepod-water was extremely important.

Because there can be two kinds of chemical signals to warn prey of the proximity of predators: substances released directly by the predator (kairomones) and substances released by an injured prey (alarm substances) (Brönmark & Hansson 2000), the copepods were fed with the algae of the experiments and after 24 hours the residence-water was added to the cultures.

The C-content of the diatoms fed to the copepods was such that the copepods were well fed. There is however no way of knowing the concentrations of alarm substances` via the quantities of ingested algae.

Only in the case of the Drebes-medium August *T. rotula* the copepod-water treatments showed a development different from that of the non-copepod-water treatments. The mean cell numbers per chain decreased in the copepod-water treatments, which the statistical test confirmed with significant differences on day six and day eight. This is a different effect to other studies where predators increase the size of the algal colonies (e. g. van Donk 1997, Lürling 1998, Lürling 2003, Verschoor et al. 2004). It could be explained by the fact that in a chain-forming diatom it would be more detrimental to have a longer chain as the copepod would, once having gripped an end, eat its way along the chain as implied by publications on copepod foraging strategies (Brandl 1998) anatomical studies.(Paffenhöfer & Loyd 1999) and models and observations of the orientation of prey particles in copepod feeding currents (Visser & Jonsson 2000) The *T. nitzschioides* cultures of the experiment reacted only weakly to the copepod-water, except for the chain numbers. The latter are the highest in the copepod-water treatments on day four. The mean cell numbers per chain are lower in the copepod-water treatments on this day. As in the case of the Drebes-medium August *T. rotula* strain, this does not point to a classical potential defence mechanism where larger aggregates prevent ingestion. This influence of the copepod-water on the *T. nitzschioides* cultures was only noticeable on day four.

In the *T. rotula* experiment, it was also investigated whether any of the treatments were influenced by bacteria numbers (Fig. 3.3) or vice versa. The ambivalent and complex character of bacteria-phytoplankton relationships is well known. Bacteria are not only important in nutrient cycling but also provide algae with growth stimulating

substances like e.g. vitamins (Parker, 1977, Donderski & Strzelczyk 1987, Strzelczyk & Donderski 1987), release oxygen tension to enhance photosynthesis (Mouget et al. 1995) while using algal excretion products like glycolate and other organic substances as substrate (Nalewajko et al. 1980, Jones & Cannon 1988). On the other hand, diatoms and bacteria are known to compete for the same nutrients (Caron et al. 1988, Brussaard & Riegman 1998, Danovaro 1998), diatoms also produce antibiotic substances that inhibit bacterial growth (Kellam & Walker 1989, Adolph et al. 2004) as well as bacteria showing algicidal activity (Fukami et al. 1997, Kim et al. 1999). Regarding the induction of cyclomorphosis, fish-associated chemicals enhanced, for example, phototactic downward swimming in *Daphnia*, but it was not the fish, but bacteria associated with the fish, that produced the kairomone (Ringelberg & Van Gool 1998). The role of the bacteria in the copepod-diatom interaction in this work can only be speculated upon, because the algae treatments themselves show no proven reactions regarding the results of the bacterial investigations.

It is conspicuous that the bacterioplankton was less abundant in the copepod-water treatments than in the treatments without copepod-water of both the *T. rotula* f/2-medium strains. Especially the abundances in the stirred copepod-water treatment were distinctly lower. It is interesting that the copepod-water influenced the bacteria in this way as one would assume that due to the presence of urea and ammonium as well as other physiological waste products in copepod water the bacterial numbers should have gone up as demonstrated in experiments by Roman et al. (1988). It may be the case that some substances in the copepod-water inhibit the bacterial growth and that this reaction is partially intensified by stirring.

If the f/2-medium *T. rotula* algae of the experiment became more robust and healthy by means of a substance in the copepod-water they may have been less susceptible to bacterial degradation than the non-copepod-water algae. This may have caused the smaller bacterial abundances in the copepod-water treatments. Based on this hypothesis it would be plausible that the stirred copepod-water treatment has the lowest bacterial abundances, because of the better dispersion of the copepod related products due to the stirring. If the copepod-water leads to better health while the algae are in a period of growth, this will explain why the copepod-water did not influence the bacterial abundances of the Drebes-medium experiment as well. The extremely high cell abundances of the *T. rotula* algae, which peaked at the end of the

Drebes-medium experiment, will have resulted in large amounts of algal degradation products having been available to bacteria. Thus, the abundances of the bacteria would have been higher compared to the f/2-medium experiment where the algae were not yet in the death phase.

All results suggest that neither *T. rotula* nor *T. nitzschioides* were significantly influenced by the presence of copepods in our experiments although both species showed reactions to the grazer-water treatments that are consistent with recent studies like the effect of shorter chains that would fit with observations on copepod feeding behaviour where long colonies would be a disadvantage since they do not keep predators from ingesting the algae. Also, the effect of the grazer presence on the bacteria abundances in the *T. rotula* f/2 experiments indicates interactions between these two trophic levels.

Diatoms are known to respond sensitively to environmental factors (Krawiek 1982). One of these factors, turbulence, was also investigated in this work.

The influence of the stirring was clearly observable in the *T. rotula* in f/2-medium. Here it caused lower cell abundances per litre (and lower biovolume), the algae cultures grew equally well and the stirred cultures (especially the April strain) had fewer chains per litre in comparison to the non-stirred cultures. Because of the low growth rates, the building of new chains was also low in comparison to the non-stirred cultures. In contrast to this, the *T. rotula* Drebes-medium cultures do not show the same reactions. A reason for these differences could be the higher initial cell numbers in the Drebes-medium cultures. This could have made a development of differences in the treatments impossible, because the growth of the cultures stagnated rapidly and almost stopped after day four. Another factor could have been the higher amount of trace elements in the f/2-medium and their facilitated dispersion by stirring that led to the different results, especially as the differences between the treatments of the f/2-medium became more pronounced from day four on.

It has been shown by Schöne (1970) that shaking the media for even a short period influenced the rhythm of cell division of *Skeletonema costatum* positively. In his work it may have been the case that all cultures were influenced by the slight daily shaking by hand which prevented the algae from sticking to the bottom. Regarding the

outcome of the results, this should have had no serious effect. Even if the daily shaking caused higher cell numbers in all cultures, the stirring provided for the S and SC treatments was much longer and stronger leading to the inhibition in the stirred *T. rotula* cultures.

Schöne (1970) as well as Sommer (1998), Arin et al. (2002) and Maar et al. (2002) also mention possible effects of the water turbulence on the cell itself. The shifting of the thin water film surrounding the cell could affect the exchange of ambient nutrients and thereby shift the outcome of the competition for nutrients with bacteria. Schöne also pointed out that by shaking cultures it is very easy to break diatom chains by mechanical force. Powerful turbulence caused by the sea disturbance in the Kieler Förde had a similar influence on the diatom chains. In his investigations (with *Chaetoceros curvisetus*, *Cerataulina bergonii* (Peragallo) Schütt, *Guinardia flaccida* Peragallo and *Skeletonema costatum*) he observed that the chains became shorter with increasing turbulence, and concluded that this probably happened due to mechanical chain breaking.

It is evident that there is no positive influence on the growth caused by better availability of nutrients during our experiments. Regarding the mean cell number per chain only the stirred August *T. rotula* in f/2-medium evinced decreasing chain lengths. Considering possible disruptions of the diatom chains caused by the stirring, a smaller mean cell number per chain seems likely, although it is unclear why this happened only with the August strain of the *T. rotula*. It is speculation to suggest a higher sensitivity of the August algae in this state of growth, and should be further investigated.

Schöne (1970) also showed in his study on *Skeletonema costatum* that the ageing of a population can influence the chain length and the chains can become smaller with increasing age. In our work, this may have happened at the end of the *T. nitzschioides* experiment and the *T. rotula* in the Drebes-medium experiment.

Generally, the chain formation of the *T. nitzschioides* cultures appears not to be influenced by stirring. However, the stirred cultures had more chains per litre. This leads to the conclusion that the stirring led to disruptions of the chains of *T. nitzschioides* and thus the number of chains increased, while the colonies became smaller.

These results demonstrate that both the *Thalassiosira rotula* and the *Thalassionema nitzschioides* algae are influenced by stirring. *T. rotula* is inhibited in growth and a reduction of the chain length is apparent in both the *T. rotula* f/2-medium August and the *T. nitzschioides* cultures.

Regarding all results of our experiment, there remain many questions for further studies. It would be of great interest to investigate the reactions of the diatoms to direct grazing by copepods under different nutrient concentrations and in return look for any effects of algal products on the predators. Also, relations between diatoms, copepods and bacteria would be worth studying. And finally, the influence of more physical factors like turbulence or mechanical stress in general should be taken into account, especially when laboratory experiments are compared to outdoor data.

Conclusions

The use of two different culture media in the experiments with *T. rotula* showed, that the choice of medium, even if both provide all major nutrients, does have an effect on the results. Regarding physical stress, it can be concluded that the stirring influenced the development of the *Thalassiosira rotula* and the *Thalassionema nitzschioides* cultures regarding growth rates and chain lengths. The grazer presence simulated by the use of copepod water led to shorter chains in the case of *T. rotula* which is consistent with recent research on copepod foraging behaviour indicating that smaller colonies are of advantage when to avoid grazing. The bacterial growth is inhibited in the copepod water of *T. rotula* f/2 medium cultures. Overall, although isolated at different times of the year, the two *T. rotula* clones show little physiological variability under the experimental conditions.

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Algal Growth in Long-term Batch Cultures: Pattern Definitions

The phytoplankton community at Helgoland is one of the best monitored worldwide. But despite the long time of observation and the amount of data archived in the Helgoland Roads Long-term Series, it is difficult to find the mechanisms that shape the algal community since almost nothing is known on the physiological characteristics of the algae involved. This is a common problem in the literature where often either outdoor data or laboratory studies are presented. In addition, some foodweb interactions like the relationships between algae and bacteria are difficult to be observed in the ocean.

The aim of this study was to obtain insights into the nutrient requirements, growth, and physiological characteristics of important microalgal species as well as their associated bacterial community of the Helgoland foodweb. In batch culture experiments the development of nutrient concentrations, algal growth, cell size and photosynthetic efficiency were followed over a period of eight weeks. Apart from the similar development of photosynthetic efficiency and its relation to nitrate concentrations, different responses to the culturing with respect to growth rates and cell sizes were observed, showing a diversity of strategies and pointing to species specific adaptations. The results can now be reapplied to field data, aiding in the interpretation of the observed processes in the phytoplankton community.

Introduction

Phytoplankton succession and foodweb interactions are important subjects in aquatic ecology. The literature is highly diverse with reports describing all kinds of marine and freshwater environments, ranging from small artificial systems (Beulker et al. 2003, Kim et al. 2003) and extreme habitats (e.g. McMinn et al. 2000) to studies on changes in phytoplankton communities for large areas, being lakes (Salmaso 2003) or coastal as well as offshore waters of marine environments (Tillmann et al. 1999, Bernardi Aubry et al. 2004, Feuerpfeil et al. 2004). As diverse as the areas and locations of these sites are the chemical and hydrographical parameters influencing the plankton communities. There are studies in highly acidic lakes (Beulker et al. 2003) and environments with all kinds of trophic levels (e.g. Scheffler & Padisak, Roelke & Buyukates 2002, Feuerpfeil et al. 2004) as well as reports focusing on the effects of turbulence, stratification (Lindenschmidt & Chorus 1998, Merico et al. 2004), changes in salinity, wind, tides (Eslinger & Iverson 2001, Joordens et al. 2001) and temperature (Mei et al. 2002, Wiltshire & Manly 2004). Despite all of this variety, the common bottom line of all these studies is that beyond mere description, it is very difficult to find the mechanisms behind the observed phytoplankton succession in the multitude of interactions in natural environments. Even in such intensively monitored systems as the plankton community of Helgoland, where for more than 40 years, samples have been taken work-daily and the results of the cell counts been documented in the Helgoland Roads Long-term Series. From this data set, the key species of the system were already identified, based on abundance and number of blooms (Mieruch et al. 2006). But besides the large amount of data on abundances and oceanographical parameters, no literature on physiological and morphological characteristics of these species or foodweb interactions exists, following the general problem that studies often either present outdoor data or laboratory experiments for specific systems or species. Therefore it is necessary to obtain detailed insight or “fingerprints” of the involved species to better understand and interpret outdoor measurements. In the laboratory, biotic and abiotic factors influencing the growth of algae can be studied under controlled conditions, providing important background information. The results can then be applied to other data from the same community, providing a basis for interpretation. Furthermore, there are important ecological relationships that are difficult to study in nature, such as the complex interactions between phytoplankton species and bacteria.

The influential role of bacteria for the phytoplankton community is well known. Apart from the degradation of dead diatom cells and nutrient cycling, relationships range from parasitism (Fukami et al. 1997, Lovejoy et al. 1998) to competition for nutrients (Grossart 1999) and to symbiosis (Stewart et al. 1997, Croft et al. 2005). This is especially noticeable regarding algal cultures. As several studies report, diatoms tend to grow less in axenic environments (e.g. Grossart 1999, Sanders et al. 2001), lacking essential nutrients produced by bacteria as for example vitamins (Donderski & Strzelczyk 1987) or growth promoting substances like proline (Stewart et al 1997), while bacteria profit from algal extracellular products, e.g. glycolate (Nalewajko et al 1980) and other organic carbon exudates (Myklestad et al. 1972, Darley 1977, Percival et al. 1980, Bell 1984).

The bacteria associated with diatoms can either be attached to the cells themselves or free-living in the area around algae defined as the “phycosphere” (Bell & Mitchell 1972). Several studies (Hold et al. 2001, Schäfer et al. 2002) have shown a general specificity of bacteria associated with diatom species, leading to the assumption that the algae have a certain selecting influence while the bacteria themselves seem to be generalists regarding their choice of hosts (Michel 2006).

The aim of this study was to obtain first insights into growth, the nutrient requirements and physiological characteristics of algae regularly found in the phytoplankton community during blooms. These traits were unknown so far but are crucial to the understanding of the mechanisms behind phytoplankton succession, not only for the interpretation of existing data but also for foodweb models and predictions with regard to climate change.

To assess the population development, photosynthetic efficiency and cell sizes of seven phytoplankton species under increasing nutrient depletion, laboratory experiments with batch cultures were performed. Additionally, the relationships of growth rates and photosynthetic quantum yield with bacterial abundances were investigated. Up to date, the majority of studies on phytoplankton ecology has been carried out examining freshwater systems. Here, we present results from a marine environment.

Material and methods

Algal cultures

The single cells for all diatom and dinoflagellate cultures were isolated in 2002 from samples taken with 20 µm and 80 µm meshed plankton nets at Helgoland. After isolation, the cultures were kept in Guillard's f/2-medium (Guillard 1975) at 16 °C and a light-dark cycle of 12:12 hours with 40.0 µmol photons m² sec⁻¹ during illumination periods.

For the experiment, six key species of the Helgoland Roads phytoplankton community were chosen: *Guinardia delicatula* (Cleve) Hasle, *Pseudonitzschia pungens* Grunow, *Rhizosolenia pungens* Cleve-Euler, *Thalassiosira rotula* Meunier (two strains, one isolated in April, one in August), *Ceratium horridum* (Cleve) Gran and *Akashiwo sanguinea* (Hirasaki) Hansen & Moestrup. The algae were incubated as batch cultures in Guillard's f/2 medium (Guillard, 1975) in 2 L glass bottles at 16 °C and a light-dark cycle of 12:12 hours with a light intensity of 40.0 µmol photons m² sec⁻¹. To start the experiment, two litres of medium were inoculated with 10ml of the respective algae culture in the early stationary phase. For each species, three replicates were incubated for eight weeks with samples taken from the original culture (0) and after 7, 14, 21, 28 and 56 days.

To determine cell numbers, the samples were counted in Sedgewick-Rafter chambers using the method introduced by Lund et al (1958). For each chamber a minimum of 400 cells or colonies was counted with colonies being noted as single units. In the case of colony formation, the cell number of a colony was recorded separately. Degrading and dead cells were also counted to gain a more detailed insight into the overall population development of each species. The three physiological states were determined by the condition of the chloroplasts and the cytoplasm. Cells with few chloroplasts and significantly reduced cytoplasm were recorded as dying, empty valves as dead. Growth rates were calculated as

$\mu = \frac{\Delta LNn}{\Delta t}$ (Sommer 1998). Cell size was determined using the formulas given in

Hillebrand et al (1999).

Measurements of nutrient concentrations

In order to monitor the nutrient depletion, ammonium, nitrite, nitrate, silicate and phosphate were measured photometrically according to Grasshoff & Johannsen

(1974) and Grasshoff et al (1999) with three replicates each. For the statistical analysis, changes in nutrient concentrations $\Delta_{\text{conc}} = \frac{\text{conc}_2 - \text{conc}_1}{t_2 - t_1}$ between measurements were used in parallel to algal growth rates.

Photosynthetic efficiency

To determine the effective quantum yield of photochemical energy conversion the PAM (Pulse Amplitude Modulation) method of fluorescence measurement was applied. Since an energy conversion of 100 % is impossible, a small fraction of the light energy absorbed by a cell is released, usually as chl a fluorescence originating in Photosystem II. These fluorescence emissions are highest when the yield of photochemistry and heat dissipation, the two other pathways of de-excitation, are lowest. Therefore, fluorescence yields directly reflect the efficiency of a plant's photosynthetic performance. To determine the quantum yield of Photosystem II the first initial fluorescence (F_0) is induced by low levels of actinic light after a period of dark adaptation. When the emission reaches a stable level, a strong pulse of light (Saturation Pulse) is applied. This leads to a quick reduction of the electron transport chain between the two photosystems, forcing the cells to emit all energy as fluorescence. This maximum fluorescence value (F_m) is recorded and the effective quantum yield calculated from $F = \frac{(F_m - F_0)}{F_m}$ for dark adapted and $F' = \frac{(F'_m - F'_0)}{F'_m}$ for previously light exposed samples (Genty et al. 1989). It reflects directly the electron flow through Photosystem II at the time of the measurement (Heinz Walz GmbH 1999)

The quantum yield was measured using a Xenon-PAM (Walz, Germany). It uses a Xe-flash light emitting a broad spectrum of light that can be adjusted to the desired wavelength by optical filters, in this case a SP 695 (short-pass filter, Schott) to avoid interfering longer wavelengths. Samples were dark-adapted for five minutes, then transferred into a 10x10 mm quartz cuvette and the fluorescence yield measured.

Bacteria counts

For the enumeration of bacteria 100 μl of fixed (1 % paraformaldehyde) and prefiltered (10 μm) samples were filtered through 0.2 μm membrane filters (millipore) and stained with Acridine Orange as described by Zimmermann and Meyer-Reil

(1974) with the modifications of Hobbie (1977) and counted using epifluorescence microscopy. All samples were counted under a Zeiss Axioskop 2 equipped with an Acridine Orange filter set).

Statistical analyses

The statistical methods applied were correlation matrices, ANOVAs with a Tukey's HSD as post-hoc test (STATISTICA 7, StatSoft, Inc., Tulsa, OK, USA), regression trees (Fig. 4.1 & 4.2) calculated in PS-Explore (VGSPS mbH, Neustadt/Wied, Germany)

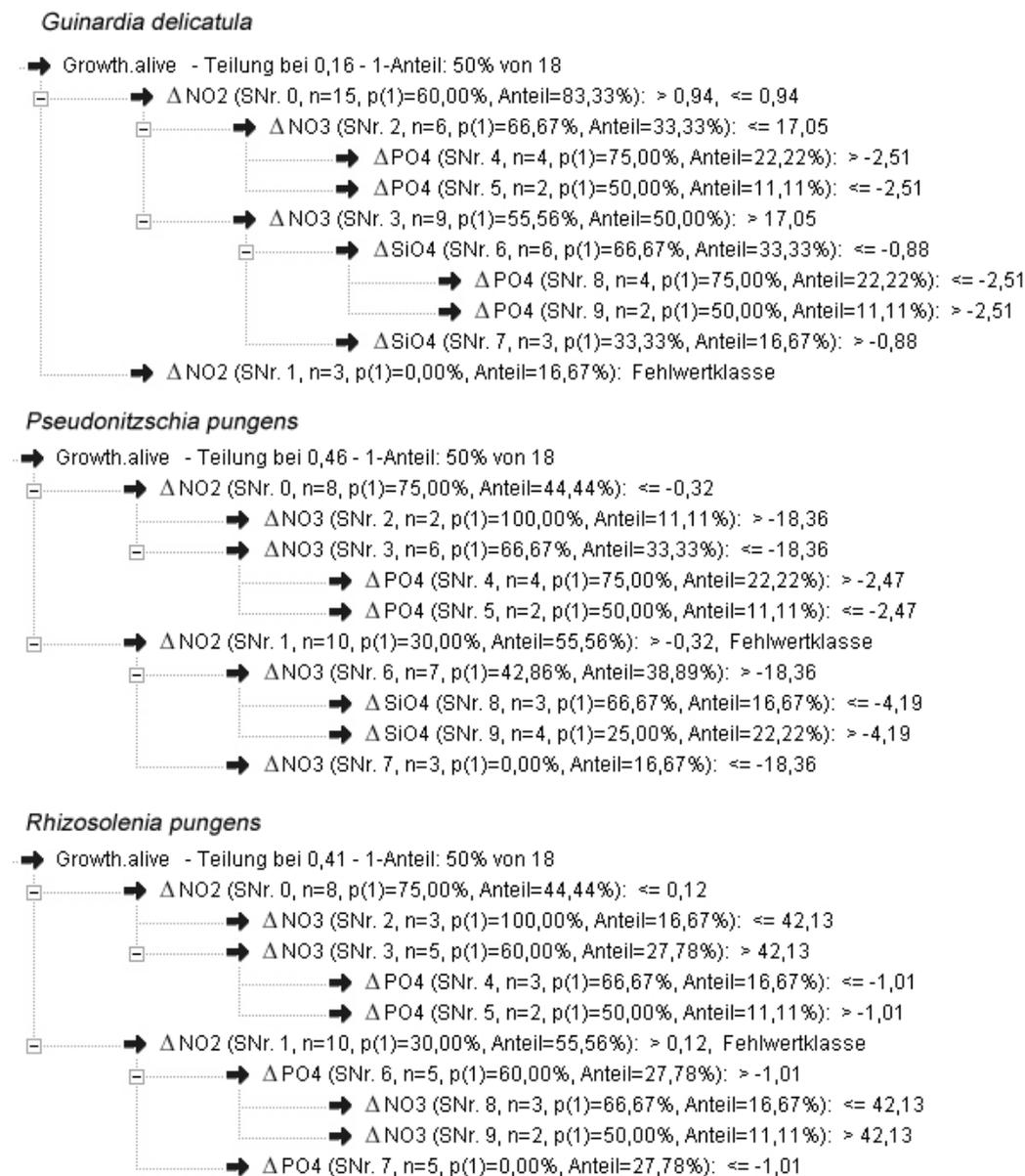


Fig. 4.1: Regression trees of the diatom data (PS-Explore). SNr: segment number, n: number of cases in a segment, p(1): probability that cases of this segment have that given or a higher growth rate, Anteil: percentage of the whole data set.

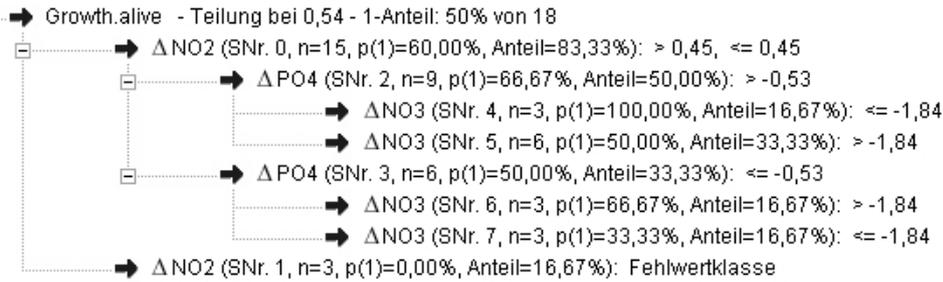
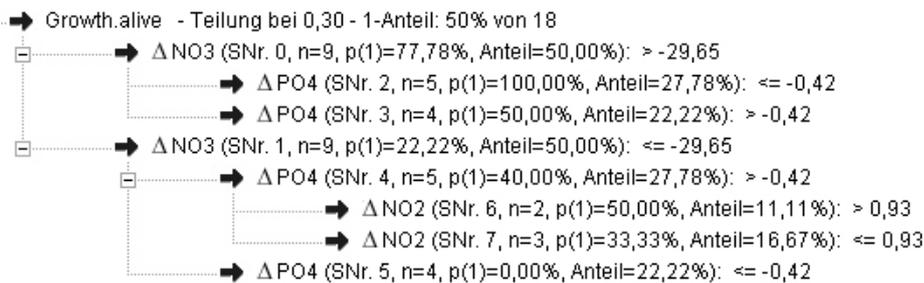
Thalassiosira rotula (spring)*Thalassiosira rotula* (summer)

Fig. 4.1 cont.: Regression trees of the diatom data (PS-Explore). SNr: segment number, n: number of cases in a segment, p(1): probability that cases of this segment have that given or a higher growth rate, Anteil: percentage of the whole data set

and PCAs to test for patterns and mutual correlations between all parameters (Fig. 4.3). For these analyses, Brodgar (Highland Statistics Ltd., Newburgh, UK) was used. Before the analyses, the datasets were examined for outliers and square root or logarithmic transformations applied when necessary. Since a majority of samples showed an arch effect, an artefact typical for ordination methods and frequently encountered in biological data sets or experiments dealing with long gradients, the chord-distance transformation was applied before resuming the analysis (Zuur & Ieno 2006).

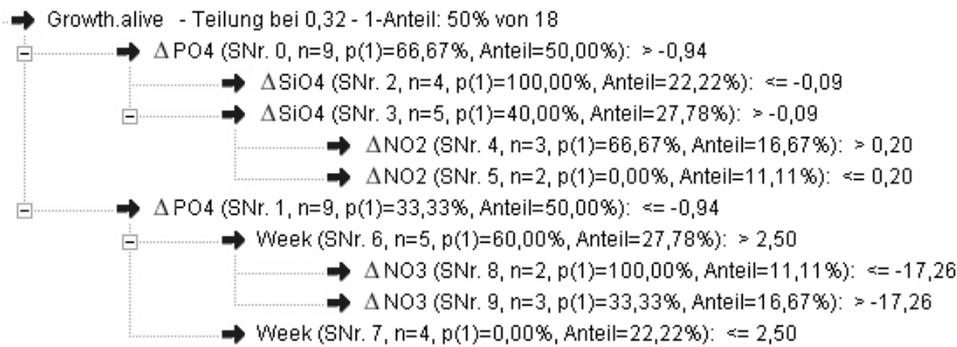
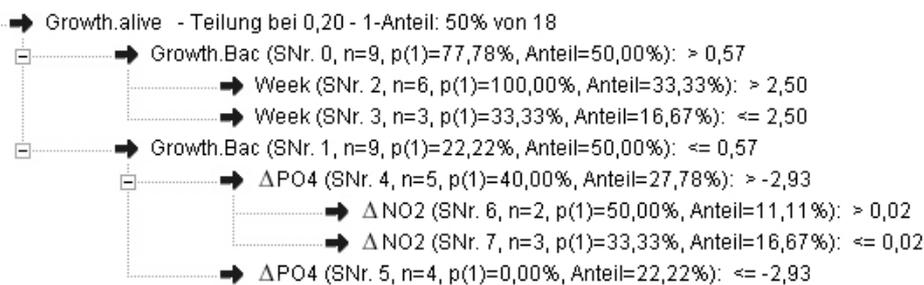
Akashiwo sanguinea*Ceratium horridum*

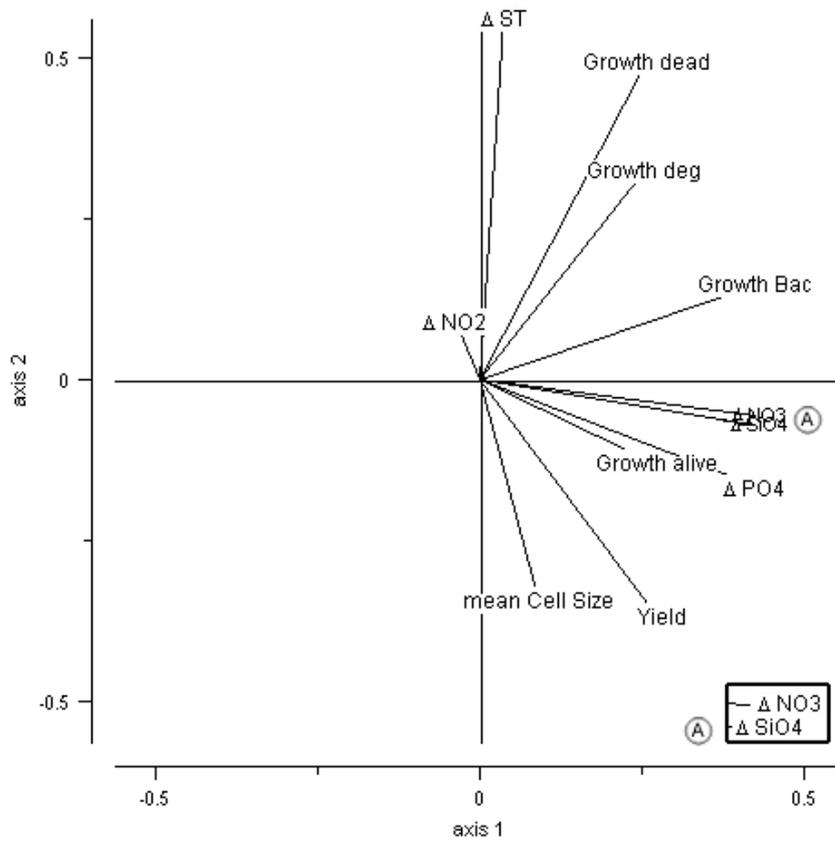
Fig. 4.2: Regression trees of the dinoflagellate data (PS-Explore). SNr: segment number, n: number of cases in a segment, p(1): probability that cases of this segment have that given or a higher growth rate, Anteil: percentage of the whole data set

Percentages of variances explained by the first two axes are given in Table 4.1.

Table 4.1: Cumulative percentages of the variance explained by the first two axes of the PCA biplot (cumulative percentage of the sum of all canonical eigenvalues).

Species	Data Transformation	Variance explained by the first two Axes
A. sanguinea	Chord Distance	68,21%
C. horridum	Chord Distance	48,60%
G. delicatula	Chord Distance	59,93%
P. pungens	Chord Distance	59,12%
R. pungens	Chord Distance	54,82%
T. rotula (spring)	Chord Distance	70,76%
T. rotula (summer)	Chord Distance	62,83%

A. sanguinea



C. horridum

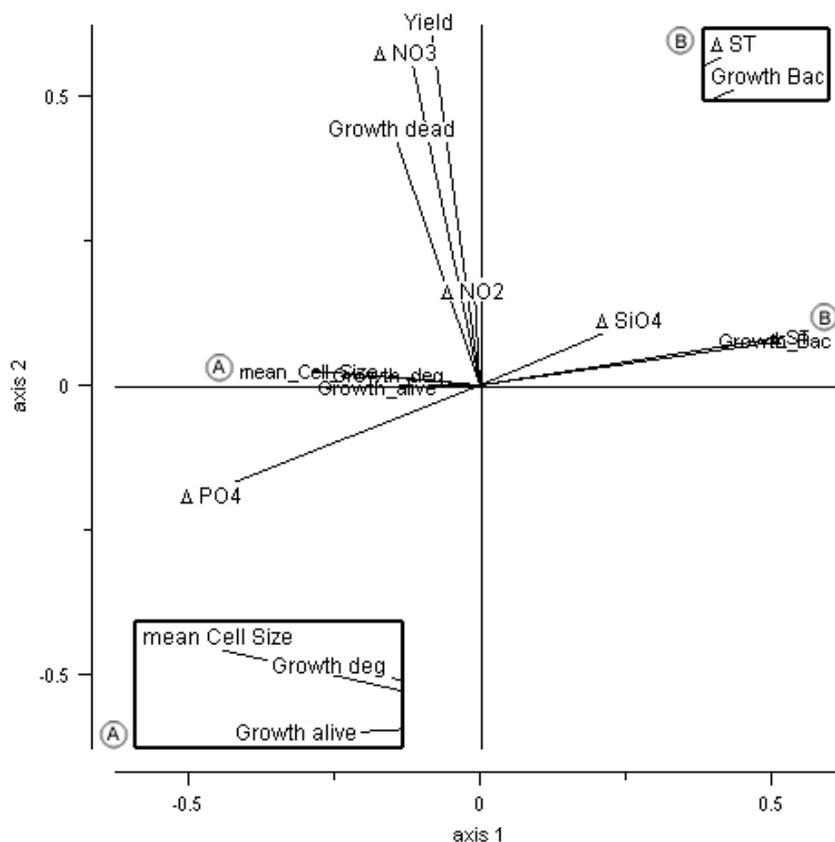
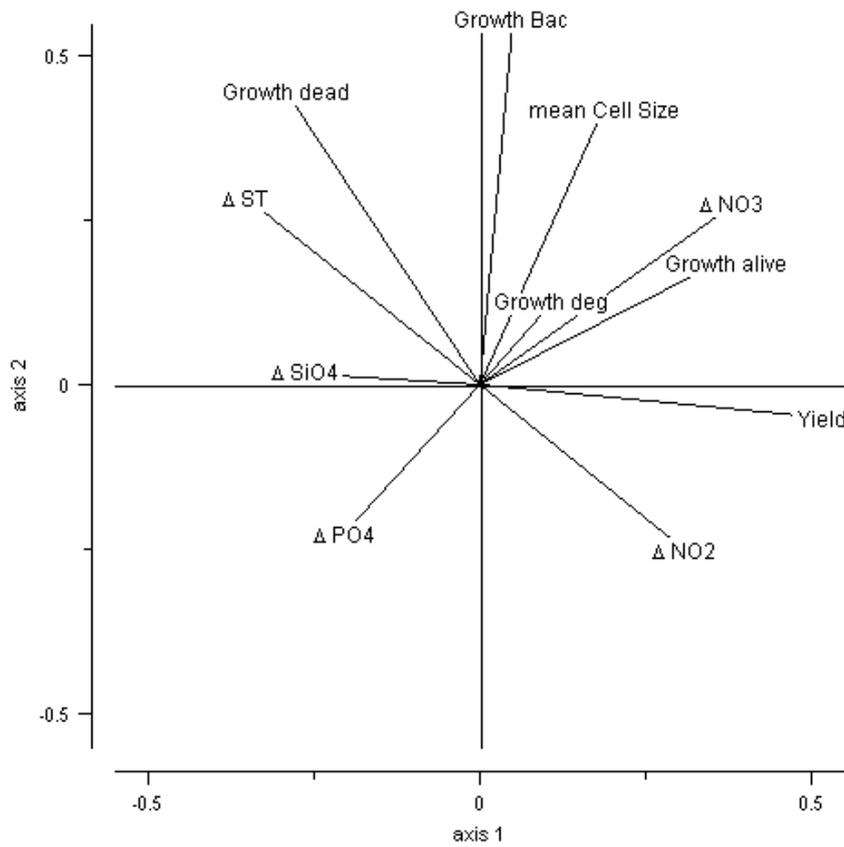


Fig. 4.3: PCA plots (Brodgar). The longer a line, the better it is represented by an axis. Correlations are indicated by the angles between variables. Small angles show positive correlations, 90° are neutral relations and angles above 90° indicate negative relationships.

G. delicatula



P. pungens

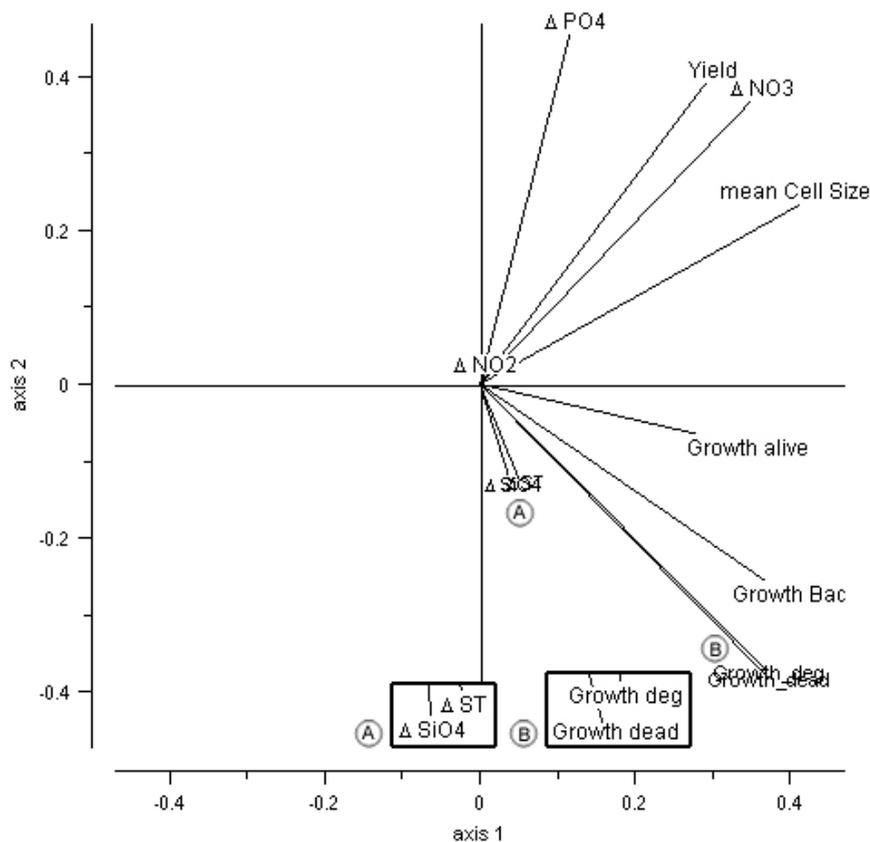
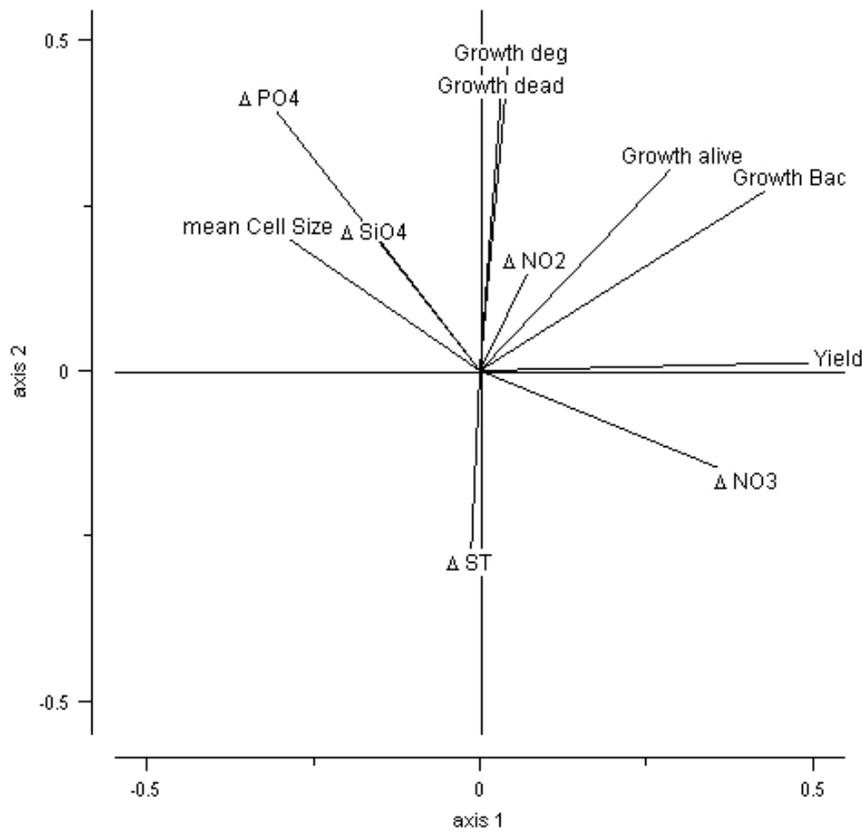


Fig. 4.3 continued: PCA plots (Brodgar). The longer a line, the better it is represented by an axis. Correlations are indicated by the angles between variables. Small angles show positive correlations, 90° are neutral relations and angles above 90° indicate negative relationships.

R. pungens



***T. rotula* (spring)**

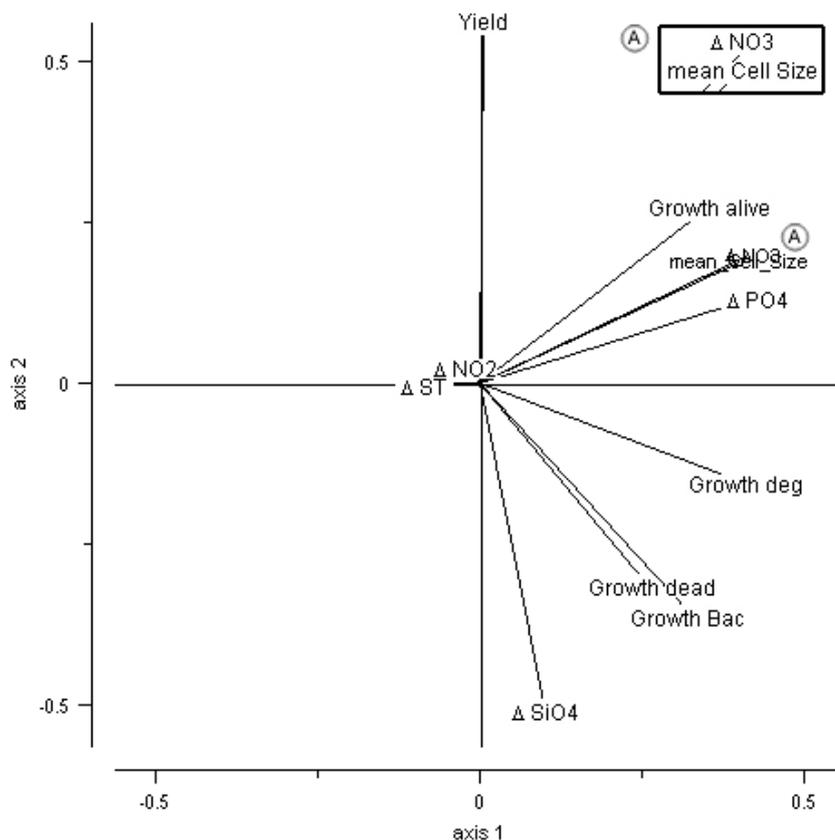


Fig. 4.3 continued: PCA plots (Brodgar). The longer a line, the better it is represented by an axis. Correlations are indicated by the angles between variables. Small angles show positive correlations, 90° are neutral relations and angles above 90° indicate negative relationships.

***T. rotula* (summer)**

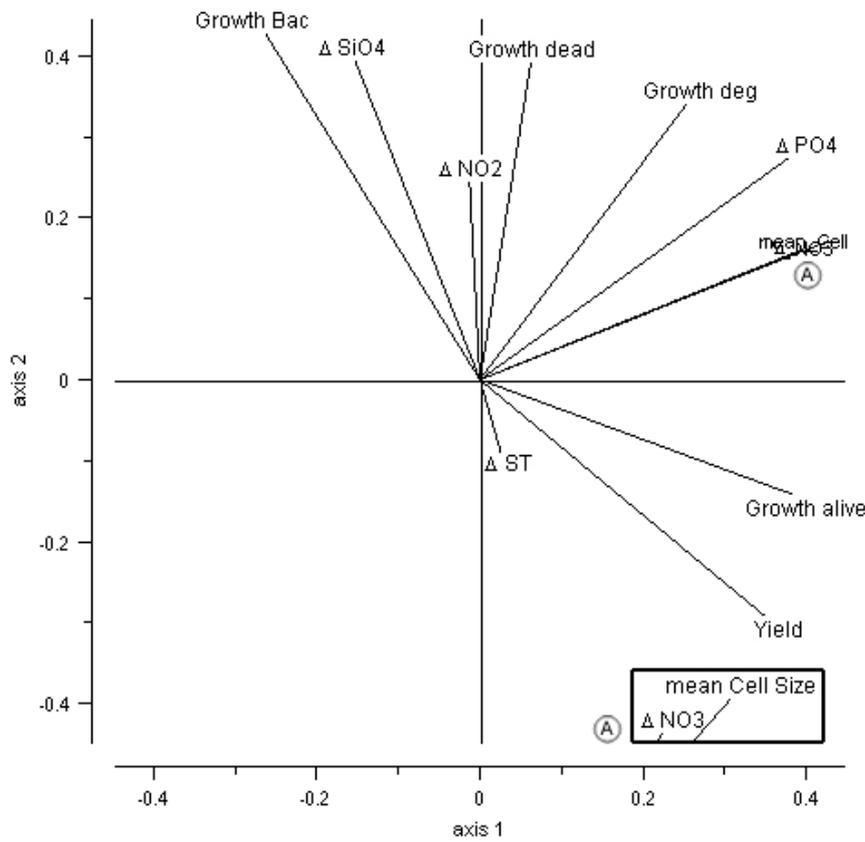


Fig. 4.3 continued: PCA plots (Brodgar). The longer a line, the better it is represented by an axis. Correlations are indicated by the angles between variables. Small angles show positive correlations, 90° are neutral relations and angles above 90° indicate negative relationships.

Results

Nutrient concentrations

Generally, nutrient concentrations varied substantially between species and replicates. Nitrite concentrations (Fig. 4.4) in all cultures had initial mean values

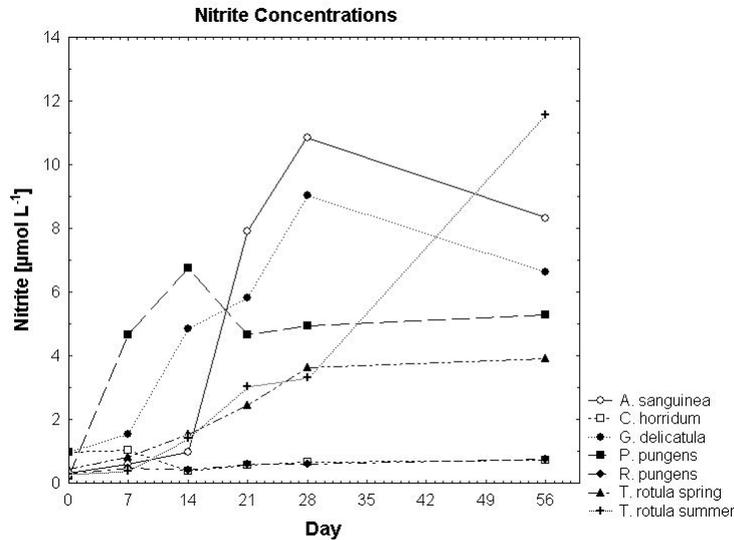


Fig. 4.4: Mean nitrite concentrations. Open symbols: dinoflagellates, filled symbols: diatoms. Standard deviations are given in table 4.2.

between 0.2 and 1.0 $\mu\text{mol L}^{-1}$. With the exception of *C. horridum* and *R. pungens*, where values remained at the same level for the whole experiment, the concentrations started to rise with the highest levels generally observed between days 14 and 21. Only in the cultures of *T. rotula* (summer), the concentrations reached their highest mean value of 11.6 $\mu\text{mol L}^{-1}$ at the end of the experiment, which also was the overall highest concentration observed. For most species, the curves matched either the growth rates of living cells, the rates of dying or dead cells. But only in the case of the dinoflagellate *A. sanguinea* were these correlations found to be significant where nitrite concentrations had a positive relationship with the number of dead cells ($r^2 = 0.27$; $p = 0.028$) and a negative one with growth rates of living dinoflagellates ($r^2 = 0.24$; $p = 0.042$) for *A. sanguinea*. However, with the exception of *C. horridum*, changes in nitrate concentration were significantly negatively correlated with growth rates of living cells of all species ($0.26 \leq r^2 \leq 0.77$; $0.00 \leq p \leq 0.05$). Although the PCA biplots could not confirm those correlations, the regression trees also showed the strong influence of changes in nitrite concentrations on diatom growth rates.

Although nitrate concentrations (Fig. 4.5) varied over the course of the experiment with mean values between $135.8 \mu\text{mol L}^{-1}$ and $379.6 \mu\text{mol L}^{-1}$ without clear general trends or becoming limiting, correlation matrices, regression trees and PCA plots showed the strong positive influence of changes in nitrate concentrations on the photosynthetic efficiency of all species ($0.26 \leq r^2 \leq 0.87$; $0.00 \leq p \leq 0.031$) as well as on the growth rates in cultures of *G. delicatula*, *R. pungens*, *T. rotula* (spring) and *T. rotula* (summer) ($0.33 \leq r^2 \leq 0.74$; $0.00 \leq p \leq 0.05$).

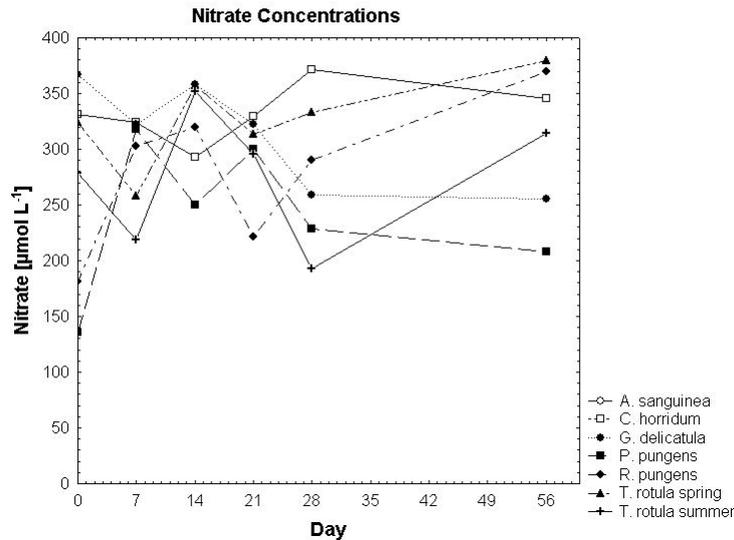


Fig. 4.5: Mean nitrate concentrations. Open symbols: dinoflagellates, filled symbols: diatoms. Standard deviations are given in table 4.2.

In all diatom cultures, silicate (Fig. 4.6) was almost completely taken up within the first two weeks of the experiments, decreasing from an initial range between $44.9 \mu\text{mol L}^{-1}$ and $52.5 \mu\text{mol L}^{-1}$ to concentrations between $0.1 \mu\text{mol L}^{-1}$ and $0.3 \mu\text{mol L}^{-1}$ at day 14. The only minor exception were the cultures of *P. pungens*, where a level between $3.5 \mu\text{mol L}^{-1}$ and $6.1 \mu\text{mol L}^{-1}$ was maintained and concentrations even rose again to $23 \mu\text{mol L}^{-1}$ at day 21. But towards the end of the experiment, concentrations had also decreased to $0 \mu\text{mol L}^{-1}$. Except for *G. delicatula*, silicate concentrations were significantly negatively correlated with the growth rate of living cells in diatom cultures ($0.30 \leq r^2 \leq 0.73$; $0.000 \leq p \leq 0.018$). Correlations with changes in silicate concentration generally also followed this pattern.

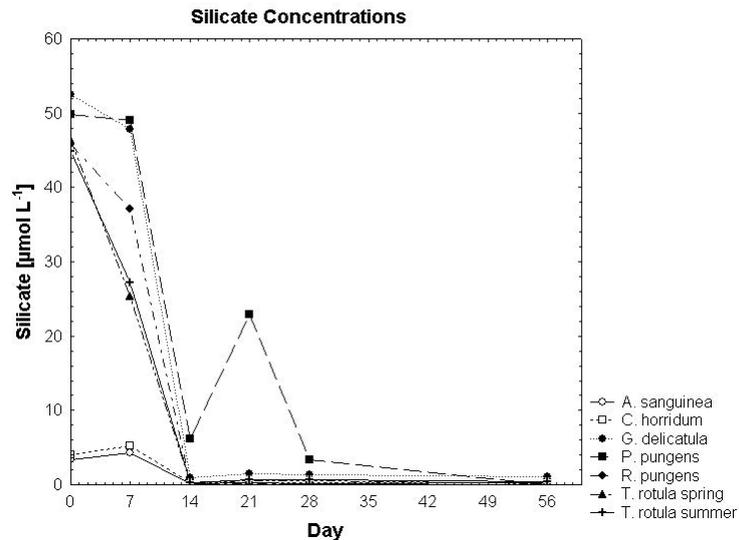


Fig. 4.6: Mean silicate concentrations. Open symbols: dinoflagellates, filled symbols: diatoms. Standard deviations are given in table 4.2.

For phosphate concentrations (Fig. 4.7), a general declining trend was observed, although the rates varied between species. The highest initial concentration was

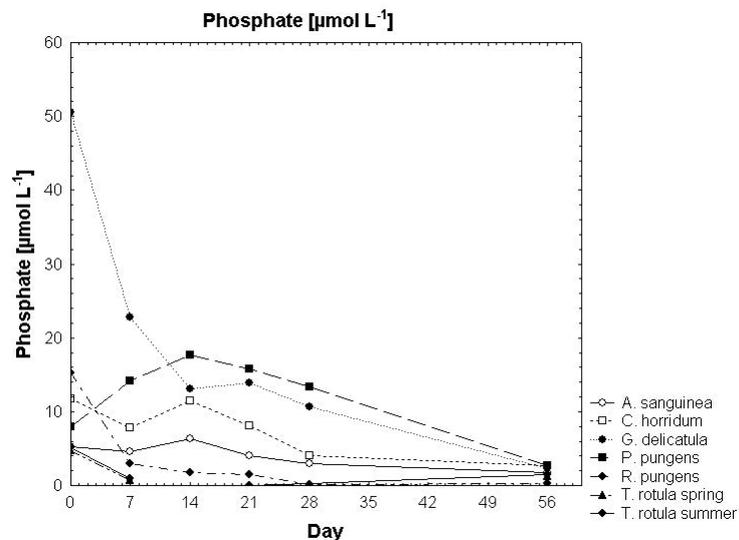


Fig. 4.7: Mean phosphate concentrations. Open symbols: dinoflagellates, filled symbols: diatoms. Standard deviations are given in table 4.2.

measured in the cultures of *G. delicatula* with $50.6 \mu\text{mol L}^{-1}$ while for all other species, levels between $4.7 \mu\text{mol L}^{-1}$ and $15.3 \mu\text{mol L}^{-1}$ were found. The concentrations in *G. delicatula*, *R. pungens* and both *T. rotula* strains declined continuously. In the case of

both dinoflagellates a rise between days seven and 14 was observed after an initial decline before a steady decrease towards the end. In the cultures of *P. pungens*, the

values rose from the beginning until day 14 and then also declined towards the end of the experiment. On day 56, the final concentrations of all cultures showed mean values between $0.3 \mu\text{mol L}^{-1}$ and $2.7 \mu\text{mol L}^{-1}$. Generally, changes in phosphate concentrations were positively correlated with algal growth rates and in the cases of *A. sanguinea*, *R. pungens* and both strains of *T. rotula* this relationship was significant ($0.26 \leq r^2 \leq 0.77$; $0.00 \leq p \leq 0.03$). For both dinoflagellates, the regression trees showed changes in phosphate concentrations to be the most important influence on growth rates which, especially in the case of *A. sanguinea*, was also confirmed by the PCA. Positive relationships with cell sizes were found by the PCA for *A. sanguinea*, *C. horridum*, *P. pungens*, *R. pungens* and both strains of *T. rotula*.

Table 4.2: Nutrient concentrations

Variable	Species	Day 0		Day 7		Day 14		Day 21		Day 28		Day 56	
		mean	±	mean	±	mean	±	mean	±	mean	±	mean	±
NO_2^- [$\mu\text{mol L}^{-1}$]	<i>A. sanguinea</i>	0,3	0,1	0,6	0,3	1,0	0,4	7,9	12,1	10,9	10,2	8,3	8,7
	<i>C. horridum</i>	1,0	0,2	1,0	0,4	0,4	0,3	0,6	0,5	0,7	0,6	0,7	0,5
	<i>G. delicatula</i>	1,0	0,7	1,5	0,5	4,8	0,7	5,8	4,7	9,0	1,2	6,6	1,6
	<i>P. pungens</i>	0,2	0,2	4,7	3,8	6,7	1,9	4,6	4,0	4,9	3,7	5,3	4,1
	<i>R. pungens</i>	0,3	0,1	0,5	0,1	0,4	0,1	0,6	0,2	0,6	0,2	0,7	0,6
	<i>T. rotula</i> spring	0,4	0,0	0,8	0,1	1,5	0,4	2,4	0,5	3,6	0,8	3,9	0,5
	<i>T. rotula</i> summer	0,3	0,1	0,4	0,4	1,4	0,4	3,0	0,6	3,3	0,4	11,6	9,7
NO_3^- [$\mu\text{mol L}^{-1}$]	<i>A. sanguinea</i>	331,0	0,2	324,1	6,9	292,7	18,6	329,8	3,1	371,6	6,6	345,4	4,9
	<i>C. horridum</i>	325,6	0,2	323,2	2,3	349,1	0,5	299,9	44,8	283,8	58,4	365,2	1,0
	<i>G. delicatula</i>	3,7	1,2	322,0	2,7	358,5	4,8	322,3	0,6	259,1	139,6	255,2	220,0
	<i>P. pungens</i>	135,8	0,2	318,2	21,7	250,3	196,4	299,9	9,3	228,8	85,4	208,2	78,0
	<i>R. pungens</i>	181,6	1,0	303,1	27,8	320,0	25,9	221,4	14,9	290,7	29,4	370,0	6,9
	<i>T. rotula</i> spring	324,6	0,2	258,4	79,5	356,9	0,5	313,6	13,6	332,9	1,3	379,6	0,8
	<i>T. rotula</i> summer	278,9	3,5	219,1	169,9	352,0	2,3	295,3	45,6	192,6	21,6	314,2	71,9
SiO_2^- [$\mu\text{mol L}^{-1}$]	<i>A. sanguinea</i>	3,4	0,0	4,3	1,0	0,1	0,0	0,2	0,1	0,1	0,1	0,2	0,1
	<i>C. horridum</i>	4,0	0,0	5,2	2,2	0,2	0,1	0,3	0,2	0,2	0,2	0,3	0,2
	<i>G. delicatula</i>	52,5	0,0	47,9	0,9	0,9	0,5	1,5	0,0	1,3	0,0	1,1	0,2
	<i>P. pungens</i>	49,8	0,0	49,0	0,7	6,1	3,4	23,0	8,6	3,4	3,5	0,0	0,0
	<i>R. pungens</i>	45,8	0,0	37,1	4,5	0,2	0,1	0,1	0,1	0,0	0,0	0,0	0,0
	<i>T. rotula</i> spring	46,4	0,0	25,3	6,5	0,3	0,1	0,6	0,0	0,6	0,0	0,1	0,1
	<i>T. rotula</i> summer	44,9	0,0	27,2	8,0	0,3	0,1	0,7	0,2	0,7	0,1	0,5	0,1
PO_4^{3-} [$\mu\text{mol L}^{-1}$]	<i>A. sanguinea</i>	5,3	0,9	4,6	1,4	6,3	1,9	4,0	0,4	3,0	2,0	1,8	1,2
	<i>C. horridum</i>	6,1	0,1	7,8	3,8	11,4	7,5	8,2	2,2	4,1	1,2	2,7	0,9
	<i>G. delicatula</i>	50,6	14,3	22,7	17,8	13,1	0,6	13,8	1,3	10,7	0,0	2,2	0,1
	<i>P. pungens</i>	11,8	0,2	7,8	3,3	11,4	6,5	8,2	3,1	4,1	1,1	2,7	1,0
	<i>R. pungens</i>	15,3	1,5	3,0	1,9	1,8	1,4	1,5	1,7	0,2	0,4	0,3	0,4
	<i>T. rotula</i> spring	4,7	0,4	0,7	0,2	-0,2	0,1	0,0	0,4	-0,2	0,1	1,2	0,5
	<i>T. rotula</i> summer	5,1	0,2	0,9	0,2	-0,1	0,1	0,1	0,2	0,2	0,1	1,5	1,2

Algal growth rates

Of all the species (Fig. 4.8), *P. pungens* had the highest growth rate. The fastest starters were *R. pungens* and both strains of *T. rotula*. While *R. pungens* and *T. rotula* (spring) maintained high growth rates for the first 14 days, the growth of the summer culture of *T. rotula* began to decrease after day seven and growth rates became negative between days 14 and 28 and slightly increasing again towards day 56. *R. pungens* and *T. rotula* (spring) both maintained their fast growth until day 14 when in both cultures, the growth rates became negative until day 28 before reaching a plateau with a slightly negative tendency in the case of *R. pungens* and a slightly positive one in the cultures of *T. rotula* (spring). This pattern was also shown by the dinoflagellate *A. sanguinea*. Of all diatoms, *G. delicatula* had the slowest growth rates showing the highest increase in living cells in the first week and rates becoming negative after day 21. The longest lag phase of three weeks was shown by the dinoflagellate *C. horridum* whose cell numbers then continuously increased until the end of the experiment. Apart from the aforementioned correlations with nutrient concentrations, the number of bacteria had a significantly negative influence ($r^2 = 0.29$; $p = 0.021$) in the cultures of *T. rotula* (summer).

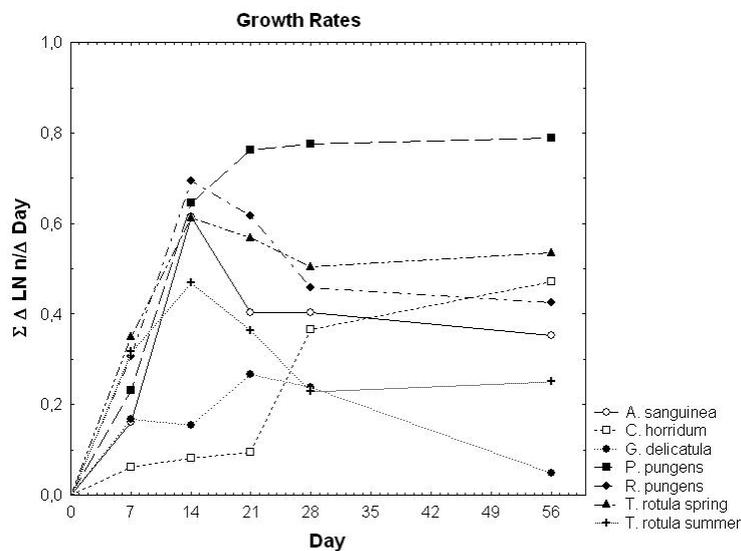


Fig. 4.8: Mean growth rates. Open symbols: dinoflagellates, filled symbols: diatoms. Standard deviations are given in table 4.3.

In the cultures with the highest initial growth rates (*R. pungens* and both *T. rotula* strains), degrading cells started to appear after the first week of the experiment, their numbers increased fast between day seven and 14 and then reaching a plateau. Apart from *G. delicatula*, where dying cells were observed from day 21 onwards, dying individuals were observed from day 14 onwards in all other cultures. In the case of *P. pungens*, which also had a high initial growth rate, their numbers increased quickly until day 28 and then reached a plateau. In all remaining species, only few degrading cells were observed. Aside from the correlations with nutrients, a significant positive correlation ($r^2 = 0.28$; $p = 0.024$) with the number of bacteria was found in the cultures of *A. sanguinea*.

The highest mortality was shown by the dinoflagellate *A. sanguinea*. In these cultures, a fast increase in dead cells between days 14 and 21 was observed, slowing only little until day 28 and then reaching a plateau with a slightly negative slope until the end of the experiment. With the exception of *G. delicatula* and *C. horridum*, empty valves were observed from day 14 on in all other cultures, but in no case their increase in numbers was as fast as in the samples of *A. sanguinea*. While *P. pungens* showed a continuous increase in mortality although slowing down after day 28, mortality reached stable plateaus after day 28 for *R. pungens* and both strains of *T. rotula*. For *G. delicatula* and *C. horridum*, the species with the lowest growth rates, dead cells began to appear only from day 28 onwards. Again, the growth rate of bacteria was significantly positively correlated with the rate of increase of dead cells in the cultures of *A. sanguinea* ($r^2 = 0.51$; $p = 0.001$)

Mean cell size

Initially, cell sizes of all species dropped during the first week of the experiment (Fig. 4.9). Compared to the initial value, the decrease ranged from 2 % to more than 80 %. Except for *R. pungens*, biovolumes increased again between days seven and 14. During the following weeks, the development differed between species. While only little variance in cell size was observed in the cultures of *P. pungens* and *C. horridum*, the spring strain of *T. rotula* showed a maximum increase of the biovolume of 140 % of the initial value decreasing then again to about 110 %. The other species showing an increase of cell size over 100 % for more than two weeks was *G. delicatula* with a maximum size of $435 \mu\text{m}^3$ (126 %) at day 28. But in this case, the mean biovolume then decreased to about 70 % of the initial volume at the end of the

experiment. The cells of *T. rotula* (summer) shrunk to about 45 % (3405 μm^3) of the initial volume (7633 μm^3) and the cell size remained below 100 % over the whole experiment, with a final size of 68 % (5192 μm^3). The greatest drop in cell size was displayed by *R. pungens*. From an initial volume of 4674 μm^3 , it decreased to 790 μm^3 (16 %) and remained at this low level for the rest of the experiment. Apart from correlations with nutrients as described above, positive correlations with the overall number of living cells in the cultures were observed for *G. delicatula* ($r^2 = 0.23$; $p = 0.042$) and the growth rate of *P. pungens* ($r^2 = 0.31$; $p = 0.02$). In the case of *G. delicatula* the number of bacteria was also classified as significantly positive relationship ($r^2 = 0.29$; $p = 0.022$).

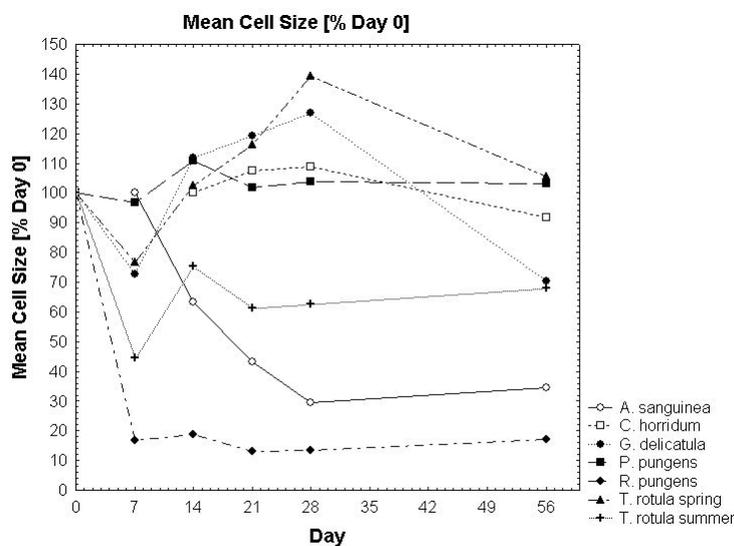


Fig. 4.9: Mean cell size. Open symbols: dinoflagellates, filled symbols: diatoms. Standard deviations are given in table 4.3.

In contrast, the numbers bacteria had a negative influence on the cell size of *R. pungens* as well as the growth rate of living cells in these cultures. The latter correlation was found to be significant with $r^2 = 0.60$ and $p = 0.0002$.

Photosynthetic efficiency

All species showed the same general pattern (Fig. 4.10) with regard to photosynthetic efficiency. They started with low to medium quantum yields at day 0 and showed a strong increase within the first seven days. Afterwards, quantum yields declined again over the next 14 days and then remained at medium levels. Exceptions were *A. sanguinea* and *G. delicatula* where the yield continued to decline until day 56. In the case of *C. horridum*, the curve of the photosynthetic efficiency

matched the growth rates, remaining low until day 21 and then showing the same pattern as the other species with a strong increase followed by a slow decline. Apart from correlations with nitrogen and other nutrients, the growth rates of living cells had a significantly positive influence on the photosynthetic efficiency in the cultures of *G. delicatula*, *A. sanguinea* and *C. horridum* ($0.26 \leq r^2 \leq 0.50$; $0.001 \leq p \leq 0.03$). Cell size on the other hand was found to be of significantly negative influence for *R. pungens* ($r^2 = 0.28$; $p = 0.024$) and *T. rotula* (spring) with $r^2 = 0.29$ and $p = 0.022$. For this strain of *T. rotula*, the growth rate of bacteria also had a significantly negative relationship with the quantum yield ($r^2 = 0.23$; $p = 0.044$).

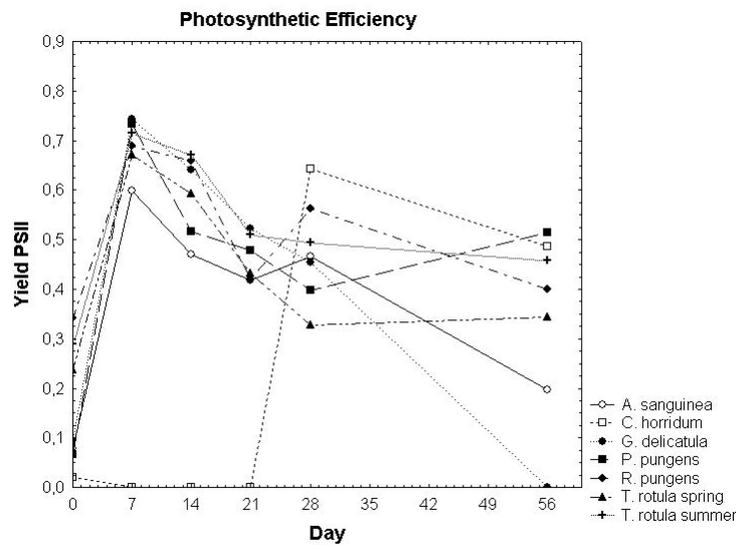


Fig. 4.10: Mean photosynthetic efficiency. Open symbols: dinoflagellates, filled symbols: diatoms. Standard deviations are given in table 4.3.

Bacterial growth rates

Bacterial growth was found to form three distinct groups (Fig. 4.11) depending on the phytoplankton species cultured. The lowest growth was found in the cultures of *P. pungens* and the spring strain of *T. rotula* where almost no increase in cell numbers was observed. Nevertheless, significantly positive correlations were found with the abundance of degrading ($r^2 = 0.39$; $p = 0.005$ and $r^2 = 0.64$; $p = 0.000$) and dead cells ($r^2 = 0.51$; $p = 0.001$ and $r^2 = 0.60$; $p = 0.000$). In the cultures of both dinoflagellate species, *A. sanguinea* and *C. horridum*, medium growth rates were found with the fastest increase during the first week of the experiment. In the case of *A. sanguinea*, bacterial abundance continued to rise until day 21 and then declined again slightly while in the cultures of *C. horridum*, the growth rate became negative between days seven and 14 and then slowly increased until the end of the experiment. For *A.*

sanguinea, significantly positive correlations were found with the rates of degrading ($r^2 = 0.26$; $p = 0.031$) and dead cells ($r^2 = 0.51$; $p = 0.001$). High initial growth rates during the first week were only found in the cultures of *G. delicatula*, *R. pungens* and *T. rotula* (summer), those diatom cultures where the abundance of living cells either never showed high growth rates or started to decline fast early during the experiment. For the bacterial population in the cultures of *G. delicatula* significantly positive correlations were only found with the mean cell size of the diatoms ($r^2 = 0.29$; $p = 0.022$) while the regression tree also identified the duration of the experiment as important factor. The abundance of bacteria in *R. pungens* cultures was again significantly positively correlated with degrading ($r^2 = 0.45$; $p = 0.002$) and dead diatom cells ($r^2 = 0.70$; $p = 0.000$). Dead diatom cells were also the main positive factor for bacterial growth in the case of *T. rotula* (summer) with $r^2 = 0.24$ and $p = 0.042$.

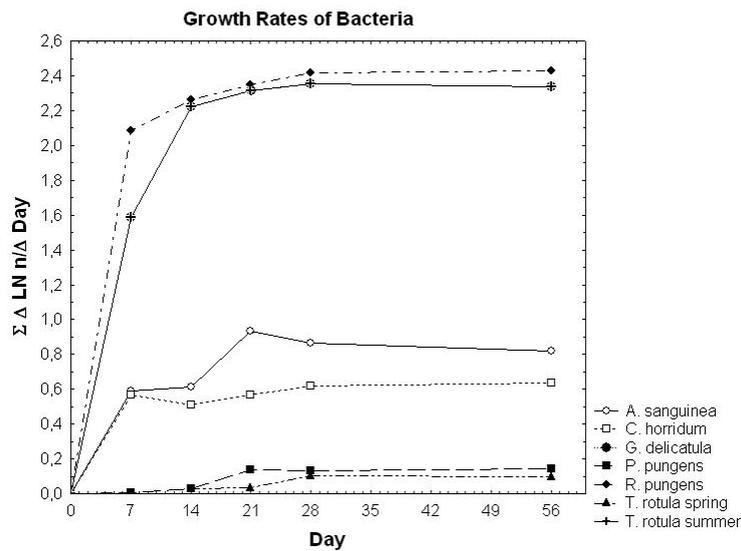


Fig. 4.11: Mean growth rates of bacteria. Open symbols: bacterial growth rates in dinoflagellate cultures, filled symbols: bacterial growth rates in diatom cultures. Standard deviations are given in table 4.3.

Table 4.3: Photosynthetic efficiency and cell counts. * = no standard deviation because of absent replicates.

Variable	Species	Day 0		Day 7		Day 14		Day 21		Day 28		Day 56		
		mean	±	mean	±	mean	±	mean	±	mean	±	mean	±	
Yield PSII	<i>A. sanguinea</i>	0,08	*	0,60	0,03	0,47	0,03	0,42	0,26	0,47	0,13	0,20	0,34	
	<i>C. horridum</i>	0,02	*	0,0	0,0	0,0	0,0	0,0	0,0	0,64	0,08	0,49	0,03	
	<i>G. delicatula</i>	0,10	*	0,74	0,02	0,64	0,02	0,52	0,18	0,45	0,05	0,0	0,0	
	<i>P. pungens</i>	0,07	*	0,73	0,02	0,52	0,06	0,48	0,07	0,40	0,03	0,52	0,05	
	<i>R. pungens</i>	0,34	*	0,69	0,01	0,66	0,01	0,42	0,04	0,56	0,08	0,40	0,07	
	<i>T. rotula spring</i>	0,24	*	0,67	0,02	0,59	0,02	0,43	0	0,33	0,01	0,34	0,03	
	<i>T. rotula summer</i>	0,29	*	0,71	0	0,67	0,01	0,51	0,02	0,49	0,17	0,46	0,14	
Cell counts	alive [cells ml ⁻¹]	<i>A. sanguinea</i>	3,75	*	12,92	6,88	282,50	64,55	322,50	337,25	200,83	227,64	247,50	426,52
		<i>C. horridum</i>	2,50	*	18,75	32,48	6,67	6,88	7,50	7,50	33,33	9,38	1105,87	762,24
		<i>G. delicatula</i>	81,25	*	263,13	53,13	235,63	8,13	561,25	255,00	462,78	238,04	0	0
		<i>P. pungens</i>	3,75	*	49,17	73,57	5821,25	9020,85	5493,06	5224,39	7191,67	6093,35	14058,33	13360,11
		<i>R. pungens</i>	87,50	*	815,00	360,31	11775,00	4340,15	7041,67	3483,20	2575,00	1855,57	1941,67	2843,89
		<i>T. rotula spring</i>	77,50	*	1651,88	1678,58	6750,00	5152,00	4161,11	546,30	2810,42	1118,84	7779,17	3067,58
		<i>T. rotula summer</i>	217,50	*	2255,42	1208,16	7151,39	5188,88	3110,83	1509,17	1859,72	1894,19	4250,83	4711,01
	degrading [cells ml ⁻¹]	<i>A. sanguinea</i>	0	*	0	0	0	0	3,33	2,89	2,50	2,17	6,67	5,77
		<i>C. horridum</i>	0	*	0	0	0,42	0,72	2,08	3,61	0,42	0,72	13,27	12,12
		<i>G. delicatula</i>	0	*	0	0	0	0,00	46,25	46,25	338,61	127,29	146,88	128,13
		<i>P. pungens</i>	0	*	0	0	6,25	10,83	406,94	284,38	1977,08	1405,82	3750,00	3415,86
		<i>R. pungens</i>	0	*	900,00	1014,89	4,25E+04	4,11E+04	1,26E+05	1,66E+04	7,33E+04	1,03E+04	9,98E+04	3,49E+04
		<i>T. rotula spring</i>	0	*	21,25	9,01	712,50	666,97	618,06	58,97	1078,47	477,44	1579,17	582,60
		<i>T. rotula summer</i>	48,75	*	40,42	21,52	680,56	695,85	550,42	110,68	931,94	591,91	941,67	685,72
	dead [cells ml ⁻¹]	<i>A. sanguinea</i>	0	*	0	0	0	0	41,67	14,43	166,67	112,73	455,83	763,55
		<i>C. horridum</i>	0	*	0	0	0,83	0,72	0	0	0	0	11,75	6,59
		<i>G. delicatula</i>	0	*	0	0	0,00	0,00	0	0	291,53	8,27	3309,38	559,38
		<i>P. pungens</i>	0	*	5,83	10,10	20,00	34,64	490,28	549,69	1531,25	899,28	11341,67	3516,95
		<i>R. pungens</i>	0	*	483,33	500,83	19666,67	27038,55	189166,67	36477,16	242000,00	107038,54	274166,67	141444,63
		<i>T. rotula spring</i>	0	*	1,04	1,80	447,92	525,53	626,39	172,01	1134,03	533,47	1787,50	1053,19
		<i>T. rotula summer</i>	11,25	*	9,17	13,77	491,67	496,45	552,92	246,72	1364,58	367,23	1977,50	1179,36
	Bacteria [cells ml ⁻¹]	<i>A. sanguinea</i>	7,45E+04	*	4,64E+06	*	5,48E+06	*	5,06E+07	*	3,22E+07	*	7,35E+06	*
		<i>C. horridum</i>	7,98E+04	*	4,29E+06	*	2,86E+06	*	4,26E+06	*	5,97E+06	*	1,20E+07	*
		<i>G. delicatula</i>	0,00E+00	*	6,62E+04	*	5,62E+06	*	1,08E+07	*	1,46E+07	*	7,57E+06	*
		<i>P. pungens</i>	9,99E+06	*	1,03E+07	*	1,23E+07	*	2,60E+07	*	2,49E+07	*	3,70E+07	*
<i>R. pungens</i>		5,87E+06	*	2,21E+06	*	7,48E+06	*	1,37E+07	*	2,22E+07	*	3,07E+07	*	
<i>T. rotula spring</i>		4,50E+06	*	3,87E+06	*	5,51E+06	*	5,71E+06	*	9,29E+06	*	8,01E+06	*	
<i>T. rotula summer</i>		3,05E+07	*	6,59E+06	*	9,24E+06	*	1,58E+07	*	3,49E+07	*	1,63E+07	*	

Discussion

Growth rates between species differed substantially. In most cultures, the maximum numbers of living cells was already reached at day 14. As the growth rates were negatively correlated with phosphate and silicate concentrations and positively with concentration changes, this matches the depletion of these nutrients within the first two weeks of the experiment although all species must have been able to store both silicate and phosphate inside their cells for later growth. This strategy is already known for diatoms (Martin-Jézéquel et al. 2000, Claquin et al. 2002), especially for algae living in unstable environments and having to deal with nutrient pulses (Spijkerman & Coesel 1998) which would be the general assumption for ecosystems. In contrast to silicate having a possible limiting effect on growth rates already at early stages, phosphate limitation is known to affect the final cell yield, resulting in lower overall abundances (Guerrini et al. 2000). A time span of about two weeks of growth until a crash of diatom populations has also been reported by Chai et al. (2002) for model runs. Apart from increasing grazing pressure, the authors also attributed the decline of diatom biomass to the exhaustion of the silicate pool. The highest initial growth rates were shown by both *T. rotula* cultures as well as *R. pungens*. From the

Helgoland Roads Long-term data, *R. pungens* is known to follow a “boom and bust” strategy during spring bloom events, producing high biomasses but only within a narrow window of favourable conditions. The longest lag phase was observed for the dinoflagellate *C. horridum* whose cell numbers started to rise after day 21. Because the cells of this species are fairly large, the strategy of *C. horridum* may be to take up as much nutrients as possible for storage before starting to reproduce. In field data, mixotrophy might be a factor for delayed responses to changes in nutrient concentrations since *Ceratium* species are known to prey on ciliates (Smalley & Coats 2002). However, since the strain used in this experiment had already been in culture for several weeks, growing on f/2 medium, this should not have been the case.

Degrading cells began to appear in cultures as early as day seven for *T. rotula* (both strains) and *R. pungens*, which had high initial growth rates. In the other cultures, the number of degrading cells started to grow from day 14 onwards, coinciding with the depletion of silicate and phosphorus.

The highest mortality was observed in the cultures of *A. sanguinea* with a fast increase in the abundance of dead cells already from day seven on. For the other species, dead cells started to appear at day 14, again with possible relations to the depletion of silicate and phosphorus pools.

With the exception of *P. pungens* and *C. horridum*, all algae showed a high variation of the mean cell volume over the course of the experiment. The cell size differed by as much as 80 % (*R. pungens*) from the initial volume. Apart from the dinoflagellate *C. horridum*, cell size was always more or less closely correlated with silicate and phosphate concentrations. As Masojídék et al. (2000) and Claquin et al. (2002) found in their studies on nutrient limitation with *T. pseudonana*, that both nitrogen and phosphorus limitation can result in an increased cell size. Additionally, in an environment with nutrient pulses, larger species can have the advantage of storing enough of the limiting nutrients to sustain several generations (Lean 1984, Suttle et al. 1987). On the other hand, a decrease in cell volume may also be a reaction to nutrient depletion as the resulting smaller diffusion boundary layers allow smaller cells to utilize resources more efficiently and thus to outcompete larger species under prolonged nutrient limitation (Lean 1984, Spijkerman & Coesel 1998). A decline in cell size seems to be a general trend in phytoplankton evolution (Raven 1998, Jiang

et al. 2005). Regarding our results, both uptake strategies may have been followed to deal with nutrient depletion.

Apart from the dinoflagellate *C. horridum*, whose cell numbers and photosynthetic reaction did not begin to increase before day 21 and *G. delicatula*, which showed a continuous downward trend after the optimum at day seven, all species followed a similar pattern. From initially low values due to the handling stress at the first day of the experiment, all algae reached an optimum quantum yield between 0.6 and 0.75 at day seven. Afterwards, yields slowly declined to medium levels and remained there for the rest of the experiment. In most cases, photosynthetic efficiency was closely related to nitrogen concentrations. The close coupling between photosynthesis, carbon and nitrogen metabolism and its significance for algal physiology, as many interactions depend on respiratory processes, has already been described in several studies (e.g. Turpin 1991). N-limitation can affect photosynthesis in many different ways, e.g. resulting in a decrease of nitrogenous pigments like chlorophylls and phycobilins (Allen & Smith 1969, de Loura et al. 1986, Falkowski et al 1989, Herzig & Falkowski 1989, Masojídek et al. 2000), selective inactivation of Photosystem II (Berges et al. 1996) and a decline in enzymes of photosynthetic carbon metabolism, e.g. Rubisco (Kuppers & Weidner 1980, Falkowski et al. 1989, Geider et al. 1998). Even under non-limiting conditions, photosynthetic quantum yield follows fluctuations in nitrogen concentrations (Lippemeier et al. 2001). Although these results cannot always be reproduced to the full extent in outdoor experiments (Babin et al. 1996), the important influence of nitrogen on algal photosynthesis is evident.

In most cultures, the number of bacteria was positively correlated with the abundances of degrading and dead cells. Bacteria had only little influence on growth rates of living cells, cell sizes and photosynthetic efficiency. However, since only the overall bacterial abundances were used in these analyses, the results also reflect only general trends in relationships. In a parallel investigation of the species-specific communities and interactions between bacteria and microalgae by Sapp et al. (2006), it was shown that the composition of the bacterial communities varied to different extents between algal species with the communities of *T. rotula* (spring) and *A. sanguinea* being most different from each other whereas the compositions in *P.*

pungens and *C. horridum* cultures were most similar. In the case of *G. delicatula*, bacteria showed a succession of species during the experiment with completely separated attached and free-living communities at the end of the experiment. In contrast, the bacterial community in *A. sanguinea* cultures did not vary over time. To identify the most prominent phylotypes in the different algal cultures, sequence analyses from excised DGGE bands were performed. The sequence data revealed the presence of two phyla of bacteria with most sequences being related to *Alpha-* and *Gammaproteobacteria* (43 % and 37 %). Additionally, members of the *Flavobacteria-Sphingobacteria* group within the *Bacteroidetes* phylum were found (16 % of all phylotypes). All of these groups have already been found to be important in the interactions between microalgae and bacteria in previous studies (Bidle et al. 2001, Hold et al. 2001, Wichels et al. 2004, Grossart et al. 2005).

The aim of this study was to obtain first insights into growth, the nutrient requirements and physiological characteristics of algae considered to be key species of the Helgoland foodweb. These traits were previously unknown but are crucial to the understanding of the mechanisms behind phytoplankton succession, not only for the interpretation of existing data but also for foodweb models and predictions with regard to climate change.

Apart from similar patterns, e.g. the development of photosynthetic efficiency and its relationship with nitrate concentrations, different responses to the experimental setup regarding growth rates and cell size were observed. Differences between strains and clones as reported by Krawiec (1982) and Liang et al. (2005) were also found in this study between the two strains of *T. rotula*. Except for *P. pungens*, growth rates did not follow the predicted curves and showed considerable differences between species. Substantial variances were also observed in the development of cell sizes, revealing different strategies in reaction to changing nutrient concentrations. The results may now be applied and compared to other studies and field data. Nevertheless, it is necessary to further investigate the autecological characteristics of key species under various experimental conditions to obtain a more precise set of “fingerprints”, especially for the use in models.

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Spring Bloom in a Bottle: Trying to understand the Mechanisms behind Phytoplankton Succession

Phytoplankton blooms are a well documented phenomenon. However, while the succession of species is also usually well documented, the mechanisms that drive it still remain unknown to a large extent. Correlations between algal behaviour and biotic and abiotic factors can provide valuable information. But, as long as little continues to be known about the requirements of individual species such correlations remain somewhat speculative. To obtain a better insight, we cultured three important diatom species from different phases during the 2004 spring bloom at Helgoland, under five different nutrient regimes at two grazer-related stress levels and at three temperatures. Photosynthetic efficiency, growth rates, individual biovolumes and colony sizes were measured. The results of this experiment can be linked to data from the 2004 spring bloom as well as the Helgoland Roads Long-term Series and show different life strategies, preferences and reaction patterns that might explain the observed succession during blooms.

Introduction

Whether looking at whole ecosystems, at parts of a foodweb or at single species, a multitude of factors and processes influence morphological and physiological reactions of individuals as well as their interactions with other organisms. Moreover, factors vary with time, in regularity, impact or may depend on each other. While some follow regular patterns related to seasonality with the rise and fall of temperatures and changes in day length, others occur as discrete events like the decreases in salinity or shifts in nutrient concentrations at Helgoland Roads by freshwater from the river Elbe (Hickel et al. 1993). These events in turn depend on other factors such as wind and currents.

The complexity and flexibility of a system is perhaps best seen in long-term series. However, while being able to follow the shifts and changes of a system together with biotic and abiotic parameters over time, long-term sets also accumulate a lot of background noise and scatter that can blur otherwise significant interactions. Therefore, while the Helgoland Roads Long-term Series is an excellent resource for documentation of long-term changes and general patterns, extracting information on the behaviour of individual species beyond trends is difficult.

One of these trends is the observation that the late blooming species *Guinardia delicatula* has increased in abundance on the expense of earlier species like *Thalassionema nitzschioides* and *Odontella aurita* alongside the documented rise in mean water temperatures of 1.1 °C since the beginning of sampling in 1962 (Wiltshire & Manly 2004, Wiltshire et al. 2005, Wiltshire et al. 2006 *in review*). Temperature is known to be of great importance for the timing of spring bloom events and the succession of species (e.g. Goffart et al. 2002, Murell & Lores 2004, Abrantes et al. 2006). But as mentioned before, species are influenced by many interacting factors. Therefore it is necessary to gain knowledge of the basic requirements of each species that can then serve as a basis for interpretation of existing data and provide insights into the mechanisms of the observed phytoplankton succession.

To find these basic requirements, laboratory experiments were used to study growth rates, cell sizes, chain lengths and photosynthetic efficiency of three key species of the Helgoland phytoplankton community at three different temperatures, simulated grazer presence and five nutrient treatments. The results were then compared to the

data from the spring bloom 2004 and the Helgoland Roads Long-term Series to see whether they were able to explain and supplement the patterns found in the outdoor data.

Material and Methods

Algae

During the spring bloom 2004, cultures of all species found in 25 μm net samples from Helgoland Roads were made from single cells isolated once per week by micropipetting (Daste et al. 1983) and subsequent washing. Cultures were maintained in Guillard's *f/2* medium (Guillard & Ryther 1962) and transferred to new bottles with fresh medium every two weeks. *T. nitzschioides* was isolated on March 4th at 4.2 °C, *T. rotula* on March 11th from a water temperature of 3.7 °C and *G. delicatula* on May 13th at 10.5 °C. Together with the plankton samples, 15 litres of water were taken at each sampling day for later use in the experiments. The water was passed through a 15000DA filter to remove any organisms and stored in darkness at 4 °C.

Setup

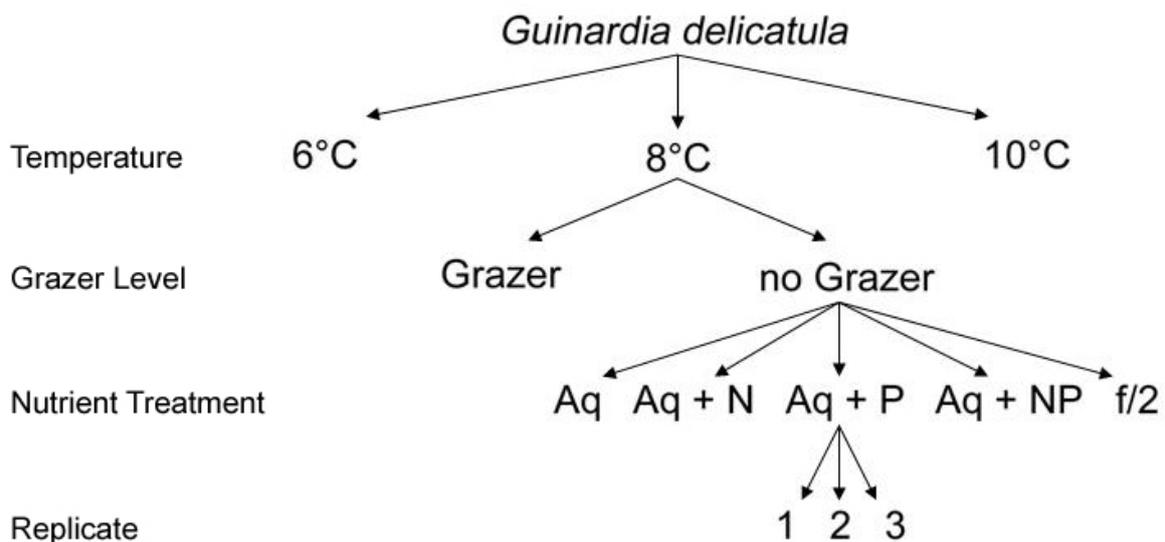


Fig. 5.1: Experimental setup

Experiments were carried out at 6 °C, 8 °C and 10 °C (Fig. 5.1). For each temperature, the water taken at the corresponding temperature outside was used as basis for the media. Grazer presence was simulated by previously incubating the

water samples for 24h with *Acartia tonsa* from continuous copepod cultures maintained at a density of app. 400 individuals per litre. Five nutrient levels were defined (Table 5.1) and added to the treatment categories water (Aq*) and copepod water (Cop*).

Table 5.1: Concentration of nutrients added to the filtrated water samples.

Treatment	added nutrients [$\mu\text{mol L}^{-1}$]		
	N	P	Si
Aq	-	-	-
Aq + N	80	-	5
Aq + P	-	80	5
Aq + NP	80	80	5
f/2	full medium (Guillard & 1976)		
Cop	-	-	-
Cop + N	80	-	5
Cop + P	-	80	5
Cop + NP	80	80	5
Cop f/2	full medium (Guillard & 1976)		

Before experimental runs, the algal abundances in the cultures were counted to ensure approximately the same starting densities at each temperature where possible. 100 ml Erlenmeyer flasks were filled with 10 ml of algae cultures and 65 ml of medium with three replicates of each treatment. The cultures were kept under a light-dark cycle of 10:14 hours at a light intensity of $20 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$. A total sample of 4 ml for cell counts, biovolume determination and measurements of photosynthetic efficiency was taken under sterile conditions at day 0, 4, 7, 10 and 13. The samples for cell counts and biovolume measurement were preserved in Lugol's solution.

Cell numbers, colony size and biovolume

To determine algal cell numbers (Table 5.2), three replicates were counted in Sedgewick-Rafter chambers using the method described by Lund et al. (1958). For each sample a minimum of 400 cells was counted with colonies being noted as single units. In the case of colony formation, the number of cells in a colony was recorded separately. Growth rates were calculated as $\mu = \frac{\Delta LN_n}{\Delta t}$ (Sommer 1998).

For biovolume determination (Table 5.3), 40 cells of the first replicate were measured and the cell size calculated using the formulas in Hillebrand et al. (1999).

Photosynthetic efficiency

The quantum yield during photosynthesis is a very sensitive indicator of a plant's physiological state and stress level (Durako & Kunzelman 2002, Juneau et al. 2003). To determine the effective quantum yield (Table 5.4) of photochemical energy conversion the PAM (Pulse Amplitude Modulation) method of fluorescence measurement was applied. To acquire the quantum yield of Photosystem II the first initial fluorescence F_0 and F'_0 for light exposed samples respectively is induced by low levels of actinic light after a period of dark adaptation. When the emission reaches a stable level, a strong pulse of light, the Saturation Pulse, is applied. This leads to a quick reduction of the electron transport chain between the two photosystems, forcing the cells to emit all energy as fluorescence. The maximum fluorescence value F_m and F'_m for light exposed samples respectively is recorded and

the effective quantum yield calculated from $F = \frac{(F_m - F_0)}{F_m}$ for dark adapted and

$F' = \frac{(F'_m - F'_0)}{F'_m}$ for previously light exposed samples (Genty et al. 1989). This reflects

directly the electron flow through Photosystem II at the time of the measurement and is regarded as an indicator for the fitness of the algae cells. The quantum yield was measured using a Xenon-PAM, Walz, Germany (Walz 1999). Samples were dark-adapted for five minutes, then transferred into a 10x10 mm quartz cuvette and the fluorescence yield measured.

Statistical analyses

To compare the differences between growth rates for different sampling days, factorial ANOVAs with Tukey's HSD as post hoc tests were carried out using the software package STATISTICA 7 (StatSoft, Inc., Tulsa, OK, USA). The classification trees to analyse the relative importance of the factors chosen in the experiments were calculated in PS-Explore (VGSPS mbH, Neustadt/Wied, Germany). To compare the results of this study to the data sets of the spring bloom 2004 and the long-term series, correlation matrices and redundancy analyses (RDA, calculated in Brodgar, Highland Statistics Ltd., Newburgh, UK) were used. While for the correlation analyses the whole series was used, the RDA was performed on a subset of the long-term data from 1991 to 2001. Temperature, salinity and the concentrations of ammonium, nitrite, nitrate, silicate and phosphate were chosen as explanatory

variables for the abundances of the three species. In a second run, a partial RDA was performed, using temperature as a covariable to eliminate its strong influence on the cell numbers and to detect underlying correlations.

Results

Growth rates

Of all species, *T. nitzschioides* showed the highest variations in growth rates between treatments at the given temperatures (Fig. 5.2).

The most consistent growth was found at 6 °C and the largest differences at 8 °C with abundances in Cop and Cop f/2 cultures declining from day eight on and then crashing during the final days of the experiment. At 10 °C, only both full media treatments showed the expected population development while in most of the other cultures, abundances varied. But despite this variation, it was at this temperature, where there was a trend of separation between Aq* and Cop* treatments with Aq* cultures having higher final abundances. Neither at 6 °C nor at 8 °C was such a trend found. Apart from the full media treatments, the influence of nutrients depended also on the temperatures. At 6 °C, Aq + N had the highest final abundance but the importance of phosphate increased with higher temperatures and at 10 °C, Aq + NP cultures had the highest cell numbers.

The influence of grazers was less important with no significant differences between Aq* and Cop* treatments apart from the population crash in Cop and Cop f/2 at the end of the 8 °C experiment. The regression tree found the day the sample was taken to be most important for the abundance, followed by the temperature and the different treatments.

For *T. rotula* (Fig. 5.3), temperature had fewer effects on the growth rates than for the two other species and cultures generally followed the expected development with no evident lag phases in most cases. Nonetheless, significant differences regarding nutrient treatments could be observed. In the second weeks of the 6 °C and 8 °C experiments, significant grouping of growth rates occurred with phosphate treatments reaching higher abundances than those only enriched with nitrogen ($0.0002 \leq p \leq 0.0273$ and $0.0004 \leq p \leq 0.0181$). At 10 °C, this effect was less pronounced but still detectable. The regression tree classified the sampling day as most important for the number of cells followed by temperature and the kind of nutrient treatment.

The growth rates of *G. delicatula* (Fig. 5.4) increased with each temperature level and showed the strongest reactions to the simulated grazer presence. At 6 °C, only in both full media treatments f/2 and Cop f/2 a positive growth rate was observed. At this temperature there were no significant differences between the Aq* and Cop* group. But at 8 °C, Cop* treatments had higher growth rates than Aq* cultures while at 10 °C the pattern was reversed and Aq* treatments reached the highest abundances ($0.0000 \leq p \leq 0.0472$). Apart from the full media treatments, nitrogen enriched cultures generally showed higher growth rates than the ones treated with phosphorus. According to the regression tree, temperature had the highest effect on cell numbers followed by the sampling day and the treatments.

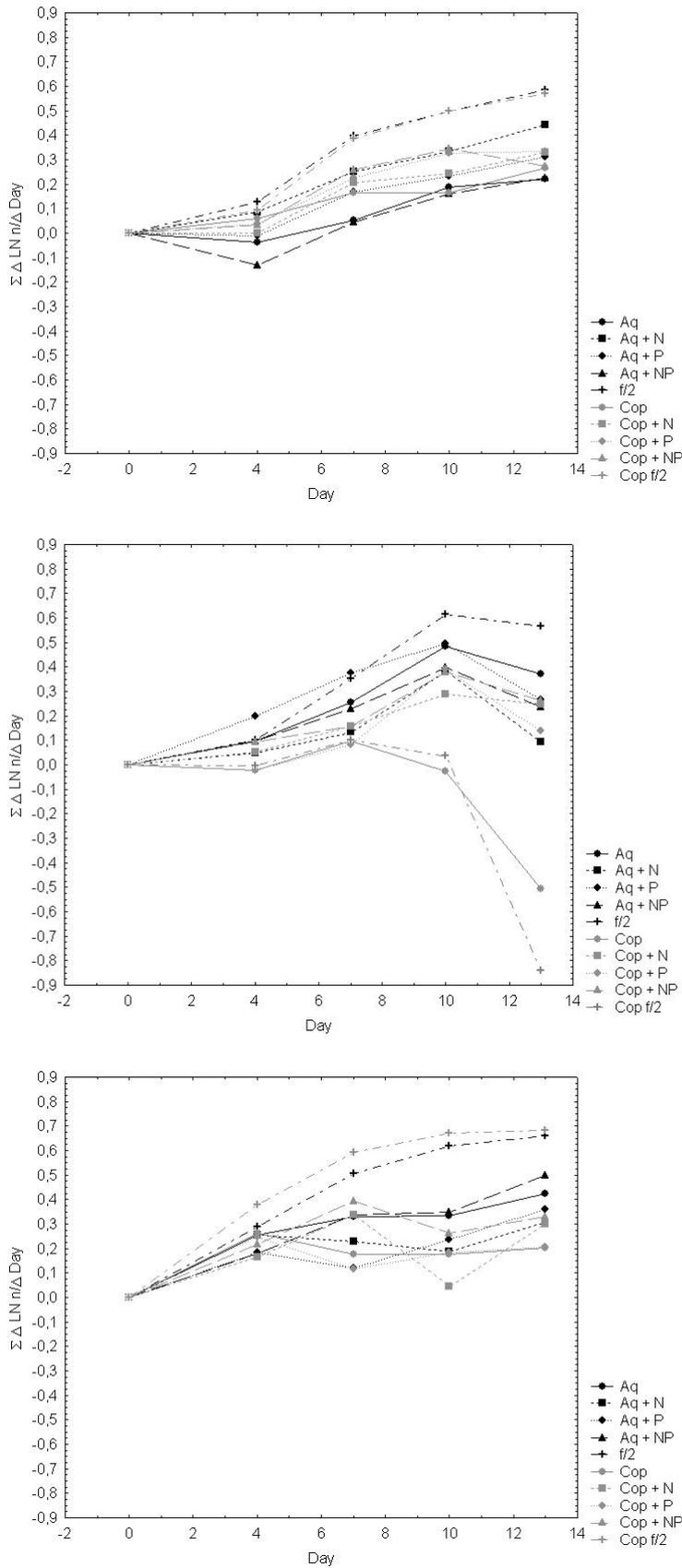


Fig. 5.2: Mean growth rates of *T. nitzschioides* at 6 °C, 8 °C and 10 °C. Black: Aq* treatments, grey: Cop* treatments. Original abundances and standard deviations are given in table 5.2.

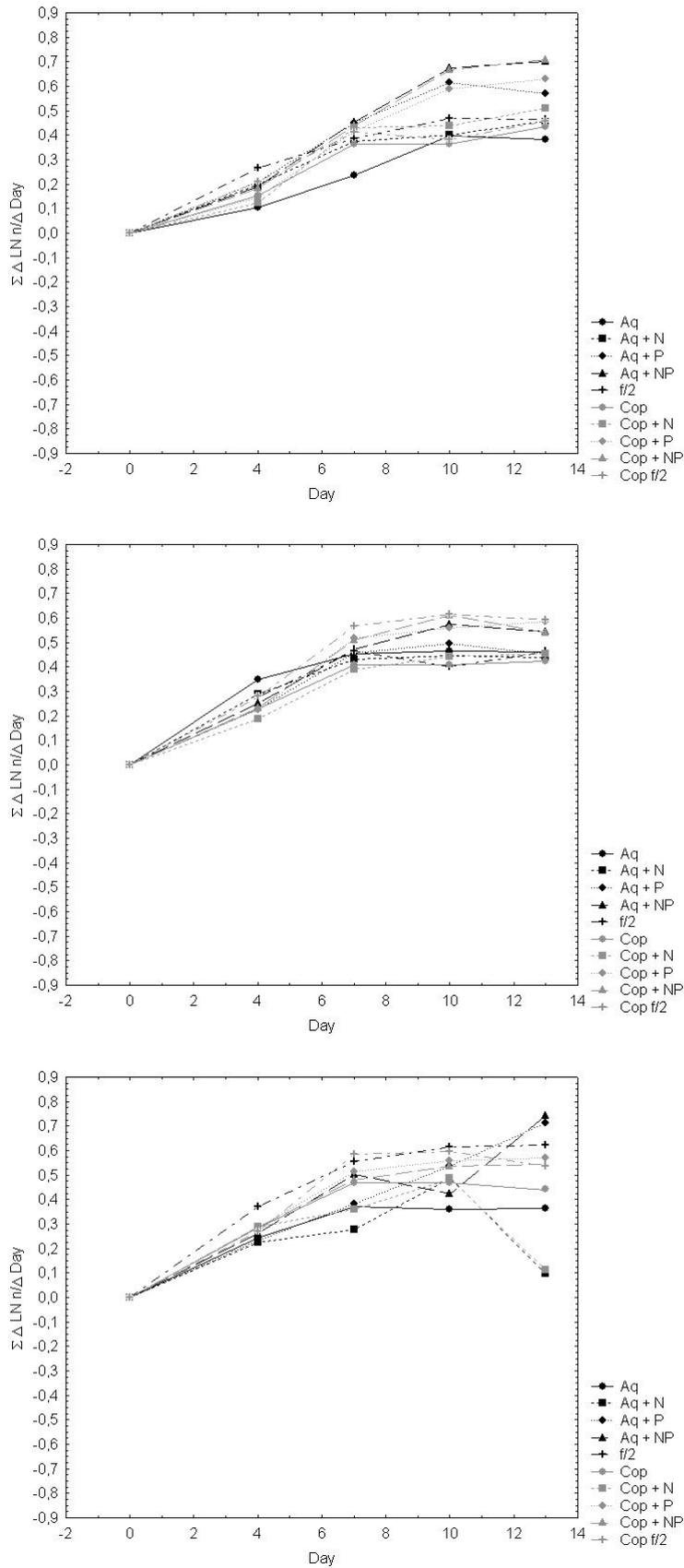


Fig. 5.3: Mean growth rates of *T. rotula* at 6 °C, 8 °C and 10 °C. Black: Aq* treatments, grey: Cop* treatments. Original abundances and standard deviations are given in table 5.2.

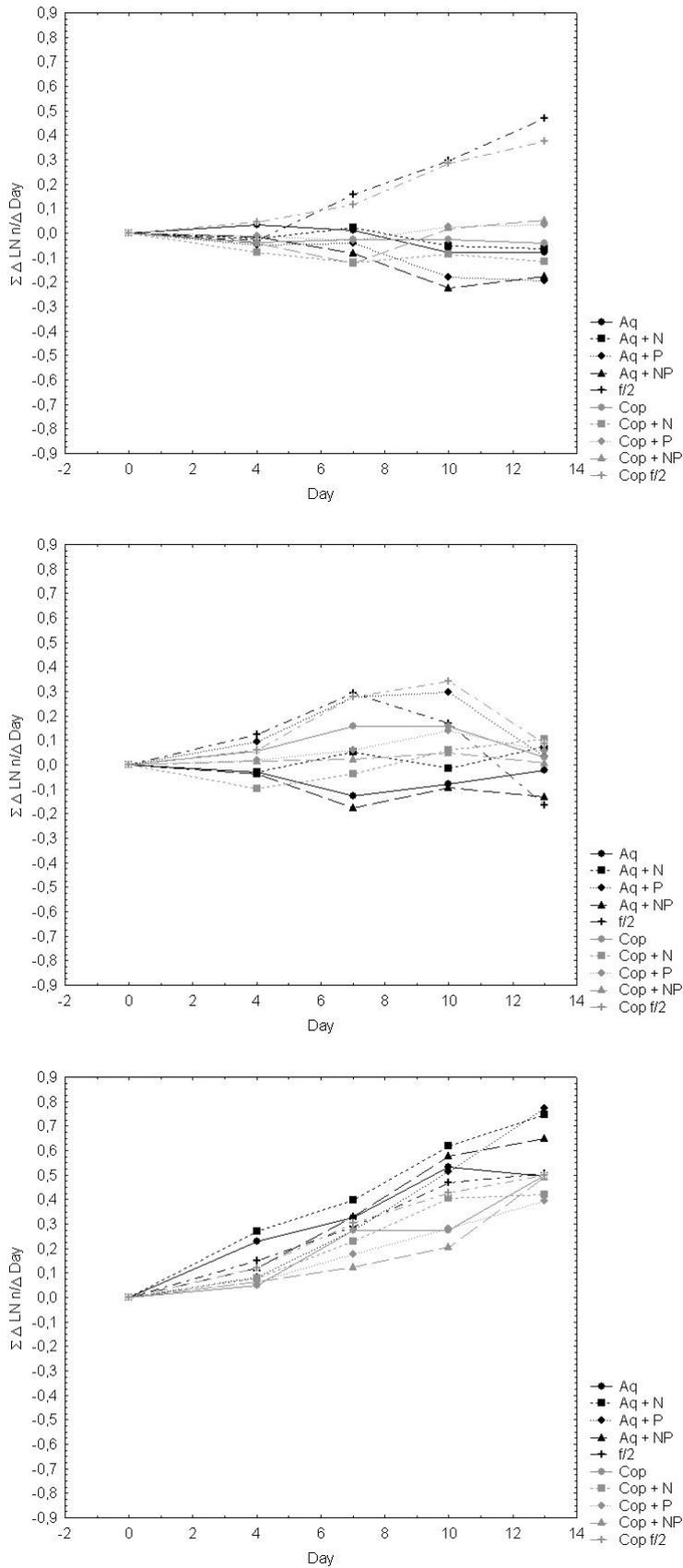


Fig. 5.4: Mean growth rates of *G. delicatula* at 6 °C, 8 °C and 10 °C. Black: Aq* treatments, grey: Cop* treatments. Original abundances and standard deviations are given in table 5.2.

Cell size

Changes in cell sizes also varied to a great extent between species, temperatures and treatments. For *T. nitzschioides* (Fig. 5.5), the smallest species, variance in cell sizes increased with temperature and in contrast to the growth rates, a significant grazer effect on cell sizes at higher temperatures was observed ($0.0000 \leq p \leq 0.0055$ at 8 °C and $p = 0.0000$ at day 13 at 10 °C). Cells in Cop* treatments reached up to 257 % of the initial cell volume of $304.69 \mu\text{m}^3$ at day 13 of the 10 °C experiment. Differences between treatments were significant in most cases. According to the regression tree, most important for the determination of the cell size was the treatment followed by temperature and day of sampling.

Cell sizes of *T. rotula* (Fig. 5.6) showed the same reactions across all temperatures and treatments with the mean biovolumes decreasing to about 40 % of the initial sizes during the first four days and remaining at this level. The regression analysis found the sampling day to be most important. For the first seven days, temperature followed while in the second week, treatments became the second most important factor.

In the case of *G. delicatula* (Fig. 5.7), again temperature had the highest influence on the results. At 6 °C, cells sizes varied with the nutrients added having a greater impact than the treatment category although Aq* treatments generally developed larger cells. The alternating pattern observed for growth rates continued for cell sizes at 8 °C with significant differences between treatments ($p = 0.0002$ after day four) and a trend towards larger cells for Cop* cultures. Within categories, N and NP enriched cultures had the largest cells at day 13. At 10 °C all cultures showed the same pattern of decreasing sizes. At the end, Cop* treatments had significantly larger cells than Aq* cultures ($p = 0.0003$). For this species, cell sizes were mostly determined by temperature with time (day the sample was taken) as second highest influence. In the 10 °C experiments, treatments had no further influence according to the regression tree but mattered in the 6 °C and 8 °C runs.

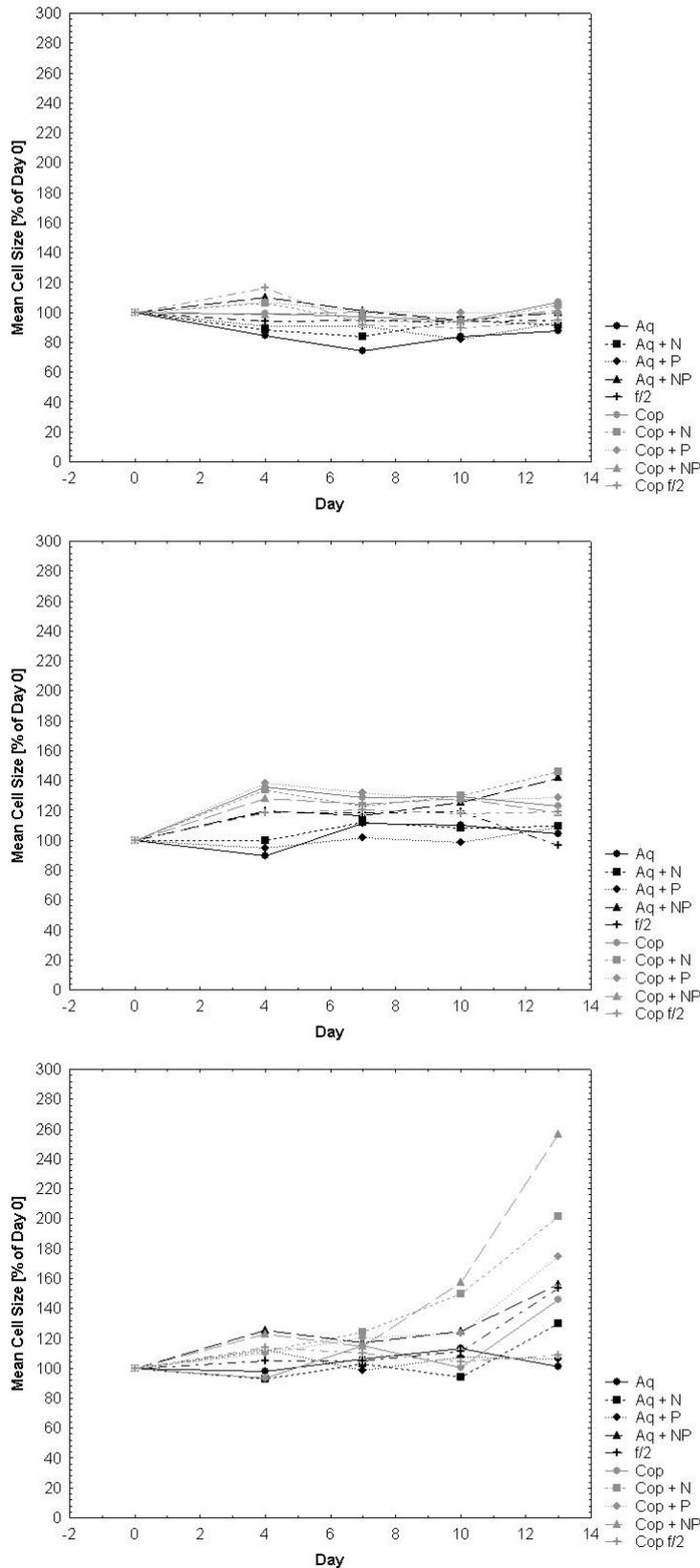


Fig. 5.5: Mean cell sizes of *T. nitzschioides* at 6 °C, 8 °C and 10 °C. Black: Aq* treatments, grey: Cop* treatments. Original values and standard deviations are given in table 5.3.

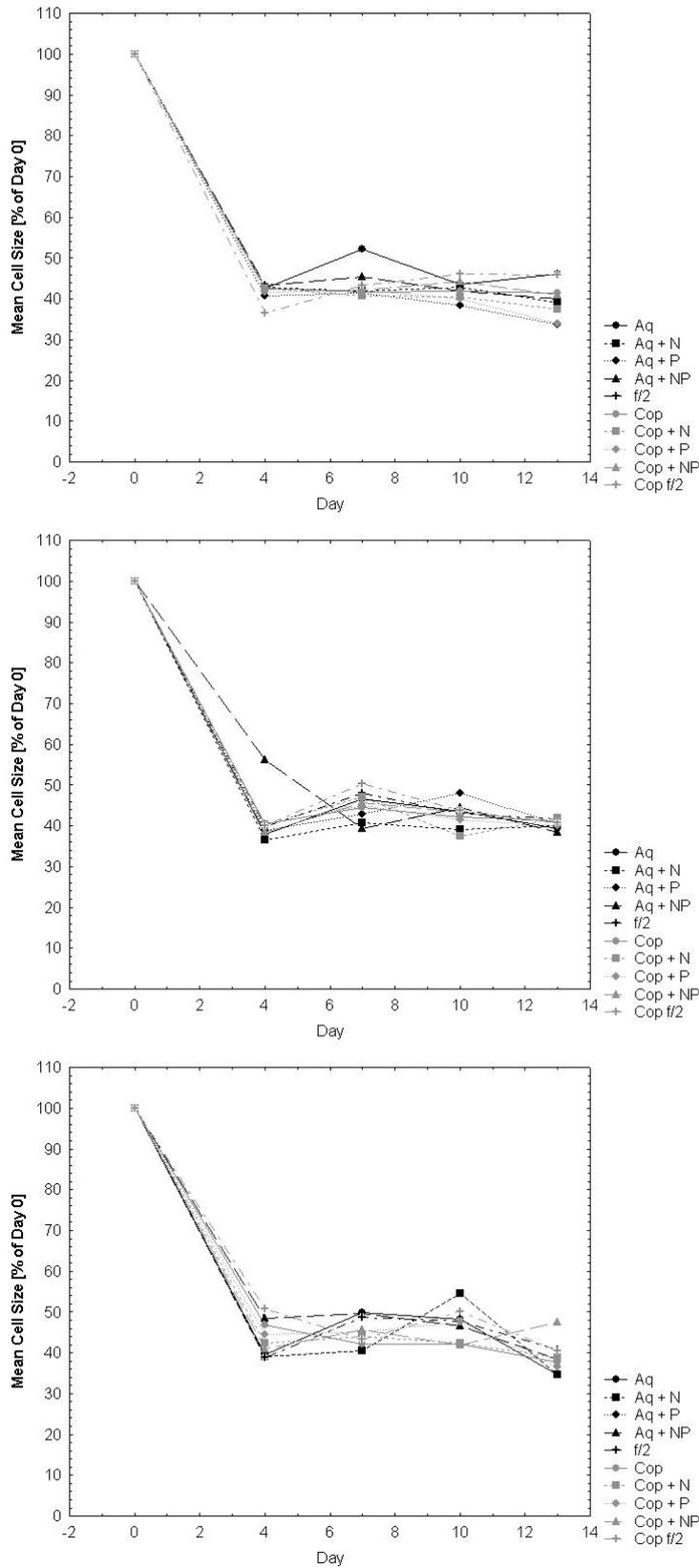


Fig. 5.6: Mean cell sizes of *T. rotula* at 6 °C, 8 °C and 10 °C. Black: Aq* treatments, grey: Cop* treatments. Original values and standard deviations are given in table 5.3.

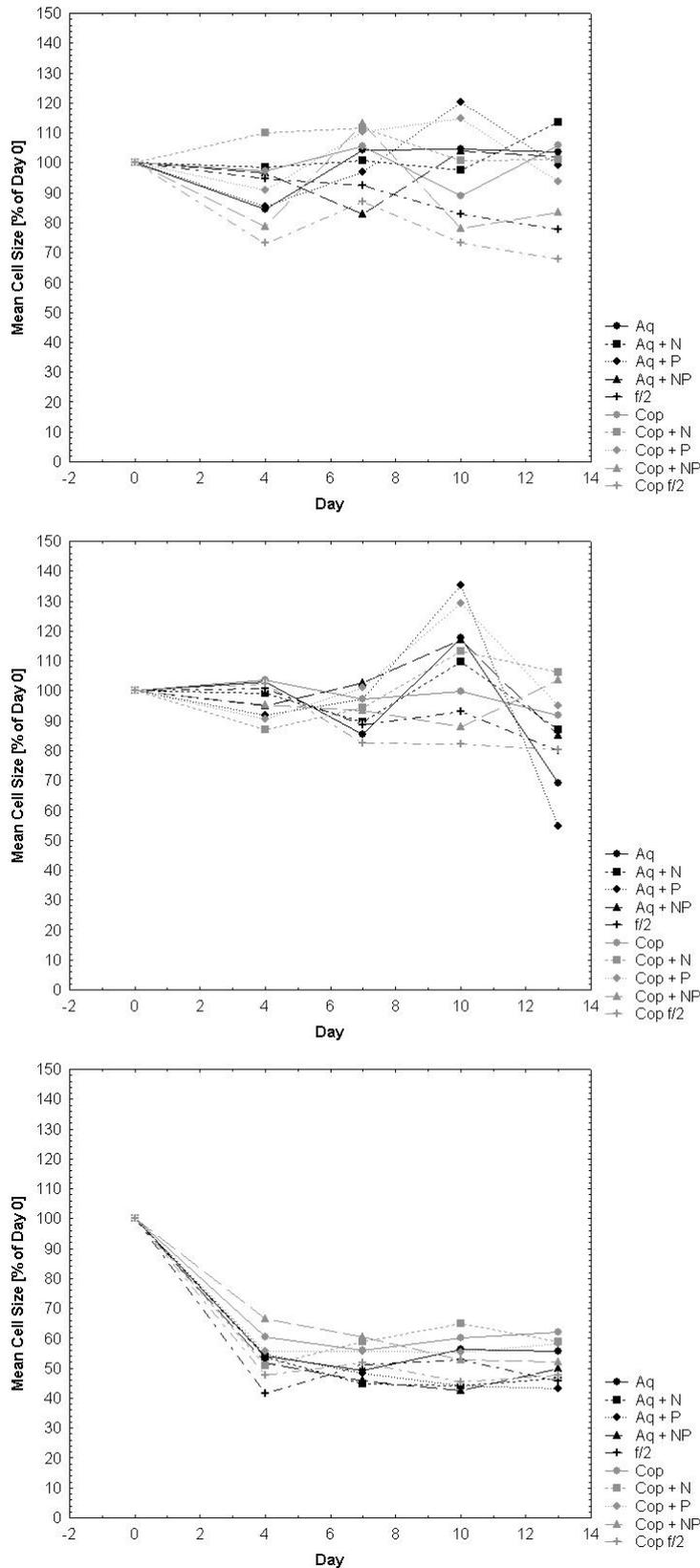


Fig. 5.7: Mean cell sizes of *G. delicatula* at 6 °C, 8 °C and 10 °C. Black: Aq* treatments, grey: Cop* treatments. Original values and standard deviations are given in table 5.3.

Chain length

The most interesting development regarding chain lengths of *T. nitzschioides* colonies was observed at 10 °C where Aq* cultures generally had longer chains than Cop* cultures. Viewed together with cell sizes, Cop* treatments therefore had larger cells but shorter chains while in Aq* cultures the opposite effect was observed.

Regardless of temperatures or treatments, the vast majority of *T. rotula* cells were found to be single.

For *G. delicatula*, colony sizes were rather small with Aq* cultures generally having larger chains than their Cop* counterparts.

Photosynthetic efficiency

For all three species, photosynthetic efficiency generally was only little influenced by the experimental conditions. Exceptions were *G. delicatula* at 8 °C where Aq* and Cop* cultures differed significantly and the Aq + N treatment of *T. rotula* at 8 °C.

T. nitzschioides showed only little variations between treatments at all temperatures and cells were able to stay close to the optimum level of 0.7 (Tab. 3). In the cultures of *T. rotula* (Tab. 3), the least variation between treatments was found at 8 °C and the highest at 10 °C where the photosynthetic quantum yield in Aq + N treatments dropped to 0.26 at day 13. In contrast, *G. delicatula* cells (Tab. 3) showed the highest variation at 8 °C where, except for f/2, all Aq* cultures maintained high levels of photosynthetic efficiency while Cop* cultures showed an almost continuous decrease. These differences were significant both at the treatment category ($0.0000 \leq p \leq 0.0246$) and the treatment level ($0.0002 \leq p \leq 0.0475$) from day seven on. At the end of the experiment, the lowest quantum yield was measured in Cop + N cultures at 0.3. Least variation was found at 6 °C with all cells being able to keep levels close to 0.7 and only Aq and Cop f/2 at day 13 showing significant differences to the initial results ($0.0003 \leq p \leq 0.0466$ and $0.0002 \leq p \leq 0.0449$). At 10 °C, consistent significant differences were found only at day 13 where Aq + N ($0.0002 \leq p \leq 0.0429$) and Cop f/2 ($0.0003 \leq p \leq 0.05$) cultures were found to deviate from all other treatments.

For all three species, the regression analysis identified time as most important followed by temperature and treatments.

Comparison of the experimental results with field data

During the study of the spring bloom event (Fig. 5.8) from February 16th to May 31st, *T. nitzschioides* was present from the first day onwards until the end of April. Most observations were made at temperatures between 4°C and 7°C while the highest abundances were found between 7 °C and 9 °C as three single events. *T. rotula* was first found at 4 °C. All other observations were made between 6 °C and 11°C with the highest abundances found between 8 °C and 10 °C. *G. delicatula* was encountered in the samples from 6 °C on with numbers rising alongside water temperatures. This matches the experimental results of optimal growth ranges. The redundancy analyses (RDA, Fig. 5.9) were then run again using temperature as covariable to eliminate its overwhelming effect. For *T. nitzschioides*, nitrate was put on a positive, nitrite at a neutral and all other nutrients at negative angles. But all explanatory variables were not very well represented by the first two axes and therefore no final conclusions could be drawn. In the case of *T. rotula*, the peak at medium temperatures led to inconsistent results. But with temperature used as covariable analysing the *G. delicatula* data nitrate, nitrite and ammonium, previously put at negative angles, became positively related to the abundance of this species. This is again consistent with the results of this study.

In the long-term data (Fig. 5.8), *T. nitzschioides* was always found in high abundances at early dates with sometimes second peaks in early summer or autumn. For *T. rotula*, two distinct populations could be observed with the spring blooming strain showing the highest cell numbers between 4 °C and 6 °C. Abundances of *G. delicatula* generally became higher with rising temperatures. This succession became even clearer when using the water temperature as explanatory variable for cell numbers. The abundance of *G. delicatula* was always positively correlated with water temperature while the correlation of *T. nitzschioides* was always negative. In the case of *T. rotula*, the sign varied. Again, this can be explained by the growth rates found in this study where *G. delicatula* grew best at 10 °C, *T. rotula* had the most homogeneous growth rates at the medium temperature of 8 °C and *T. nitzschioides* at 6 °C. Using RDA (Fig. 5.9) on the Long-term data, *G. delicatula* was positively correlated with temperature while *T. rotula* was placed at a neutral angle. *T. nitzschioides* was not very well represented by the first two axes. For all three species, nutrients and salinity were classified as neutral or negative correlations. In a second partial RDA with temperature as covariable, nitrate became positively

correlated with the abundance of *G. delicatula*. In the case of *T. rotula*, the removal of the temperature had almost no effect on the correlations. As in the first run of the RDA, *T. nitzschioides* was not represented well enough to draw any conclusions.

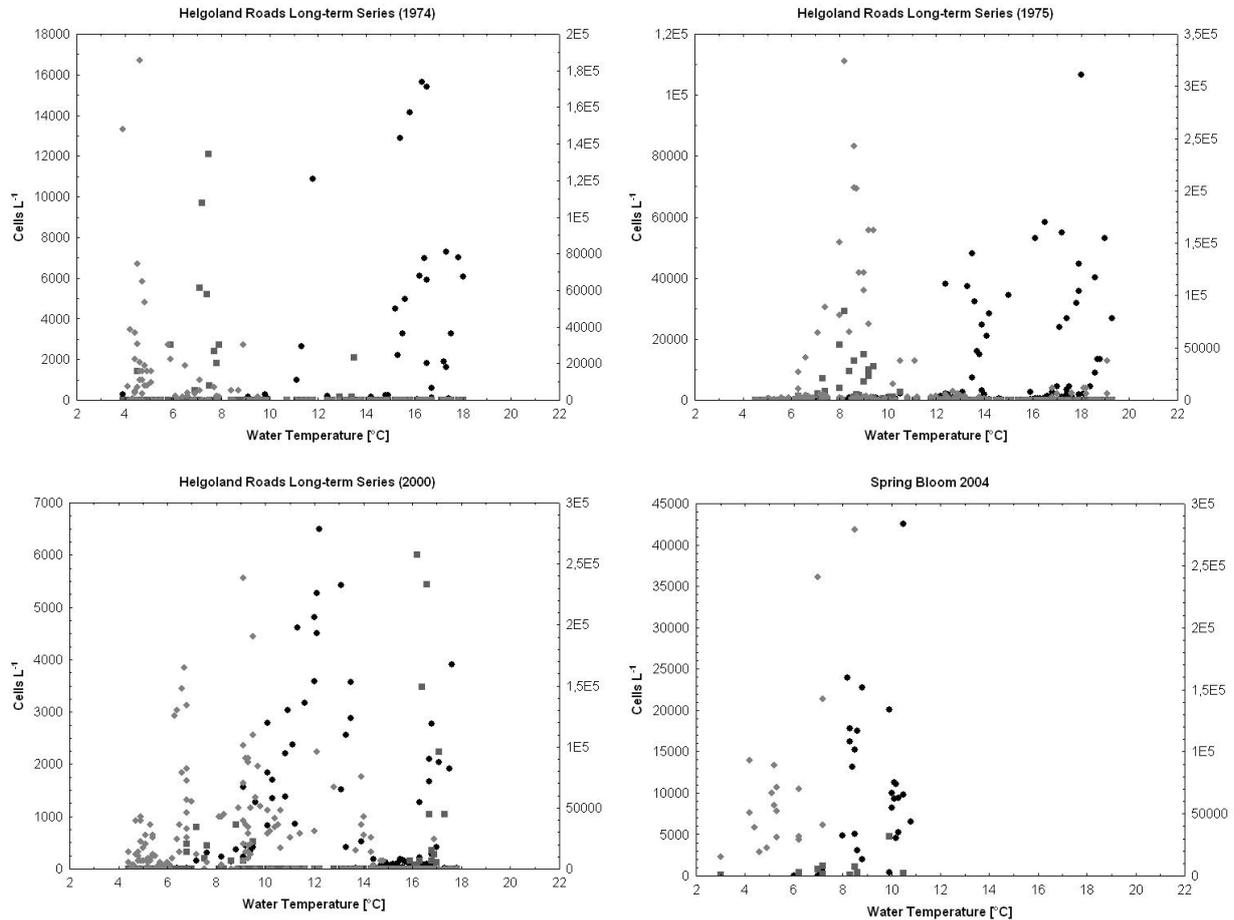
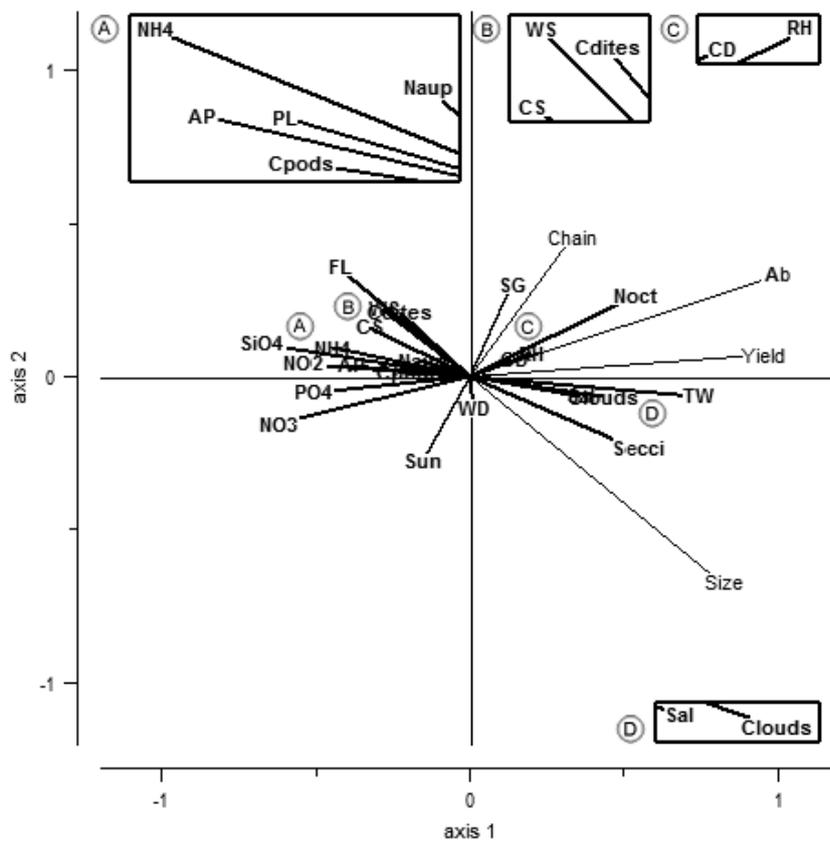


Fig. 5.8: Succession patterns from the Helgoland Roads Long-term Series (<http://www.pangaea.de>) and the Spring Bloom survey 2004. ●: Cell numbers of *Guinardia delicatula* (on the right y-axis), ■: cell numbers of *Thalassiosira rotula* and ◆: cell numbers of *Thalassionema nitzschioides*. Selection of long-term data was based on whether all three species were present in a year.

Guinardia delicatula



Abbreviations

- Ab - Abundance
- AP - Air Pressure
- CD - Currents Directions
- Cdites - Copepodites
- Chain - Chain Length
- Clouds - Cloud Cover
- Cpod - Copepods
- CS - Currents Speed
- FL - Fish Larvae
- Naup - Copepod Nauplii
- Noct - Noctiluca
- PL - Polychaete Larvae
- RH - Relative Humidity
- Sal - Salinity
- SG - Sum Grazer
- Secci - Secchi Depth
- Size - Cell Size
- Sun - Sunshine Hours
- TW - Temperature Water
- WD - Wind Direction
- WS - Wind Speed
- Yield - Yield PS II

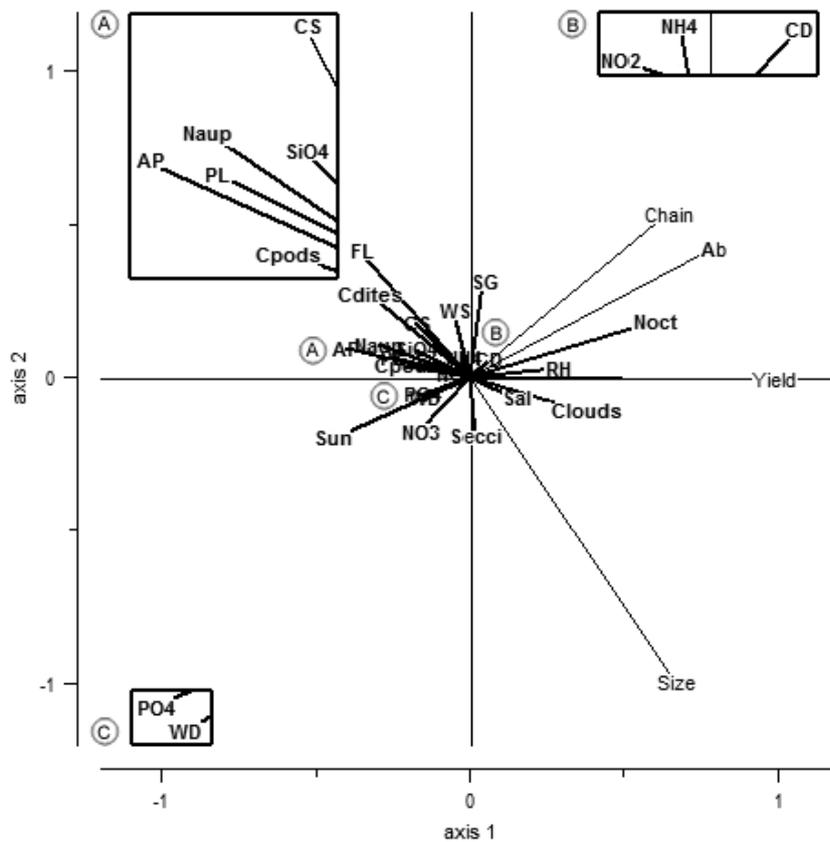


Fig. 5.9: Examples of RDA analyses: *G. delicatula* (spring bloom 2004); full RDA (upper) and partial RDA (lower plot) with water temperature as covariable.

Helgoland Roads Long-term Series 1991-2001

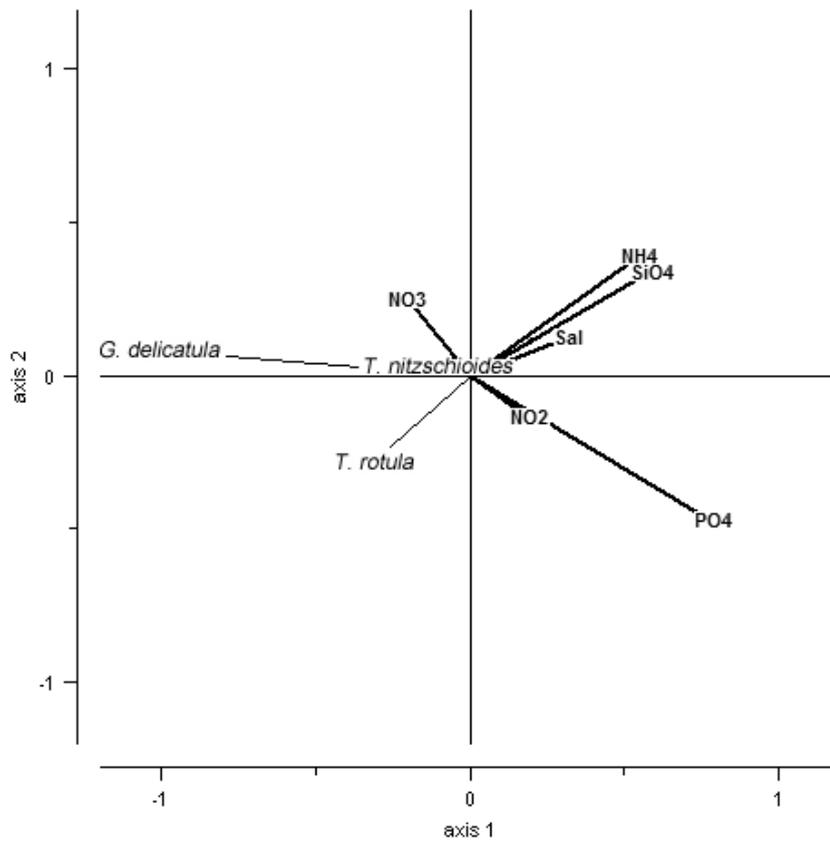
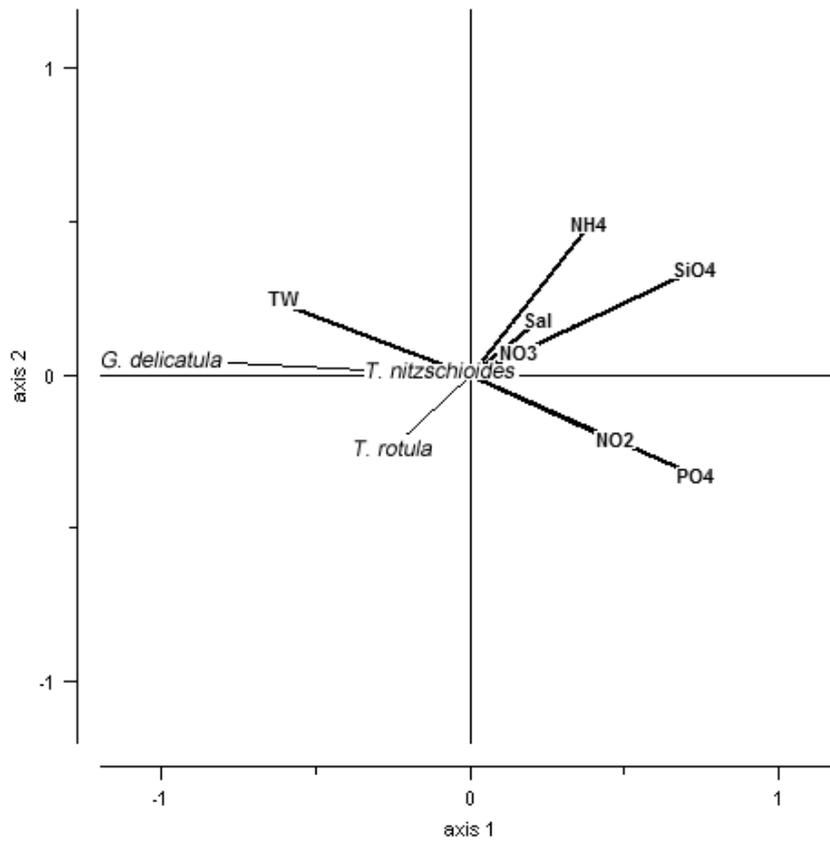


Fig. 5.9 continued:
Examples of RDA
analyses:
Helgoland Roads data;
full RDA (upper) and
partial RDA (lower plot)
with water temperature
as covariable.

Table 5.2: mean abundances [cells ml⁻¹] and standard deviations.
a.: *Thalassionema nitzschioides*, b.: *Thalassiosira rotula*, c.: *Guinardia delicatula*.

a.	Treatment	Day											
		0		4		7		10		13			
		mean abundance	±	mean abundance	±	mean abundance	±	mean abundance	±	mean abundance	±		
6°C	Aq	7253.52	0	6306.32	992.94	8563.15	2958.34	12957.01	4809.56	13704.07	2256.11		
	Aq + N	4164.56	0	6000.63	1318.85	9774.44	1377.38	12560.68	3026.32	17284.33	1911.99		
	Aq + P	6417.72	0	6537.83	3039.96	11117.25	4325.55	13458.62	4972.36	17162.50	6656.37		
	Aq + NP	8641.51	0	5326.61	1605.74	8783.80	1617.81	12377.31	315.78	14925.00	1555.89		
	f/2	5613.21	0	10442.00	5431.16	21783.80	7229.51	28644.23	4395.68	39488.13	16585.69		
	Cop	6174.42	0	8055.05	2233.30	10716.94	341.61	13711.11	2036.07	15158.53	4943.99		
	Cop + N	6428.57	0	6758.41	2539.12	12079.50	2378.74	15445.31	3198.91	17407.25	667.77		
	Cop + P	5825.00	0	6985.52	1858.94	12285.79	3704.18	16422.78	3693.41	16874.81	4601.49		
	Cop + NP	6300.00	0	7293.25	1355.40	15302.96	7533.29	18526.88	2757.99	15110.12	3891.61		
	Cop f/2	5910.89	0	8617.74	1224.36	21669.79	7053.75	29527.29	7736.56	37122.33	11162.63		
8°C	Aq	3125.00	0	4871.48	1884.42	7457.80	945.22	18045.28	11050.75	10616.27	1247.10		
	Aq + N	5063.29	0	6367.65	1718.72	9486.71	6887.28	18977.38	10317.40	8035.47	4158.98		
	Aq + P	3478.26	0	8214.44	3680.03	15704.96	9285.54	22947.39	14106.76	9698.55	3123.20		
	Aq + NP	4938.27	0	7300.84	842.63	11484.94	4905.60	21814.05	13024.07	11217.11	1920.55		
	f/2	3478.26	0	5667.02	2758.61	11426.88	3357.24	28925.83	18069.77	25194.44	19132.30		
	Cop	5128.21	0	7008.34	3564.63	10552.38	4946.19	19707.58	10900.31	11502.02	3694.43		
	Cop + N	4494.38	0	5655.28	1041.64	7767.20	2157.51	21272.55	13419.78	10241.41	2706.72		
	Cop + P	4819.28	0	4508.06	1107.14	7124.66	4904.33	18120.20	10999.00	7770.83	3511.86		
	Cop + NP	4166.67	0	6093.25	1073.58	8392.68	4886.66	20370.45	15254.63	11099.75	6003.43		
	Cop f/2	4123.71	0	7298.54	2562.46	25547.69	16371.86	40234.52	24964.30	30203.60	16803.65		
10°C	Aq	2407.83	450.42	6768.08	1927.49	8345.76	1335.08	8329.69	745.69	10899.26	825.89		
	Aq + N	2658.55	776.55	7208.25	687.47	6978.04	2545.33	5836.45	598.22	8775.64	3054.26		
	Aq + P	3112.84	178.02	6616.60	1322.61	5456.58	1004.45	7838.98	2186.88	10995.60	320.42		
	Aq + NP	2200.42	257.07	4535.49	399.18	7435.54	2204.08	7793.89	2548.58	12023.25	3211.07		
	f/2	3070.56	426.08	10181.23	4077.43	18904.81	3569.04	26080.08	2093.24	29542.12	2863.90		
	Cop	3187.41	330.88	9402.59	3264.08	7574.00	3459.57	4874.55	1595.48	7658.33	867.91		
	Cop + N	3384.40	933.42	6471.78	958.22	10920.03	2542.74	7314.60	2643.39	9710.80	1354.03		
	Cop + P	2901.64	271.22	7992.56	2302.45	5307.26	1138.64	6799.35	2754.52	6984.63	1211.04		
	Cop + NP	2817.75	512.19	6984.53	2790.52	11455.24	2278.87	7630.86	555.89	9917.81	4502.55		
	Cop f/2	2509.72	178.92	11719.89	2902.11	22025.56	5114.53	27773.83	3842.72	28799.23	5884.62		
b.	Treatment	Day											
		0		4		7		10		13			
		mean abundance	±	mean abundance	±	mean abundance	±	mean abundance	±	mean abundance	±		
		6°C	Aq	733.94	0	1133.96	155.59	1727.69	558.11	2693.92	248.40	2692.62	1000.83
			Aq + N	587.37	0	1298.99	145.21	2236.21	452.47	2386.09	133.12	2821.04	268.48
			Aq + P	567.38	0	1354.99	362.24	2786.69	773.49	4501.20	848.45	4712.95	2860.14
			Aq + NP	544.96	0	1208.83	367.99	2702.79	963.83	5327.49	2415.70	5561.22	1490.03
			f/2	526.32	0	1545.48	194.37	2203.33	311.78	2821.82	115.03	2792.52	242.54
			Cop	656.81	0	1216.59	114.66	2319.26	481.71	2868.25	359.95	2812.29	54.34
			Cop + N	566.57	0	950.70	240.49	2397.75	571.10	2509.05	311.89	2870.89	341.67
Cop + P	597.91		0	1077.00	170.13	2425.30	326.91	4158.96	1160.61	4613.42	752.15		
Cop + NP	479.62		0	1012.71	222.13	2280.12	967.53	4442.57	1357.29	4952.93	1387.50		
Cop f/2	618.24		0	1468.43	353.91	2658.45	290.77	2426.04	207.07	3011.07	31.75		
8°C	Aq	455.58	0	1851.11	284.44	2512.38	173.86	2620.97	139.11	2607.60	359.33		
	Aq + N	514.14	0	1630.69	140.12	2524.18	501.72	2623.09	315.86	2571.21	140.75		
	Aq + P	671.14	0	1692.43	266.85	3424.17	939.67	3763.49	587.34	3526.65	1637.35		
	Aq + NP	522.88	0	1429.50	59.47	2821.96	524.53	3893.73	1339.23	3472.94	630.23		
	f/2	516.80	0	1605.94	481.06	2930.02	1258.67	2281.48	243.83	2747.47	312.12		
	Cop	510.86	0	1315.43	389.71	2209.94	239.09	2448.38	496.16	2308.53	362.80		
	Cop + N	565.77	0	1218.20	241.13	2258.05	551.16	2297.84	412.85	2819.05	1070.80		
	Cop + P	503.14	0	1242.13	57.40	3193.87	1335.77	3450.92	864.19	3800.63	1445.29		
	Cop + NP	455.58	0	1162.22	103.27	2686.96	513.04	3662.96	758.16	3093.14	1218.30		
	Cop f/2	457.14	0	1422.48	324.07	3350.54	536.82	3873.33	591.64	3592.34	296.16		
10°C	Aq	380.33	52.48	1006.87	110.80	1528.33	510.72	1416.41	52.69	1434.58	75.74		
	Aq + N	388.63	36.17	966.56	178.53	1115.75	86.89	2137.04	459.05	655.97	54.20		
	Aq + P	376.81	73.48	944.61	113.75	1528.63	439.66	2382.18	549.79	4067.38	1013.68		
	Aq + NP	390.14	50.45	1131.41	271.78	2381.15	857.13	1828.63	398.11	4824.87	1269.79		
	f/2	342.00	46.29	1600.07	594.74	2619.78	145.10	3117.68	60.91	3258.37	688.12		
	Cop	392.33	51.86	1252.44	337.79	2168.00	534.92	2260.07	434.74	1982.51	425.99		
	Cop + N	403.36	19.13	1311.54	303.93	1617.43	375.97	1370.48	190.96	780.80	227.29		
	Cop + P	377.93	84.81	1215.92	443.78	2678.06	1905.31	2648.64	216.58	2733.56	343.06		
	Cop + NP	372.50	129.04	1139.88	134.87	2078.02	588.49	2427.48	560.32	2458.26	371.03		
	Cop f/2	350.33	29.01	1089.70	428.03	2705.31	694.98	2786.60	652.96	2315.71	370.71		
c.	Treatment	Day											
		0		4		7		10		13			
		mean abundance	±	mean abundance	±	mean abundance	±	mean abundance	±	mean abundance	±		
		6°C	Aq	559.00	0	663.27	223.91	606.86	104.64	468.42	133.41	472.47	140.57
			Aq + N	573.00	0	529.14	55.02	633.28	220.35	495.21	160.53	464.67	60.50
			Aq + P	795.30	0	664.36	113.38	676.48	76.64	555.83	350.78	467.32	225.64
			Aq + NP	769.44	0	728.19	113.24	617.73	222.44	428.76	242.30	511.11	314.32
			f/2	598.86	0	567.51	249.80	989.90	409.22	1438.03	336.02	2395.30	423.63
			Cop	766.86	0	665.67	60.93	686.35	17.93	645.53	413.55	739.46	441.11
			Cop + N	730.96	0	554.30	169.94	500.39	211.31	656.41	565.93	525.71	256.93
Cop + P	646.27		0	623.14	29.02	622.20	273.61	764.92	390.86	778.44	415.80		
Cop + NP	804.04		0	682.07	85.79	586.12	272.29	854.92	278.81	965.80	384.19		
Cop f/2	579.00		0	724.15	250.90	880.63	221.59	1589.42	872.25	1885.25	349.49		
8°C	Aq	674.80	0	611.33	132.79	454.67	84.10	557.67	228.30	623.19	144.74		
	Aq + N	731.89	0	617.40	193.71	567.87	219.40	802.34	467.10	605.65	266.99		
	Aq + P	863.45	0	728.46	177.22	1096.07	441.23	1975.99	941.85	2276.41	1338.86		
	Aq + NP	675.86	0	605.96	209.77	424.67	242.55	576.00	407.07	441.33	17.62		
	f/2	656.17	0	965.68	194.20	1604.19	457.40	2839.51	1570.81	1789.20	186.33		
	Cop	643.00	0	571.60	175.37	698.68	29.21	974.28	339.89	706.54	47.16		
	Cop + N	838.79	0	580.88	148.20	686.01	75.40	1010.50	587.79	1061.36	243.78		
	Cop + P	523.00	0	570.56	117.29	640.82	31.95	837.32	293.56	636.36	68.30		
	Cop + NP	656.18	0	702.18	15.96	724.19	158.48	772.88	14.18	690.12	86.18		
	Cop f/2	934.62	0	719.61	306.69	953.32	513.12	1726.39	524.05	2074.72	595.02		
10°C	Aq	461.08	86.76	1165.22	272.48	1549.91	295.94	2856.06	366.36	2548.28	254.76		
	Aq + N	383.90	104.37	1107.24	119.72	1623.04	238.30	3221.36	817.08	4915.79	2251.17		
	Aq + P	571.66	150.59	787.69	171.35	1421.50	360.65	2845.59	406.85	6204.86	1173.28		
	Aq + NP	513.04	192.30	805.74	114.80	1516.20	318.0						

Table 5.3: mean cell size [μm^3] and standard deviations. a.: *Thalassionema nitzschioides*, b.: *Thalassiosira rotula*, c.: *Guinardia delicatula*.

a.	Treatment	Day										
		0		4		7		10		13		
		mean cell size	±									
6°C	Aq	343,75	0	290,43	42,49	254,88	7,48	288,48	42,21	301,56	48,45	
	Aq + N	343,75	0	302,73	43,95	287,89	42,05	326,56	36,19	313,67	40,97	
	Aq + P	343,75	0	311,52	45,23	311,33	42,98	282,23	77,57	320,70	37,55	
	Aq + NP	343,75	0	378,37	71,26	348,51	37,52	326,56	15,74	343,75	0,00	
	f/2	343,75	0	323,37	15,22	325,72	15,74	323,44	15,10	325,52	15,74	
	Cop	343,75	0	340,00	10,36	334,24	14,70	323,44	15,10	366,41	65,01	
	Cop + N	343,75	0	365,89	58,58	322,52	24,00	321,09	14,13	359,93	39,37	
	Cop + P	343,75	0	366,82	62,62	345,51	53,85	343,29	27,61	340,91	9,20	
	Cop + NP	343,75	0	339,06	11,45	344,35	37,10	321,09	14,13	350,52	41,17	
	Cop f/2	343,75	0	400,49	78,81	314,99	110,04	307,14	16,05	326,09	15,84	
	8°C	Aq	283,89	41,47	254,38	35,50	315,82	40,76	312,09	51,33	296,88	18,97
		Aq + N	283,89	41,47	283,16	53,37	317,97	39,88	306,64	42,63	310,16	34,49
Aq + P		283,89	41,47	268,55	28,78	288,32	47,75	280,47	45,26	309,38	20,02	
Aq + NP		283,89	41,47	339,29	11,21	331,52	15,59	355,14	58,08	402,34	85,56	
f/2		283,89	41,47	337,80	12,57	336,96	13,18	337,80	15,99	273,59	28,19	
Cop		283,89	41,47	384,67	72,53	365,00	52,45	366,72	50,64	348,23	39,88	
Cop + N		283,89	41,47	379,83	69,61	348,21	14,94	369,14	63,89	414,00	82,66	
Cop + P		283,89	41,47	392,86	66,65	375,00	50,94	359,00	52,05	364,40	52,68	
Cop + NP		283,89	41,47	363,19	45,77	351,81	38,36	362,48	52,12	336,31	13,64	
Cop f/2		283,89	41,47	336,65	16,51	341,88	44,62	334,38	14,69	338,07	12,34	
10°C		Aq	304,69	71,97	298,05	44,18	324,41	36,34	344,14	7,50	308,50	37,29
		Aq + N	304,69	71,97	282,81	38,97	313,67	41,51	286,72	41,39	399,41	82,57
	Aq + P	304,69	71,97	343,75	0,00	300,88	45,82	328,13	15,82	324,02	103,48	
	Aq + NP	304,69	71,97	382,62	86,57	356,45	72,53	381,03	95,24	476,56	210,56	
	f/2	304,69	71,97	320,40	52,56	320,58	37,71	339,45	38,11	468,75	337,80	
	Cop	304,69	71,97	285,40	41,88	350,89	62,68	306,45	60,55	444,97	80,49	
	Cop + N	304,69	71,97	339,87	69,44	377,66	93,00	456,45	98,60	613,67	146,49	
	Cop + P	304,69	71,97	335,16	56,64	366,99	71,81	377,18	108,34	531,25	93,84	
	Cop + NP	304,69	71,97	375,02	96,05	349,80	62,01	480,37	77,15	782,42	152,00	
	Cop f/2	304,69	71,97	347,56	55,11	334,96	45,18	317,77	50,92	330,53	18,06	

b.	Treatment	Day										
		0		4		7		10		13		
		mean cell size	±									
6°C	Aq	58043,38	2104,02	24816,40	1094,41	30423,75	5870,31	25287,98	4474,27	26839,07	1722,40	
	Aq + N	58043,38	2104,02	24870,57	2149,11	24447,97	478,60	24999,59	4323,72	22788,23	3404,82	
	Aq + P	58043,38	2104,02	23711,34	1883,77	24144,86	1379,77	22359,92	5271,07	19619,61	4639,92	
	Aq + NP	58043,38	2104,02	25157,28	1001,99	26469,35	4440,58	24372,50	4503,82	23295,65	2731,16	
	f/2	58043,38	2104,02	21306,76	2591,11	25213,07	2295,14	26808,46	4048,63	26754,47	3102,97	
	Cop	58043,38	2104,02	24736,54	1214,74	24321,63	1516,02	24380,48	4573,04	24136,88	3574,28	
	Cop + N	58043,38	2104,02	24798,33	1857,84	23749,37	2412,28	23550,90	2747,63	21748,78	3184,02	
	Cop + P	58043,38	2104,02	24201,83	858,06	24543,69	0	23303,62	5668,96	19714,72	3822,37	
	Cop + NP	58043,38	2104,02	24630,09	1911,62	24543,69	1592,61	25789,28	3863,04	23778,54	2958,77	
	Cop f/2	58043,38	2104,02	24175,54	880,50	24712,79	1246,06	24981,18	3683,67	24304,39	736,55	
	8°C	Aq	51650,97	3825,20	22054,96	1959,99	27090,66	2884,11	25365,74	2316,30	22902,19	3106,92
		Aq + N	51650,97	3825,20	21192,31	2456,98	23710,62	1942,46	22717,91	2342,78	23337,75	2811,07
Aq + P		51650,97	3825,20	22667,39	1483,14	24912,32	1898,25	27970,46	3970,29	23596,31	1833,35	
Aq + NP		51650,97	3825,20	32658,27	4343,95	22874,11	2744,69	25878,40	3121,38	22287,95	3181,85	
f/2		51650,97	3825,20	23172,87	1753,38	27955,27	1874,10	25012,79	1613,26	24201,83	858,06	
Cop		51650,97	3825,20	23512,27	1446,39	25938,69	3127,06	24543,69	1447,51	23939,30	1757,20	
Cop + N		51650,97	3825,20	23466,84	1670,87	27372,88	2952,45	21754,30	829,07	24357,16	1982,46	
Cop + P		51650,97	3825,20	23404,16	1224,66	26183,40	3145,13	24069,92	1677,35	23249,01	1875,41	
Cop + NP		51650,97	3825,20	22350,18	3322,13	26591,52	3168,59	25234,28	1504,13	23679,09	2045,33	
Cop f/2		51650,97	3825,20	23267,42	1235,75	29312,11	3451,75	25539,99	1973,75	23622,77	3059,87	
10°C		Aq	58192,48	2237,94	23066,05	2315,33	29011,57	3662,96	28048,84	2925,99	20183,23	1425,51
		Aq + N	58192,48	2237,94	22767,34	1680,68	23547,53	5668,10	31754,94	9568,94	20183,23	1425,51
	Aq + P	58192,48	2237,94	23564,73	1204,25	28416,99	4734,70	24490,92	9865,46	23932,12	2767,24	
	Aq + NP	58192,48	2237,94	28246,42	3584,50	28878,11	5181,12	27082,74	7780,17	22409,62	2462,92	
	f/2	58192,48	2237,94	24549,03	1271,15	28288,45	3919,00	27788,37	2383,58	23736,93	1509,59	
	Cop	58192,48	2237,94	27291,97	4506,92	24544,00	3923,58	23230,06	3041,49	21916,12	2159,40	
	Cop + N	58192,48	2237,94	24672,85	2988,14	25603,37	5643,18	24639,72	3320,29	22616,31	2214,57	
	Cop + P	58192,48	2237,94	25837,45	3263,62	26250,09	3764,46	27641,72	7313,58	21204,67	2407,98	
	Cop + NP	58192,48	2237,94	25474,82	2818,03	26495,84	3366,56	24325,87	7745,07	27632,02	3006,59	
	Cop f/2	58192,48	2237,94	29605,22	3654,56	25214,35	2651,16	29147,48	2765,02	23582,56	2348,83	

c.	Treatment	Day										
		0		4		7		10		13		
		mean cell size	±									
6°C	Aq	15907,90	4065,99	13413,07	4476,35	16566,26	2638,81	16645,68	3145,63	16465,22	4165,75	
	Aq + N	15907,90	4065,99	15650,32	2880,07	16034,39	3272,40	15518,78	4938,11	18067,34	6376,22	
	Aq + P	15907,90	4065,99	13568,85	4098,15	15419,42	4338,35	19134,53	3700,59	15772,06	4243,92	
	Aq + NP	15907,90	4065,99	15359,02	4067,21	13199,65	3540,90	16536,21	5112,07	16231,10	5935,82	
	f/2	15907,90	4065,99	15085,49	4545,75	14728,72	4292,63	13183,25	3368,37	12375,71	2858,44	
	Cop	15907,90	4065,99	15460,21	2803,15	16806,90	4633,75	14130,92	3663,21	16843,46	6016,95	
	Cop + N	15907,90	4065,99	17485,88	2763,28	17745,60	4742,96	16005,26	5252,06	16091,27	4840,84	
	Cop + P	15907,90	4065,99	14433,57	3144,97	17574,80	4090,87	18273,63	5040,63	14912,85	4636,91	
	Cop + NP	15907,90	4065,99	12521,98	1999,16	18000,57	3021,25	12441,64	4070,31	13294,40	3866,81	
	Cop f/2	15907,90	4065,99	11627,81	2574,03	13817,84	3165,84	11645,14	2397,89	10766,46	2562,81	
	8°C	Aq	14138,89	3525,54	14582,14	4260,15	12087,93	5195,37	14619,71	4851,54	9770,18	5052,16
		Aq + N	14138,89	3525,54	14011,18	3433,53	12673,92	3684,78	13537,88	3782,81	12290,16	4774,24
Aq + P		14138,89	3525,54	12966,91	3916,69	13740,72	5286,16	11479,40	2507,96	7754,33	3082,32	
Aq + NP		14138,89	3525,54	13440,21	2666,19	14507,01	3194,80	15049,73	4728,98	12015,54	3490,42	
f/2		14138,89	3525,54	14229,04	3572,90	12531,18	3148,22	11697,27	2909,86	11296,03	2560,17	
Cop		14138,89	3525,54	14657,27	2114,52	13770,77	2871,01	14552,09	2818,92	12983,16	4801,00	
Cop + N		14138,89	3525,54	12313,31	2420,85	13335,03	3264,13	13815,85	2893,98	15016,19	5450,17	
Cop + P		14138,89	3525,54	12779,09	2648,76	14274,12	2608,25	14093,82	2820,49	13420,43	2989,20	
Cop + NP		14138,89	3525,54	13477,78	2604,30	13192,29	2909,40	13733,21	2786,13	14659,26	5353,43	
Cop f/2		14138,89	3525,54	14483,86	4636,78	11674,73	2920,32	11967,72	4967,25	11333,44	5204,35	
10°C												

Table 5.4: mean photosynthetic efficiency [yield PSII] and standard deviations. a.: *Thalassionema nitzschioides*, b.: *Thalassiosira rotula*, c.: *Guinardia delicatula*.

a.	Treatment	Day										
		0		4		7		10		13		
		mean yield PSII	±									
6°C	Aq	0.69	0.00	0.70	0.01	0.67	0.01	0.68	0.02	0.67	0.02	
	Aq + N	0.70	0.01	0.70	0.01	0.68	0.01	0.68	0.01	0.68	0.01	
	Aq + P	0.69	0.02	0.69	0.01	0.68	0.01	0.67	0.02	0.67	0.02	
	Aq + NP	0.72	0.04	0.71	0.01	0.68	0.00	0.68	0.01	0.67	0.03	
	f/2	0.68	0.01	0.69	0.00	0.66	0.02	0.66	0.02	0.63	0.04	
	Cop	0.70	0.02	0.70	0.01	0.68	0.01	0.67	0.02	0.66	0.01	
	Cop + N	0.69	0.00	0.70	0.00	0.68	0.00	0.67	0.01	0.66	0.00	
	Cop + P	0.69	0.00	0.71	0.00	0.68	0.00	0.68	0.02	0.66	0.01	
	Cop + NP	0.69	0.01	0.70	0.01	0.68	0.02	0.68	0.02	0.65	0.01	
	Cop f/2	0.67	0.01	0.68	0.00	0.67	0.02	0.66	0.01	0.64	0.01	
8°C	Aq	0.70	0.03	0.70	0.01	0.67	0.01	0.67	0.01	0.66	0.01	
	Aq + N	0.69	0.01	0.68	0.01	0.61	0.13	0.62	0.06	0.60	0.07	
	Aq + P	0.70	0.00	0.69	0.01	0.68	0.01	0.67	0.00	0.65	0.02	
	Aq + NP	0.69	0.03	0.69	0.01	0.67	0.01	0.65	0.02	0.63	0.01	
	f/2	0.68	0.02	0.69	0.02	0.66	0.01	0.66	0.01	0.64	0.01	
	Cop	0.71	0.00	0.70	0.01	0.69	0.02	0.68	0.02	0.67	0.01	
	Cop + N	0.69	0.04	0.70	0.01	0.69	0.00	0.68	0.01	0.65	0.01	
	Cop + P	0.70	0.01	0.64	0.11	0.66	0.04	0.67	0.01	0.66	0.00	
	Cop + NP	0.70	0.01	0.70	0.01	0.67	0.03	0.66	0.01	0.65	0.01	
	Cop f/2	0.68	0.00	0.70	0.01	0.68	0.01	0.67	0.01	0.65	0.01	
10°C	Aq	0.68	0.01	0.67	0.01	0.64	0.01	0.63	0.02	0.62	0.01	
	Aq + N	0.68	0.01	0.67	0.01	0.63	0.01	0.61	0.03	0.63	0.01	
	Aq + P	0.68	0.01	0.67	0.01	0.63	0.01	0.64	0.00	0.63	0.01	
	Aq + NP	0.69	0.01	0.67	0.00	0.63	0.01	0.63	0.01	0.64	0.01	
	f/2	0.67	0.00	0.69	0.01	0.66	0.01	0.64	0.02	0.61	0.03	
	Cop	0.69	0.00	0.69	0.00	0.66	0.01	0.64	0.01	0.63	0.01	
	Cop + N	0.69	0.01	0.69	0.01	0.67	0.01	0.64	0.02	0.66	0.00	
	Cop + P	0.70	0.01	0.70	0.00	0.65	0.01	0.64	0.01	0.64	0.00	
	Cop + NP	0.69	0.01	0.68	0.01	0.66	0.01	0.63	0.00	0.64	0.01	
	Cop f/2	0.68	0.00	0.69	0.01	0.66	0.01	0.63	0.00	0.61	0.01	
b.	6°C	Aq	0.65	0.01	0.70	0.01	0.68	0.01	0.66	0.02	0.66	0.02
		Aq + N	0.67	0.01	0.70	0.01	0.66	0.03	0.63	0.03	0.65	0.00
		Aq + P	0.66	0.02	0.69	0.02	0.70	0.01	0.69	0.01	0.60	0.03
		Aq + NP	0.66	0.04	0.71	0.00	0.68	0.00	0.68	0.04	0.57	0.05
		f/2	0.64	0.01	0.70	0.00	0.67	0.01	0.66	0.02	0.66	0.01
		Cop	0.66	0.01	0.70	0.00	0.67	0.02	0.65	0.01	0.65	0.01
		Cop + N	0.64	0.03	0.68	0.00	0.67	0.01	0.67	0.01	0.66	0.00
		Cop + P	0.66	0.01	0.70	0.02	0.69	0.01	0.70	0.00	0.62	0.06
		Cop + NP	0.66	0.00	0.70	0.01	0.69	0.00	0.64	0.03	0.65	0.01
		Cop f/2	0.65	0.01	0.69	0.00	0.69	0.01	0.68	0.01	0.60	0.02
8°C	Aq	0.66	0.02	0.69	0.00	0.66	0.01	0.66	0.01	0.65	0.01	
	Aq + N	0.68	0.01	0.69	0.00	0.68	0.00	0.67	0.01	0.63	0.01	
	Aq + P	0.67	0.02	0.70	0.00	0.69	0.01	0.68	0.01	0.66	0.02	
	Aq + NP	0.67	0.01	0.69	0.01	0.70	0.01	0.69	0.01	0.67	0.00	
	f/2	0.67	0.00	0.69	0.01	0.66	0.01	0.65	0.00	0.64	0.01	
	Cop	0.67	0.00	0.69	0.00	0.67	0.03	0.67	0.00	0.64	0.01	
	Cop + N	0.66	0.01	0.70	0.00	0.67	0.00	0.66	0.00	0.64	0.01	
	Cop + P	0.67	0.01	0.70	0.01	0.68	0.01	0.69	0.01	0.67	0.01	
	Cop + NP	0.66	0.01	0.70	0.01	0.67	0.02	0.67	0.02	0.63	0.03	
	Cop f/2	0.67	0.01	0.69	0.00	0.66	0.01	0.65	0.00	0.64	0.02	
10°C	Aq	0.71	0.00	0.69	0.01	0.64	0.03	0.65	0.03	0.65	0.01	
	Aq + N	0.68	0.02	0.70	0.01	0.68	0.01	0.68	0.01	0.26	0.23	
	Aq + P	0.66	0.07	0.70	0.01	0.69	0.01	0.69	0.01	0.65	0.01	
	Aq + NP	0.68	0.04	0.69	0.01	0.70	0.00	0.70	0.01	0.67	0.02	
	f/2	0.70	0.01	0.69	0.01	0.67	0.00	0.67	0.01	0.66	0.01	
	Cop	0.67	0.01	0.70	0.00	0.68	0.00	0.65	0.02	0.62	0.03	
	Cop + N	0.70	0.00	0.69	0.01	0.67	0.00	0.66	0.02	0.65	0.01	
	Cop + P	0.72	0.01	0.70	0.01	0.70	0.01	0.69	0.01	0.65	0.03	
	Cop + NP	0.66	0.01	0.70	0.00	0.68	0.03	0.67	0.00	0.64	0.02	
	Cop f/2	0.73	0.03	0.70	0.01	0.69	0.01	0.68	0.00	0.65	0.02	
c.	6°C	Aq	0.72	0.00	0.71	0.01	0.69	0.01	0.67	0.04	0.64	0.05
		Aq + N	0.70	0.02	0.72	0.01	0.68	0.02	0.67	0.03	0.68	0.01
		Aq + P	0.71	0.01	0.69	0.02	0.69	0.01	0.67	0.04	0.69	0.02
		Aq + NP	0.72	0.03	0.71	0.00	0.70	0.02	0.71	0.03	0.71	0.02
		f/2	0.73	0.03	0.67	0.01	0.71	0.00	0.70	0.00	0.65	0.01
		Cop	0.71	0.02	0.72	0.00	0.69	0.01	0.71	0.02	0.68	0.03
		Cop + N	0.75	0.02	0.72	0.00	0.70	0.00	0.70	0.01	0.67	0.01
		Cop + P	0.72	0.05	0.72	0.01	0.70	0.00	0.69	0.00	0.66	0.03
		Cop + NP	0.75	0.04	0.72	0.02	0.67	0.06	0.67	0.03	0.66	0.02
		Cop f/2	0.68	0.02	0.71	0.00	0.68	0.03	0.66	0.01	0.63	0.03
8°C	Aq	0.74	0.00	0.71	0.01	0.70	0.02	0.71	0.02	0.70	0.03	
	Aq + N	0.73	0.03	0.71	0.01	0.69	0.01	0.70	0.02	0.68	0.03	
	Aq + P	0.72	0.01	0.72	0.02	0.70	0.03	0.71	0.01	0.71	0.01	
	Aq + NP	0.77	0.02	0.71	0.02	0.66	0.03	0.72	0.02	0.70	0.03	
	f/2	0.74	0.04	0.70	0.02	0.68	0.02	0.63	0.02	0.46	0.24	
	Cop	0.71	0.02	0.65	0.01	0.63	0.03	0.56	0.01	0.49	0.10	
	Cop + N	0.74	0.03	0.66	0.03	0.60	0.05	0.53	0.02	0.30	0.26	
	Cop + P	0.72	0.03	0.65	0.02	0.45	0.18	0.50	0.04	0.50	0.12	
	Cop + NP	0.73	0.03	0.61	0.08	0.57	0.06	0.52	0.03	0.46	0.04	
	Cop f/2	0.72	0.05	0.71	0.02	0.68	0.01	0.67	0.02	0.62	0.03	
10°C	Aq	0.72	0.02	0.69	0.01	0.65	0.00	0.65	0.01	0.66	0.00	
	Aq + N	0.71	0.02	0.73	0.00	0.71	0.00	0.70	0.01	0.59	0.04	
	Aq + P	0.73	0.01	0.72	0.00	0.71	0.01	0.71	0.01	0.71	0.02	
	Aq + NP	0.75	0.01	0.74	0.01	0.73	0.00	0.71	0.01	0.70	0.02	
	f/2	0.73	0.03	0.72	0.01	0.66	0.02	0.64	0.01	0.63	0.03	
	Cop	0.74	0.01	0.73	0.00	0.71	0.02	0.68	0.00	0.65	0.01	
	Cop + N	0.74	0.00	0.74	0.00	0.72	0.01	0.73	0.00	0.64	0.04	
	Cop + P	0.74	0.00	0.74	0.02	0.73	0.01	0.72	0.01	0.71	0.01	
	Cop + NP	0.71	0.04	0.74	0.01	0.73	0.00	0.72	0.01	0.68	0.04	
	Cop f/2	0.70	0.02	0.73	0.01	0.69	0.02	0.66	0.02	0.61	0.17	

Discussion

The aim of this study was to find characteristic responses of three important phytoplankton species of the Helgoland Roads community to different nutrient treatments and grazer presence at three temperatures. The results then were compared to the data sets of the Helgoland Roads Long-term Series and the spring bloom 2004 campaign to see whether these could be used to explain the patterns found in the in situ data.

In this study, the measured parameters varied considerably between species with regards to temperature, grazer presence and nutrient treatments.

Theories on the outcome of competition predict that no more species can coexist than the number of limiting resources (e.g. Tilman 1982). However, the basic assumptions in this model are constant conditions and concentrations. In reality, this stability is never reached long enough and therefore, much more phytoplankton species can be found at any time than expected in theory leading to the “paradox of plankton” as noted by Hutchinson (1961). Especially if changes occur slowly or the distribution of resources is uneven, species can coexist along gradients.

Nitrogen and phosphorus both play vital roles in the metabolism of all beings as they are part of almost all compounds of a cell. Additionally, many metabolic pathways are closely linked as for example N and C metabolism (Huppe & Turpin 1994, Richardson & Cullen 1995, Clark 2001). Therefore, changes in the concentrations of these nutrients have a great impact and can cause fast reactions, especially in single celled life forms such as microalgae (Spijkerman & Coesel 1998, Lippemeier et al. 2001). Because of their prominent role, nitrogen and phosphorus are heavily competed for and among the major resources that are known to become limiting for phytoplankton growth. In our study, only *G. delicatula* was clearly limited by nitrogen while *T. rotula* was phosphorus limited and in the case of *T. nitzschioides*, the limiting nutrient depended on the temperature. At 6 °C, this species displayed the expected nitrogen limitation but already at 8 °C, changes to phosphorus limited growth were detectable. This effect can be explained by the early occurrence of this species when nutrient concentrations are high and therefore the limiting factor may depend on other biotic and abiotic parameters. Especially the experiment at 10 °C could have led to thermal stress and physiological changes since *T. nitzschioides* is usually found below this temperature in the North Sea. Studies from other systems (Baldauf 1985, Kopczynska et al. 1998, Romero et al. 2002) and palaeobotanical records

(Tanimura 1999, Shimada et al. 2004, Brookshire, 2005) also show an association with cold, nutrient rich waters.

It has been assumed for a long time that freshwater systems are phosphorus limited where it often is the least abundant element of macro nutrients required for algal growth (Kalff & Knoechel 1978, Wetzel 1983, Lampert & Sommer 1993) while in marine ecosystems, nitrogen plays this role. However, this is theory very simplified (Sommer 1998) and as shown in this study by the grouping of growth rates in the case of *T. rotula* and other reports on this species (Dittami 2006), phosphorus limitation can also play an important role in marine systems.

Just as found in the outdoor data presented in Chapter 2, *G. delicatula* was mainly influenced by temperature and again this behaviour matches reports from other ecosystems (Gayoso 1999, Gowen et al. 1999). The growth rates of *G. delicatula* increased with each temperature level and showed the strongest reactions to the simulated grazer presence. At 8 °C, Cop* treatments had higher growth rates than Aq* cultures while at 10 °C the pattern was reversed and Aq* treatments reached the highest abundances. Apart from the media treatments, nitrogen enriched cultures generally showed higher growth rates than the ones treated with phosphorus. While nitrogen limitation is a common phenomenon for marine phytoplankton species from almost all ecosystems (e.g. Hillebrand & Sommer 2000, Agusti et al. 2001, Corwith & Wheeler 2001, Philips et al. 2002, Thomas et al. 2003, Sommer et al. 2004), the reasons for the shift between Aq* and Cop* categories having the highest growth rates are less obvious. As seen in Chapter 3 of this thesis, diatoms can follow the strategy of high reproduction before beginning to form colonies in the presence of grazers. This may have been the case for *G. delicatula*, also, with the still suboptimal temperature at 8 °C enforcing the effect. At optimum growth temperatures, the influence of grazers then may be too small to be recognized in our experiment.

Another noteworthy result is that despite differences in cell sizes of several orders of magnitude between species, growth rates were in the same range. Usually, smaller cells should have higher growth rates (e.g. Mizuno 1991, Sommer 1998, Amato et al. 2005) but there are also studies describing only weak correlations between cell size and division rate (Banse 1982, Blasco et al. 1982, Sommer 1989) or even negative ones (Williams 1964).

Changes in cell sizes also varied to a great extent between species, temperatures and treatments. For *T. nitzschioides*, the smallest species, variances in cell sizes

increased with temperature. Cell sizes of *T. rotula* showed the same reactions across all temperatures and treatments with the mean biovolume decreasing to about 40% during the first four days and remaining at this level. As argued in chapter 3, a decrease in biovolume is a possible way to cope with nutrient depletion because smaller cells have the advantage of smaller diffusion boundary layers utilizing the remaining nutrients more efficiently (Lean 1984, Spijkerman & Coesel 1998). On the other hand, the two *T. rotula* strains used in this previous study followed the opposite strategy of increasing sizes. Since both experiments shared the f/2 treatment and *T. rotula* showed no difference between Aq* and Cop* categories, then only two explanations are possible. First, the reaction varies from strain to strain or, more likely, the observed differences result in the temperatures the experiments were conducted at since the previous study was done at 15°C.

In the case of *G. delicatula*, again temperature had the highest influence on the results. Cells from Cop* treatments tended to be larger than those from the Aq* category and in both categories, N enriched cultures produced large cells so N limited cultures may again have turned to the strategy of smaller cells to facilitate nutrient uptakes. At 10 °C all cultures showed the same pattern of decreasing sizes. Since growth rates were highest at this temperature, cells in all cultures may have chosen the same strategy to avoid early limitation. Together with the results of the other species, this would point to the conclusion, that species do not necessarily follow only one way to cope with limitation but have a certain flexibility based on a number of factors.

The most interesting development regarding chain lengths of *T. nitzschioides* colonies was observed at 10 °C where Aq* cultures generally had longer chains than Cop* cultures. Viewed together with cell sizes, Cop* treatments therefore had larger cells but shorter chains while in Aq* cultures the opposite effect was observed.

Regardless of temperatures or treatments, the vast majority of *T. rotula* cells were single while in natural samples, normally chains are found. This therefore may have been a culturing effect.

For *G. delicatula*, colony sizes were rather small with Aq* cultures generally having larger chains than their Cop* counterparts. But since *G. delicatula* normally forms long chains in natural assemblages as well as in cultures, this may have also been an artefact.

For all three species, photosynthetic efficiency generally was only little influenced by the experimental conditions. Exceptions were *G. delicatula* at 8 °C where Aq* and Cop* cultures differed significantly and the Aq + N treatment of *T. rotula* at 8 °C. While Rodríguez-Román found the photosynthetic efficiency to depend rather on the overall light history of cultures and a species' sensitivity than on the nutrient supply, other authors (Bulthuis 1987, Pierson et al. 1992, Smith et al. 1994, Major & Davison 1998, Coles & Jones 2000) highlight the importance of temperature and biomass or growth rates for photosynthetic performance and there is also evidence of nutrient effects (Lippemeier et al. 2001). In our experiments, the photosynthetic efficiency was neither correlated with temperature nor nutrient depletion or other factors. This points towards a high ability for compensation e.g. the physiological impacts of different temperatures as proposed by Smith et al. (1994).

In accordance to the field observations presented in Chapter 2, temperature was the most important factor modulating the responses to grazer and nutrient treatments and growth rates generally corresponded to the natural succession observed at Helgoland Roads with *T. nitzschioides* showing the most consistent growth across all treatments at 6 °C, *T. rotula* at 8 °C and *G. delicatula* at 10 °C. When temperature was treated as a covariable in the analyses, the abundances of *G. delicatula* became positively correlated with nitrogen, corresponding to the results of this study. For *T. nitzschioides*, no particularly strong relationship with any of the explanatory variables was found by the redundancy analyses because of poor representation either of the explanatory variables or the abundance of this species. In the case of *T. rotula* however, the strong signal of the peak at medium temperatures was still visible in the partial RDA, leading to only neutral or even negative correlations with the other factors. It therefore seems to be particularly difficult to analyse the underlying mechanisms driving the abundances of species with peaks at medium temperatures using field data. Especially in these cases, laboratory studies as the one presented here should be useful for determining their requirements.

Although the temperature range of 6 °C to 8 °C in our study may seem rather small, the clear effects observed show the impact of even small temperature deviations. This is also visible in the Helgoland Roads Long-term data, where an increase of 1.1 °C of the mean annual temperatures since the beginning of the series in 1962 is documented (Wiltshire & Manly 1994). Concurrently with the rising temperature, shifts in the abundance of important species have been observed with *G. delicatula*

becoming more numerous at the expense of species like *T. nitzschioides* and *O. aurita* (Wiltshire et al. 2005). This trend is confirmed by the results of our experiments. Other studies also report changes in the timing of spring blooms, phytoplankton composition and biomass related to rising temperatures. As shown by Goffart et al (2002) for the north-west Mediterranean, an average increase of 1 °C-1.5 °C and lower salinities between 1979 and 1998 led to drastic changes in hydrographical conditions including nutrient concentrations, a shift from diatom-dominated communities to non-siliceous species and a reduction of the phytoplankton biomass by 80 %. These changes subsequently affected higher trophic levels where especially the success of reproduction depends on the availability of food as formulated by Cushing (1974, 1975 & 1990) in his match/mismatch hypothesis. Apart from biomass, species composition of the phytoplankton community changed by rising temperatures can have other dramatic effects on the food web. The strong correlation between shifts from diatom dominated phytoplankton to cyanobacteria and temperature (Murell & Lores 2004, Abrantes et al. 2006) can lower the food quality for zooplankton species, limiting growth and reproduction (e.g. Lampert 1987, Reinikainen et al. 1994, DeMott 1999, DeMott et al. 2001). But grazers are not only affected by poor food quality. Especially in shallow lakes, the degradation of large cyanobacterial biomasses can lead to anoxia and subsequently large fish kills (Abrantes et al. 2006).

In the data from the long-term series and the spring bloom 2004 as presented in Chapter 2, the effects of temperature and seasonality may overlay other influences. For example, in Chapter 2, the abundance of *T. nitzschioides* shows a significant positive correlation with the major nutrients while for *G. delicatula* the correlation is a significantly negative one. Since *G. delicatula*, just as all organisms, needs nutrients to grow, this correlation rather depends on the sampling date as *T. nitzschioides* starts to appear already in February when nutrients concentrations are high while *G. delicatula* is a late blooming species having to deal with already depleted supplies. The positive correlation of the abundance of *G. delicatula* with nitrogen became only visible after using temperature as a covariable in the analyses. Therefore, species specific traits like the different nutrient limitations observed in our experiments are difficult to find in outdoor data. This also applies to the observed effects of grazer presence, cell size and photosynthetic efficiency.

Conclusions

The results from our experiments confirm the high impact of temperature and also revealed species-specific nutrient limitations, reactions to simulated grazer presence and provided insight regarding the interactions of these factors. Moreover, the results match the observed succession patterns and therefore can be used to evaluate conclusions derived from outdoor data. The data can also be used directly in models. Therefore they can serve as a first step to a better understanding of the mechanisms of phytoplankton succession.

The growth rates may also be useful information for culturing as they showed that the highest rates are not always found in full media like Guillard's *f/2*.

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General Discussion

Planktonic organisms have been important research objects for more than 100 years and therefore countless qualitative and quantitative studies on almost all aspects of phytoplankton biology exist. However, the main problem dealing with field data is the vast number of possible interactions and though correlations between factors may be strong, causation is difficult to find. The spatial and temporal scale of these processes influencing diatom populations range from associated bacterial communities in the “phycosphere” (Bell & Mitchell 1972) of diatoms, the influence of local nutrient concentrations, grazer presence, fluctuations in wind and currents’ speed and directions to ocean wide tidal cycles, large scale climatic shifts as for example the North Atlantic Oscillation (Ueyama & Monger 2005, Breton et al. 2006, Sharples et al. 2006) and seasonal phenomena e.g. changes in temperature and daylength. One of the most important questions underlying all laboratory experiments therefore is: “Can you bottle nature (Daehler & Strong 1996)?” Can small scale, highly controlled experiments help to understand the principles of interaction and the driving forces behind observed phenomena as for example the spring bloom succession? The same questions can also be asked regarding models and theories. In experiments, factors can be manipulated and controlled individually that are combined in field data making it difficult to assess their relative importance for an observed process in nature (Sommer 1998).

The present study focuses on the factors and mechanisms behind the succession of key species of the Helgoland Roads phytoplankton community. Based on the detailed observation of the spring bloom 2004 and existing work on the Helgoland Roads Long-term Series, laboratory experiments were conducted to find characteristic “fingerprint” reactions of the chosen species to biotic and abiotic factors. The results were then compared to the field data to explain the observed succession.

A Plankton Puzzle – Observations during the Spring Bloom 2004

In this study, presented in Chapter 2, the abundances of important phytoplankton species were followed over the course of the spring bloom 2004. In addition to the data collected for the Helgoland Roads Long-term Series, meteorological and hydrological parameters as e.g. sunshine hours, cloud cover, wind and currents as

well as biotic factors like mean cell size, photosynthetic efficiency and the influence of important grazer groups were included in the analyses of the observed patterns of the species studied. Several uni- and multivariate statistical analyses were used to identify correlations, interactions and the relative importance of the parameters selected.

Of all parameters, water temperature had the highest influence on the observed succession of species. This effect was not only seen on the species level but also found for general community parameters as for example the negative correlation with diversity. This is of particular interest with regard of the warming trend in the North Sea. According to the Helgoland Roads Long-term Series, the mean water temperature has risen by 1.1 °C since 1962 (Wiltshire & Manly 2004) with first consequences visible like delayed spring blooms (Wiltshire & Manly 2004) and a trend towards higher abundances of diatom species preferring warmer temperatures. The consequences of shifts in phenology could affect the whole food web as higher trophic levels depend on matching life cycles (Edwards & Richardson 2004). Because of its strong effect, temperature was used as a covariable in all further analyses to detect underlying patterns. From the other abiotic parameters not included in the daily measurements for the Long-term Series, wind and currents' speed and directions had the highest influences on abundances.

Generally, all diatom species studied in this survey followed known blooming behaviour as classified by Mieruch et al. (2006) and could be further characterised.

The abundance of *G. delicatula* was significantly positively correlated with water temperature. Nutrients significantly positively correlated with the abundance were nitrite and ammonium while a negative influence was found for nitrate. In Mieruch et al. (2006), *G. delicatula* was assigned to type C, species with typically narrow peaks of high abundances. This was also observed in this study where *G. delicatula* showed fast growth rates and a high peak in the middle of May with declining abundances afterwards. This pattern has also been described for other ecosystems, e.g. Bahía Blanca, Argentina (Gayoso 1999) and the western Irish Sea (Gowen et al. 1999).

Odontella aurita was present during the whole sampling campaign but with relatively low abundances and, after a period of absence from the samples, a peak in the middle of April. In Mieruch et al. (2006), *O. aurita* is classified as a type A species. In this category, species show the highest abundances the faster they start reproducing

from scratch. Other studies suggest low winter temperatures as triggers for *O. aurita* blooms (Martens 2001). Since the winter 2003/04 was rather warm with water temperatures between 7 °C and 2.6 °C (mean temperature: 5 °C) from December 21st 2003 to March 21st 2004, 2004 may just have been non *O. aurita* year. Apart from the positive correlation of the cell size with the abundance of this species, the positive correlation with copepodites, copepods and the total grazer abundance could be explained by a stronger grazing pressure by copepods on smaller cells, leaving only the upper size range for observation. The increase of smaller cells in the sample with the abundance of fish larvae could also be a top-down effect, in this case predation of fish on copepods. Chain lengths followed a reversed pattern of correlations i.e. larger cells were found in smaller colonies. This may also be connected to the grazing behaviour of zooplankton. As shown by Schnack (1983), small zooplankton like copepods in some cases of algae with large cell sizes and long chains prefer shorter colonies. Since they are preyed upon by fish larvae, the observed patterns in chain length can again be explained by top-down control. Regarding nutrients, nitrate was positively correlated with the abundance while nitrite and ammonium were placed on negative angles. This pattern is directly reversed to the nutrient-abundance relationship of *G. delicatula*.

Paralia sulcata was also found over the whole course of this study with the highest abundances during the first weeks. This may be explained by the high demands of silicate since this species has heavily silicified valves. Because *P. sulcata* is a benthic species as well, it fits that the abundance in the plankton community was also significantly influenced by turbulence connected parameters like currents and wind speed. This is confirmed by McQuoid & Nordberg (2003) who found higher abundances in well mixed, nutrient rich water columns than in stratified, nutrient depleted situations, especially with low phosphorus concentrations. In our study, nutrient concentration was of general importance for the abundance and a pronounced link with phosphorus concentrations was found for temperatures above 5.4 °C. The cell size varied over the course of time but was positively correlated with all grazers which may also be caused by a stronger predation on smaller cells. In contrast to the report of McQuoid & Nordberg (2003), no link between cell size, water temperature and phosphorus concentrations could be found. Chain length was negatively correlated to copepodites, copepods and the total grazer abundance. It is known that copepods can position diatom chains to eat the cells one by one so that

only the cells size is important for food selection, not the length of the chain (Paffenhöfer et al. 1982). It is therefore possible that in the case of *P. sulcata*, long chains with small cells were preferred.

Rhizosolenia pungens was another species present over the whole time of the observations. The abundance of this species showed a sharp peak in the end of April and was significantly positively correlated with temperature and ammonium concentrations. The strong influence of silicate concentration may be explained by the highly variable cell size with up to $75230 \mu\text{m}^3$. Wind directions being negatively correlated to cell size could also be explained by the high demand for silicate as the numerically lower first half of directions corresponds to wind coming from the mainland driving nutrient rich coastal waters into the German Bight. The same applies to the positive correlation with the directions of currents since the nomenclature is directly reversed from wind directions.

The abundance of *Skeletonema costatum* showed two peaks, one in the first half of March and the second one at the end of April. In Mieruch et al. (2006), *S. costatum* is classified as a type B bloomer. Species in this category can have varying low abundances and small growth rates at the beginning of a bloom and have several peaks in cell numbers. The results of this study match the pattern described by Mieruch et al. The highest influence found on the abundance was again the water temperature while copepodites, copepods and fish larvae were negatively correlated. Since the cells of *S. costatum* are rather small, they may be very well suited as food source for higher trophic levels and therefore be heavily grazed upon by copepods and other heterotrophs.

Thalassionema nitzschioides was found between February and the end of April with four peaks in its abundance between March 11th to April 29th. Again, this matches the classification of Mieruch et al. (2006) who classified this species as type B, alongside *S. costatum*. The significant positive correlations with all grazer groups may be a time effect of the abundances since the increasing cell size was also being positively correlated with the number of grazers, pointing again to a potential selection effect by higher trophic levels. Interestingly, colony size showed an exactly reversed pattern and was negatively correlated to the grazers' abundances. This may have the same cause as in the case of *P. sulcata* described above with grazers likely selecting cell sizes first. A preference for colonies with smaller cells can be explained with the chain formation of *T. nitzschioides* forming zigzag bands and not simple straight

chains. Therefore, not only a cell's diameter but also its length may be a factor in food selection.

Morphological Adaptations in Diatoms

As seen in Chapter 2, grazers were able to significantly influence for example chain lengths and cell sizes of some of the species studied. One aspect of the interactions between microalgae and grazers is the induction of morphological and physiological changes that can serve as defence mechanisms. Defence strategies include e.g. toxicity, swarming, diel vertical migration or changes in morphology and life history (Larsson & Dodson 1993). Predator defence mechanisms as a reason for different morphologies in diatoms is however confounded by the fact that even in culture the morphology of algae can be extremely variable. It can also be influenced by temperature, turbulence, nutrients, salinity and light. (Grover 1989, Lürling & van Donk 1999, Trainor 1992, Trainor 1993, Arin et al. 2002, Meave del Castillo et al. 2003).

One of the ways to trigger these changes are substances released by grazers, e.g. urea (Wiltshire & Lampert, 1999) serving as alarm chemicals for microalgae. The concentration and dispersion of these substances is influenced e.g. by turbulence. Turbulence has a strong impact on many aspects of diatom ecology but is another factor difficult to assess from in situ data. As Huisman et al. (2002) demonstrated, intermediate levels of turbulence can keep a diatom population in the euphotic zone even if the individual cells themselves are sinking. On the other hand, it can lead to disruption of diatom colonies by mechanical forces or may interfere with the vertical migration of species like *Rhizosolenia* spp. that are positively buoyant and known for active density regulation (Moore & Villareal 1996). All of these possible ways of influence by turbulence on the phytoplankton, may affect the development of the algae and changes of the community structure (Arin et al. 2002).

In the laboratory experiments presented in Chapter 3, the influence of chemical grazer presence and turbulence on the growth rates, cell sizes and chain lengths on two strains of *Thalassiosira rotula* and one strain of *Thalassionema nitzschioides* was studied. The experiments were performed in Guillard's f/2 and in Drebes medium to test for the influence of different culture media on the results.

As found by Krawiec (1982), strains of the same species can differ in their reactions to biotic and abiotic factors. In this study, both strains of *T. rotula* followed the same

patterns under the experimental conditions. In the f/2 medium, both strains showed continuous growth while in the Drebes medium, a classic growth curve with lag, log and plateau phase. Beginning nutrient limitations were observed from the end of the first week onwards in *T. rotula* and *T. nitzschioides* cultures.

Both the *Thalassiosira rotula* and the *Thalassionema nitzschioides* algae however were influenced by stirring. *T. rotula* was inhibited in growth and a reduction of the chain length is apparent in both the *T. rotula* f/2-Medium August and the *T. nitzschioides* cultures. As shown by Schöne (1970), it is very easy to break diatom chains by mechanical force. In his investigations (with *Chaetoceros curvisetus*, *Cerataulina bergonii* (Peragallo) Schütt, *Guinardia flaccida* Peragallo and *Skeletonema costatum*) he observed that the chains became shorter with increasing turbulence, and concluded that this probably happened due to mechanical chain breaking. In the case of *S. costatum* ageing of cells also lead to reduced chain lengths. In this study, this may have happened at the end of the *T. nitzschioides* experiment and the *T. rotula* in the Drebes-Medium experiment.

Only in the case of the Drebes-Medium August *T. rotula* the copepod-water treatments showed a development different from that of the non-copepod-water treatments. The mean cell numbers per chain decreased in the copepod-water treatments, which the statistical test confirmed with significant differences on days six and eight. This is a different effect to other studies where predators increase the size of the algal colonies (e. g. van Donk 1997, Lürling 1998, Lürling 2003, Verschoor et al. 2004). It could be explained by the fact that for a chain-forming diatom it would be more detrimental to have a longer chain as the copepod would, once having gripped an end, eat its way along the chain as implied by publications on copepod foraging strategies (Brandl 1998) anatomical studies (Paffenhöfer & Loyd 1999) and models and observations of the orientation of prey particles in copepod feeding currents (Paffenhöfer et al. 1982, Visser & Jonsson 2000) The *T. nitzschioides* cultures of the experiment reacted only weakly to the copepod-water, except for the chain numbers. The latter are the highest in the copepod-water treatments on day four. The mean cell numbers per chain are lower in the copepod-water treatments on this day. As in the case of the Drebes-Medium August *T. rotula* strain, this does not point to a classical potential defence mechanism where larger aggregates prevent ingestion. This influence of the copepod-water on the *T. nitzschioides* cultures was only noticeable on day four. All results suggest that neither *T. rotula* nor *T. nitzschioides*

were significantly influenced by the presence of copepods in our experiments although both species showed reactions to the grazer-water treatments that are consistent with recent studies like the effect of shorter chains that would fit with observations on copepod feeding behaviour where long colonies would be a disadvantage since they do not keep predators from ingesting the algae.

Algal Growth in Long-term Batch Cultures

Apart from the water temperature and grazer interactions, nutrient concentrations greatly influence phytoplankton community composition. To assess the population development of important species under increasing nutrient depletion, laboratory experiments with batch cultures of seven phytoplankton species were performed as presented in Chapter 4. To ensure non-limiting starting conditions, Guillard's f/2 medium was used. Over the course of eight weeks, growth rates, the increase of degrading and dead cells, cell sizes, chain lengths, photosynthetic quantum yield and the development of bacterial populations as well as nutrient concentrations were measured.

Freshly isolated cultures of the dinoflagellates *Akashiwo sanguinea* and *Ceratium horridum* as well as the diatoms *Guinardia delicatula*, *Pseudonitzschia pungens*, *Rhizosolenia pungens* and two strains of *Thalassiosira rotula* were used. The chosen algae are all known to be important species of the Helgoland foodweb and are found regularly in the phytoplankton community during blooms.

Growth rates between species differed to a great extent. In most cultures, the maximum numbers of living cells was already reached at day 14, matching the rapid depletion of silicate and phosphate within the first two weeks although all species must have been able to store nutrients for later growth. This strategy is already known for diatoms (Martin-Jézéquel et al. 2000, Claquin et al. 2002), especially for algae living in unstable environments and having to deal with nutrient pulses (Spijkerman & Coesel 1998) which would be the general assumption for ecosystems. The time span of about two weeks of growth until the crash of diatom population has also been found in model runs (Chai et al. 2002). Apart from increasing grazing pressure, the authors also attributed the decline of diatom biomass to the exhaustion of the silicate pool.

Both *T. rotula* strains and *R. pungens* showed the highest initial growth rates but also suffered high mortality with dead cells already appearing after the first week. From

the Helgoland Roads Long-term Data, *R. pungens* is known to follow a “boom and bust” strategy during spring bloom events, producing high biomasses but only within a narrow window of favourable conditions. In other cultures, dead cells were counted from day 14 onwards, coinciding with the depletion of silicate and phosphorus pools. The longest lag phase was found in the cultures of the dinoflagellate *C. horridum*. Cell numbers started to rise after day 21. Because the cells of this species are fairly large, the strategy of *C. horridum* may be to take up as much nutrients as possible for storage before starting to reproduce. Also, since *Ceratium* species are known to be mixotrophic (Smalley & Coats 2002), switches between feeding modes may have caused the delay.

The large differences in the development of cell sizes with deviations of as much as 80% (*R. pungens*) can also be attributed to nutrient depletion. Two strategies are known to exist for microalgae. As Masojídek et al. (2000) and Claquin et al. (2002) found in their studies on nutrient limitation with *T. pseudonana*, both nitrogen and phosphorus limitation can result in an increased cell size. Additionally, in an environment with nutrient pulses, larger species can have the advantage of storing enough of the limiting nutrients to sustain several generations (Lean 1984, Suttle et al. 1987). On the other hand, a decrease in cell volume may also be a reaction to nutrient depletion as the resulting smaller diffusion boundary layers allow smaller cells to utilize resources more efficiently and thus to outcompete larger species under prolonged nutrient limitation (Lean 1984, Spijkerman & Coesel 1998). A decline in cell size seems to be a general trend in phytoplankton evolution (Raven 1998, Jiang et al. 2005). Regarding our results, both uptake strategies may have been followed to deal with nutrient depletion.

Photosynthetic efficiency followed the same pattern in all cultures with the exception of *C. horridum* and *G. delicatula*. From initially low values due to handling stress at the start of the experiment, all algae reached an optimum quantum yield between 0.6 and 0.75 at day seven. Afterwards, yields slowly declined to medium levels and remained there for the rest of the experiment. In most cases, photosynthetic efficiency was closely related to nitrogen concentrations. The close coupling between photosynthesis, carbon and nitrogen metabolism and its significance for algal physiology, as many interactions depend on respiratory processes, has already been described in several studies (e.g. Turpin 1991). N-limitation can affect photosynthesis in many different ways, e.g. resulting in a decrease of nitrogenous pigments like

chlorophylls and phycobilins (Allen & Smith 1969, de Loura et al. 1986, Falkowski et al 1989, Herzig & Falkowski 1989, Masojídek et al. 2000), selective inactivation of Photosystem II (Berges et al. 1996) and a decline in enzymes of photosynthetic carbon metabolism, e.g. Rubisco (Kuppers & Weidner 1980, Falkowski et al. 1989, Geider et al. 1998). Even under non-limiting conditions, photosynthetic quantum yield follows fluctuations in nitrogen concentrations (Lippemeier et al. 2001). Although these results cannot always be reproduced to the full extent in outdoor experiments (Babin et al. 1996), the important influence of nitrogen on algal photosynthesis is evident.

In most cultures, the number of bacteria was positively correlated with the abundances of degrading and dead cells. In return, bacteria had only little influence on growth rates of living cells, cell sizes and photosynthetic efficiency. However, since only the overall bacterial abundances were used in these analyses, the results also reflect only general trends in relationships. In a parallel investigation of the species-specific communities and interactions between bacteria and microalgae by Sapp et al. (2006), it was shown that the composition of the bacterial communities varied to different extents between algal species and in the case of *G. delicatula*, showed a succession of species during the experiment. Therefore, details on the influence of bacteria on the development of algal populations can only be assessed by microbiological methods.

The results of this study provide deeper insights into the life patterns of microalgae and their reactions to nutrient limitations, increasing bacterial abundances and changes in population structure from high abundances of living cells to a majority of degrading and dead cells. However, since the experiments were carried out in batch cultures with ample nutrient concentrations provided by the f/2 medium and only at 15 °C, there are many questions regarding nutrient limitations and the influence of different temperatures left unanswered. Also, the question of the impact of grazer presence as additional factor was not addressed in this experiment.

Spring Bloom in a Bottle – trying to understand the patterns behind Phytoplankton Succession

For the interpretation of in situ data as well as model predictions, it is important to know the basic requirements of the species studied regarding for example possible nutrient limitations, growth rates at different temperatures and the influence of

grazers on population developments. The aim of the study presented in Chapter 5 was to find those characteristic responses of three important species of the Helgoland Roads community to different nutrient treatments and simulated grazer presence at three temperatures. Regarding population development, for *G. delicatula* these characteristics are rising growth rates with higher temperatures, nitrogen limitation and the highest influence of grazer presence for all three species. In the case of *T. rotula*, the results show most consistent growth at medium temperatures and phosphorus limitation. For *T. nitzschioides*, differences in growth rates between treatments were smallest at low temperatures. The limiting nutrient depended on the temperature the experiment was conducted at with nitrogen limitation at 6 °C and a shift to phosphorus limitation beginning at 8 °C. These results are in accordance with the field data presented in Chapter 2 and reports from other systems (Baldauf 1985, Kopczyńska et al. 1998, Gayoso 1999, Gowen et al. 1999, Romero et al. 2002).

As known from this in situ data (Chapter 2), temperature plays a major role in the succession of phytoplankton species. Temperature also was the most important factor modulating the responses to grazer and nutrient treatments in this study. Growth rates generally corresponded to the natural succession observed at Helgoland Roads with *T. nitzschioides* showing the most consistent growth across all treatments at 6 °C, *T. rotula* at 8 °C and *G. delicatula* at 10 °C. Although the temperature range may seem to be rather small, the clear effects show the impact of even small deviations. The relevance of temperature changes in the range of 1 °C - 2 °C has been reported from other ecosystems (e.g. Goffart et al. 2002) with consequences for hydrological parameters, stratification, salinity, nutrient distribution and pH that affect the entire food web by leading to delays in spring blooms, shifts from diatom to cyanobacterial-dominated plankton communities and overall reduction of phytoplankton biomass. These changes subsequently affected higher trophic levels where especially the success of reproduction depends on the availability of food as formulated by Cushing (1975, 1990) in his match/mismatch hypothesis. Temperature-related shifts in the phytoplankton community composition are also found in the Helgoland Long-term data where an increase of 1.1 °C of the annual mean water temperature over the last 40 years lead to a trend of *G. delicatula* becoming more abundant on the expense of other species like e.g. *O. aurita* and *T. nitzschioides* (Wiltshire et al. 2005). This trend is confirmed by our study.

Another factor of importance in phytoplankton succession is the concentration of major nutrients and their compounds such as nitrite, nitrate, phosphate and silicate. Apart from the individual competitive abilities, species can follow a number of strategies to protract limitation. One of them, as shown by *T. rotula* and *G. delicatula* in this study, is to decrease cell sizes. As argued in Chapter 3, a decrease in biovolume is a possible way to cope with nutrient depletion because smaller cells have the advantage of smaller diffusion boundary layers utilizing the remaining nutrients more efficiently (Lean 1984, Spijkerman & Coesel 1998). While nutrient concentrations and temperature had a noticeable impact on growth rates, the photosynthetic efficiency of all species was not influenced by temperature or treatment. This points towards a high ability for compensation e.g. the physiological impacts of different temperatures as proposed by Smith et al. (1994) and the observations of Rodríguez-Román who found the photosynthetic efficiency to depend rather on the overall light history of cultures and a species' sensitivity than on the nutrient supply.

From the results of the studies presented in this thesis, the answer to the question: "Can you bottle nature?" is a positive one. The results of the laboratory experiments confirmed and explained the in situ observations of the spring bloom 2004 and the Helgoland Roads Long-term Data. For example in the case of *G. delicatula*, the laboratory results of higher growth rates with rising temperatures and nitrogen limitation match the findings of the spring bloom 2004 where these effects were found. Also, the patterns found the Helgoland Long-term Data, e.g. the rising numbers of *G. delicatula* along the observed increase of mean water temperatures were confirmed. By using several uni- and multivariate statistical methods, patterns and mutual interactions in field data were found that would not have been detected by simple correlation matrices. Furthermore, previously unknown characteristics as e.g. the development of cells sizes in connection with nutrient concentrations were found in laboratory experiments. These "fingerprints" provide not only important background information for the interpretation of field data but can also be applied to foodweb models used for a better understanding of foodweb interactions and predictions of the consequences of for example climate change.

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Abstract

Pelagic foodwebs and phytoplankton species have been important research objects for more than 100 years. Countless studies and a lot of qualitative and quantitative data sets exist. However, because of the complex interactions in ecosystems, the factors and mechanisms that structure phytoplankton communities and lead to observed seasonal shifts of species composition still remain unknown to a large extent. Moreover, comparisons between studies are difficult. While species may be widely distributed, differences on a biological basis between ecosystems, populations and strains complicate comparisons. Technical improvements over the course of time, differences in sampling dates, sampling techniques and analyses also add to the problem even for data on one system. Also, many studies report either results from field data or laboratory work while a combination of both would improve understandings of the processes in a given system.

Based on the observations of the spring bloom 2004 and already published insights on trends and patterns in phytoplankton abundances from the Helgoland Roads Long-term Series, the aim of this thesis was to further investigate the species-specific morphological and physiological traits and requirements in order to find the mechanisms affecting and controlling the abundances of some of the most important species of the phytoplankton community at Helgoland roads.

During the spring bloom 2004, most species analysed showed known patterns of abundance. Generally, the water temperature was the most important factor for the succession of species. However, reactions to other abiotic and biotic factors as e.g. speed and directions of wind and currents, salinity, nutrient concentrations and the abundances of main grazer groups varied, pointing to different life strategies and niches.

One aspect of the interactions between microalgae and grazers is the induction of morphological and physiological changes that can serve as defence mechanisms. One of the ways to trigger these changes are substances released by grazers, e.g. urea, serving as alarm chemicals for microalgae. Reactions can range from the production of larger cells, growing of spikes or other appendices, colony formation and higher growth rates to the production of toxins. However, as all interactions, they are also influenced by other factors, one of them being turbulence. Turbulence

affects e.g. the concentration and dispersion of chemicals or can put an increased mechanical stress on colonies elongated as reaction to grazer presence. On the other hand, if a species follows the strategy of increased reproduction, it facilitates the distribution of cells and thereby can have positive effects on the survival chances of a species. In a laboratory experiment the effects of grazer presence simulated by the use of water previously incubated for 24 hrs with the common copepod species *Acartia tonsa* and turbulence on growth rates, cell size and chain length of two strains of *Thalassiosira rotula* and one clone of *Thalassionema nitzschioides* were investigated. The results show that in all three cultures, turbulence had a significantly negative influence on growth rates and chain lengths. In the case of *T. rotula*, the grazer presence also led to shorter colonies. This consistent with recent research on copepod foraging behaviour indicating that smaller colonies are of advantage when to avoid grazing.

Apart from the water temperature, nutrient concentrations greatly influence phytoplankton community composition. To assess the population development of key species under increasing nutrient depletion, laboratory experiments with batch cultures of seven phytoplankton species were performed. To ensure non-limiting starting conditions, Guillard's f/2 medium was used. Over the course of eight weeks, growth rates, the increase of degrading and dead cells, cell sizes, chain lengths, photosynthetic quantum yield and the development of bacterial populations as well as nutrient concentrations were measured. Common trends were observed regarding the development of photosynthetic efficiency and its correlation with nitrate concentrations and the coincidence of the appearance of degrading and dead cells with the depletion of silicate and phosphorus. Species-specific reactions to the experimental conditions were also found regarding individual growth rates and e.g. the increase or decrease of cell sizes in reaction to nutrient depletion. Bacterial abundances were positively correlated with the increasing numbers of degrading and dead cells but had in return no significant influence on the algal parameters measured.

The interactive effects of direct nutrient limitation, temperature and simulated grazer presence on growth rates, cell sizes, chain length and photosynthetic efficiency on the three species *Thalassionema nitzschioides*, *Thalassiosira rotula* and *Guinardia delicatula* were also investigated. Nitrogen and phosphorus were chosen as limiting nutrients and the experiments conducted with five nutrient treatments in the chemical

presence or absence of grazers at three temperatures. Growth rates at given temperatures corresponded to the patterns found during the spring bloom 2004 with *T. nitzschioides* showing the most homogenous growth across treatments at 6 °C, *T. rotula* preferring 8 °C and *G. delicatula* showing rates increasing with temperature. Additionally, *T. nitzschioides* was found to be nitrogen limited at 6 °C but limitation changed to phosphorus at 8 °C and 10 °C. *T. rotula* was phosphorus limited and in the case of *G. delicatula*, nitrogen limitation was found. These results are consistent with the observations during the spring bloom 2004, the laboratory experiments presented in this thesis and findings reported from other studies. The influence of grazer presence varied with species, nutrient concentrations and temperature level and had the highest effects on growth rates, cell sizes and chain lengths of *G. delicatula*, the late blooming species more likely to encounter higher grazer abundances than the two other diatoms.

Comparing the results to the field data, the laboratory findings were able to explain the corresponding effects found in the other data sets. E.g. in the case of *G. delicatula*, the trend of increasing abundances along the rising temperatures found in the Long-term data, the late bloom during the spring bloom 2004 being positively correlated with temperature and e.g. nitrite concentrations can now be confirmed by the laboratory results.

Zusammenfassung

Pelagische Nahrungsnetze und insbesondere das Phytoplankton sind seit mehr als hundert Jahren Gegenstände wissenschaftlicher Untersuchungen. Unzählige Studien zu den verschiedensten Aspekten der Planktonökologie mit vielen qualitativen und quantitativen Daten sind bereits vorhanden. Durch die Komplexität der Interaktionen in Ökosystemen sind jedoch viele der Faktoren und Mechanismen, die die Struktur und saisonale Zusammensetzung von Phytoplanktongemeinschaften steuern noch weitgehend unbekannt. Darüberhinaus gestalten sich Vergleiche zwischen Studien als schwierig. Obwohl viele Arten weltweit verbreitet sind, bestehen doch Unterschiede zwischen Ökosystemen, Populationen und sogar zwischen Stämmen einer Art. Hinzu kommen sowohl der technische Fortschritt als auch Unterschiede bei Probenahme und Analyse. Dadurch sind Vergleiche selbst dann schwierig, wenn es sich um Daten desselben Nahrungsnetzes handelt. Weiterhin stellen viele Studien entweder Freilanddaten oder Laborergebnisse vor. Für ein verbessertes Verständnis der Abläufe in einem System wären jedoch kombinierte Studien von Vorteil.

Ausgehend von den Beobachtungen während der Frühjahrsblüte 2004 und bereits veröffentlichten Auswertungen der Helgoland Reede Langzeitserie war es das Ziel dieser Studie, artspezifische morphologische und physiologische Charakteristika und Ansprüche zu untersuchen, um die Mechanismen hinter den saisonalen Abundanzänderungen einiger wichtiger Phytoplanktonarten des Helgoländer Nahrungsnetzes zu finden.

Während der Frühjahrsblüte 2004 folgten die meisten Arten bereits bekannten Abundanzmustern. Im Allgemeinen war der wichtigste Faktor für die Sukzession die Wassertemperatur. Die Reaktion auf andere abiotische und biotische Faktoren wie z.B. Richtungen und Geschwindigkeiten von Wind und Strömungen, Salinität, Nährstoffkonzentrationen und der Einfluss von Grazern unterschieden sich jedoch von Art zu Art und lieferten erste Hinweise auf unterschiedliche Lebensstrategien und mögliche Nischen.

Ein Aspekt in den Interaktionen zwischen Grazern und Mikroalgen ist die Auslösung von morphologischen und physiologischen Veränderungen bei Algen, die als Verteidigungsmechanismen dienen können. Zu den Auslösern solcher Veränderungen gehören von den Grazern ausgeschiedene Stoffe, z.B. Harnstoff, die

eine Signalwirkung auf Algen habe. Mögliche Reaktionen reichen von der Bildung größerer Zellen, der Ausbildung von Fortsätzen, Koloniebildung und erhöhten Wachstumsraten hin zur Bildung von Toxinen. Jedoch wird auch dieses Wechselspiel von einer Vielzahl anderer Faktoren beeinflusst. Einer dieser Einflüsse ist die Turbulenz. Turbulenzen beeinflussen z.B. Konzentration und Ausbreitung von Signalstoffen oder führen zu erhöhtem mechanischem Stress für die als Reaktion auf die Anwesenheit von Grazern vergrößerte Kolonien. Verfolgt eine Art jedoch die Strategie erhöhter Wachstumsraten, so kann die Turbulenz die Verbreitung der Zellen erleichtern und damit die Überlebenschancen erhöhen. In einem Laborversuch wurden die Effekte der von der Copepodenart *Acartia tonsa* ausgeschiedenen Stoffe und der Turbulenz auf Wachstumsraten, Zellgröße und Kettenlänge zweier Kulturen der Art *Thalassiosira rotula*, isoliert zu unterschiedlichen Jahreszeiten und einer Kultur der Art *Thalassionema nitzschioides* untersucht. In allen drei Kulturen hatte die Turbulenz einen signifikant negativen Einfluss auf Wachstumsraten und Kettenlängen. In den Ansätzen von *T. rotula* führte die simulierte Anwesenheit von Grazern ebenfalls zu reduzierten Kolonielängen. Dies stimmt mit Resultaten anderer Studien über das Fraßverhalten von Copepoden überein, die den Schluss zulassen, dass kürzere Kettenlängen von Vorteil sind, um dem Fraßdruck zu entgehen, da Copepoden in der Lage sind, Ketten passend zu drehen und eine Zelle nach der anderen zu fressen.

Neben der Wassertemperatur beeinflusst die Konzentration der Nährstoffe maßgeblich die Zusammensetzung der Phytoplanktongemeinschaft. Um die Populationsentwicklung von sieben Schlüsselarten unter zunehmender Nährstofflimitation zu untersuchen, wurden Laborexperimente mit Batch-Kulturen durchgeführt. Um nichtlimitierende Startbedingungen zu gewährleisten, wurde Guillard's f/2 Medium verwendet. Über den Zeitraum von acht Wochen wurden Wachstumsraten, die Zunahme sterbender und toter Zellen, Biovolumina, Koloniegrößen und die Quantenausbeute bei der Photosynthese verfolgt. Zusätzlich wurden Nährstoffkonzentrationen und die Entwicklung der Bakterienpopulationen erfasst. Die Ergebnisse zeigen Gemeinsamkeiten wie die Entwicklung der Photosynthese-Effizienz und ihre Korrelation mit den Nitratkonzentrationen sowie das Auftreten sterbender und toter Zellen ab dem Zeitpunkt der Aufzehrung von Silikat und Phosphat in den Kulturen. Es wurden jedoch auch artspezifische Reaktionen wie individuelle Wachstumsraten und die Entwicklung der Zellgrößen im

Zusammenhang mit der einsetzenden Nährstofflimitierung gefunden. Die Abundanzen der Bakterienpopulationen waren positiv mit dem Auftreten sterbender und toter Zellen korreliert, hatten aber keinen signifikanten Einfluss auf die gemessenen Algenparameter.

Die interaktiven Effekte direkter Nährstofflimitation, Temperatur and simulierter Anwesenheit von Grazern auf Wachstumsraten, Zellgröße, Kettenlänge und Effizienz der Photosynthese von *Thalassionema nitzschioides*, *Thalassiosira rotula* und *Guinardia delicatula* wurden in einem weiteren Laborexperiment untersucht. Stickstoff und Phosphor in den Formen Nitrat und Phosphat wurden als limitierende Nährstoffe ausgewählt und die Experimente mit fünf verschiedenen Konzentrationsabstufungen mit oder ohne die simulierte Anwesenheit von Grazern bei drei Temperaturen durchgeführt. Die Wachstumsraten der einzelnen Arten korrespondierten mit denen während der Frühjahrsblüte 2004 gefundenen Mustern. *T. nitzschioides* zeigte bei 6 °C das gleichmäßigste Wachstum aller Ansätze, *T. rotula* bevorzugte die mittlere Temperatur 8 °C und in den Kulturen von *G. delicatula* stiegen die Wachstumsraten mit der Temperatur. Bei den beobachteten Nährstofflimitationen zeigten sich die folgenden Ergebnisse: *T. nitzschioides* war bei 6 °C stickstofflimitiert, jedoch begann bei 8 °C ein Wechsel zu Phosphorlimitierung, der sich bei 10 °C fortsetzte. *T. rotula* war in allen Ansätzen phosphorlimitiert, während sich bei *G. delicatula* eine Stickstofflimitierung zeigte. Diese Ergebnisse waren ebenfalls konsistent mit den Beobachtungen während der Frühjahrsblüte 2004, den weiteren Laborexperimenten in dieser Arbeit und den Resultaten anderer Studien. Der Einfluss der Grazerpräsenz variierte zwischen Arten, Nährstoffkonzentrationen und Temperaturen. *G. delicatula* zeigte die ausgeprägtesten Reaktionen hinsichtlich Wachstumsraten, Zellgröße und Kettenlänge. Die erhöhte Sensibilität lässt sich dadurch erklären, dass es sich hierbei um eine Art handelt, die höhere Wassertemperaturen bevorzugt und zu einem späten Zeitpunkt der Frühjahrsblüte auftritt und so möglicherweise einem höheren Fraßdruck ausgesetzt ist.

Im Vergleich zwischen Laborergebnissen und Freilanddaten zeigte sich, dass die in Experimenten gewonnenen Ergebnisse in der Lage sind, die in situ beobachteten Muster zu erklären. Im Fall von *G. delicatula* etwa können die positiv mit dem Anstieg der mittleren Jahrestemperatur korrelierten Abundanzen der Langzeitserie und das

späte jährliche Auftreten sowie die Rolle des Stickstoffs als limitierender Nährstoff durch die Laborergebnisse bestätigt werden.

Scientific Contributions to the Multiple-Author Manuscripts

A Plankton Puzzle: Observations during a Spring Bloom Event

A.S. Schwaderer, S. Janisch, K. Carstens, S. Laakmann, K.H. Wiltshire

Measurements of cell sizes and photosynthetic efficiency, all analysis, text writing and graphical presentation were carried out by A.S. Schwaderer under the supervision of Prof. Dr. K.H. Wiltshire. Phytoplankton counts were done by S. Janisch and nutrient analyses by C. Karstens. S. Laakmann contributed zooplankton data.

Morphological Adaptations in Diatoms: Influence of Grazer Presence and Turbulence

A.S. Schwaderer, S. Ehmen, C-D Dürselen, M. Boersma, K.H. Wiltshire

All text writing was done by A.S. Schwaderer under the supervision of Prof. Dr. K.H. Wiltshire. The experiments and analyses were performed by S. Ehmen under the supervision of Prof. Dr. K.H. Wiltshire, Dr. C-D Dürselen and A.S. Schwaderer. PD Dr. M. Boersma provided help during the statistical analyses.

Algal Growth in Long-term Batch Cultures: Pattern Definitions

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Measurements of photosynthetic efficiency, diatom cell counts and biovolume measurements, all analyses, text writing and graphical presentation were carried out by A.S. Schwaderer under the supervision of Prof. Dr. K.H. Wiltshire. The experiment and nutrient analyses were done by A.S. Schwaderer and M. Sapp who also contributed bacterial data. Dr. A. Wichels and Dr. G. Gerdtts reviewed earlier versions of the manuscript and provided fruitful discussion.

Spring Bloom in a Bottle: Trying to understand the Mechanisms behind Phytoplankton Succession

A.S. Schwaderer, U. Sommer, K.H. Wiltshire

The experiment, all analyses, all writing and graphical presentations were done by A.S. Schwaderer under the supervision of Prof. Dr. K.H. Wiltshire. U. Sommer provided general advice and supervision.

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„... Dort wo du nur eine Spur gesehen hast, da habe ich dich getragen.“

Spuren im Sand – Margaret Fishback Powers

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Erklärung

Hiermit erkläre ich, dass die vorliegende Dissertation nach Form und Inhalt meine eigene Arbeit ist, die ich selbstständig und ohne unerlaubte Hilfsmittel angefertigt habe. Ferner habe ich weder diese noch eine ähnliche Arbeit an einer anderen Hochschule im Rahmen eines Promotionsverfahrens vorgelegt.

Die Promotion soll im Fach Biologische Meereskunde erfolgen. Als Prüfungsform wähle ich die Disputation, zu der ich Zuhörer zulasse.

Anne Schwaderer