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Investigations on the ecology of the marine centric diatom *Paralia sulcata* at Helgoland Roads, North Sea, Germany

by

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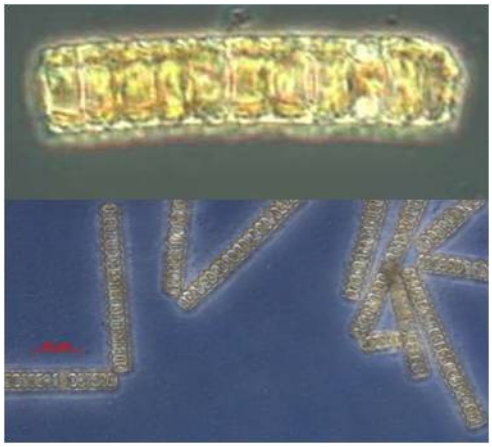
A thesis submitted in partial fulfilment
of the requirements for the degree of

Doctor of Philosophy
in Biology

Approved, Thesis Committee



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CONTENTS

GENERAL INTRODUCTION

Ecological niche and niche definition

In order to determine the ecological role of a species it is important to understand its ecological niche. Ecology in general deals with the interactions between organisms and with their environment, whereby the interactions can be both abiotic (physical and chemical factors influencing the occurrence of a species) and biotic (e.g. other organisms, competition and prey-predator interactions) nature. The environment of an organism itself consists of all those factors (biotic and abiotic) influencing the occurrence of this species (Whittaker et al. 1973, Mitchell 2005, Kearney 2006). In the following paragraph I will introduce an important concept in ecology, the niche concept.

The 'niche' is a fundamental concept of modern community ecology (Leibold 1995). The niche concept was established by G. E. Hutchinson (1957) defining the niche as an "n-dimensional hypervolume". He pointed out that the "n-dimensions" refer to all ecological factors which are important for the existence of a species and comprising all environmental conditions "which would permit the species S_1 to exist indefinitely". The "hypervolume" consists of a multi-dimensional space of resources (e.g. light, nutrients, etc.) which are available to the organism (Hutchinson 1957, Pulliam 2000, Kearney 2006). This definition considers a species in isolation, completely excluding interactions with other organisms and was referred to as *fundamental niche* (Hutchinson 1957). It includes the total range of environmental conditions which are suitable for existence of this species without the influence of biotic and abiotic interactions. However, species do not occur in isolation, but interact with a number of other species and with their environment (biotic e.g. predator-prey interactions and competition for nutrients, light availability) (McGill et al. 2006). These interactions will reduce the portion of the fundamental niche that can actually be occupied (Kearney 2006, McGill et al. 2006). In this way, the single species "n-dimensional hypervolume" is converted from the rather abstract *fundamental niche* to the *realised niche* (Hutchinson 1957) (Fig. 1a).

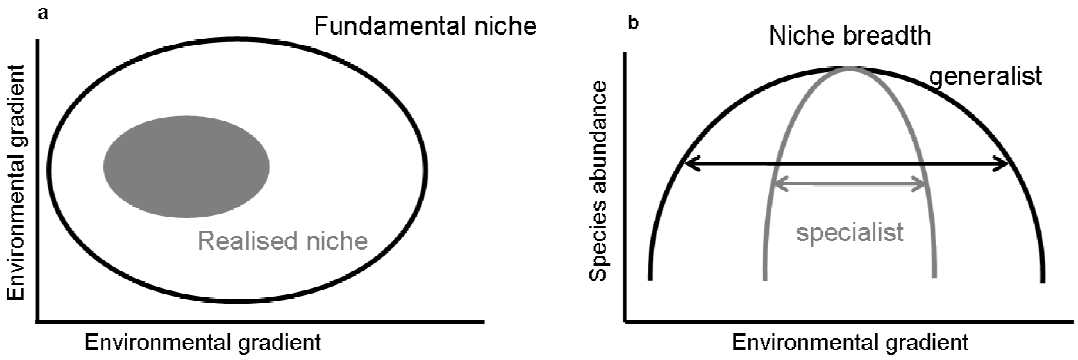

Figure 1: Schematic description of a) the fundamental and realised ecological niche due to different environmental gradients and b) of the niche breadth (species tolerance) according to a generalist or specialist species (Diagram: modified after Hutchinson (1957) and Dolédec et al. (2000)).

To describe the ecological niche it is essential to understand the species tolerance along a gradient of environmental factors as important niche parameter. The niche breadth describes the species tolerance associated with the environmental parameters (Dolédec et al. 2000). Species tolerating only narrow changes in environmental parameters are referred to as specialists. In contrast, generalists are assumed to tolerate widely varying environmental conditions and thus, should have a broader niche breadth (Dolédec et al. 2000, Heino & Soininen 2006) (Fig. 1b).

In experimental ecology the transition from the fundamental to the realised niche can be demonstrated by comparing the differences between laboratory experiments and field work. All but a few abiotic factors are kept constant during the laboratory experiment, and biotic factors are preferably excluded. In the field on the other hand all factors (competitors, predators and prey) are included, although field data are often noisy and therefore difficult to interpret. Therefore, any niche reconstructed from field data is necessarily a realised niche and may differ considerably from the simplified fundamental niche recreated from laboratory experiments (McGill et al. 2006).

Taking this into account, one focus of this thesis was to investigate the autecological behaviour of one species in more detail with laboratory experiments compared with a field sampling campaign. In the following sections the investigated species, *Paralia sulcata* and its surrounding environment (Helgoland Roads, North Sea) will be introduced.

Diatoms and their ecological role

The ecological role of microalgae, especially diatoms, is very broad. Diatoms, an extremely species rich taxonomic group, play a major ecological role in terrestrial, freshwater and marine environments (Mann 1999, Evans et al. 2007). They are dominant under naturally high nutrient concentrations, especially in the spring blooms (Miralto et al. 1999, Sarthou et al. 2005) and form the basis of the marine food web (Andersen 1992). Diatoms have an ecologically wide distribution and account for around 40% of the global primary production (Nelson et al. 1995, Mann 1999, Smetacek 1999, Sarthou et al. 2005). They are also of global significance in the carbon and silicate cycles (Mann 1999, Smetacek 1999, Sarthou et al. 2005) as they are a major source of biogenic silica (Mann 1999). A considerable amount of silicate is re-mobilised in the upper layers of the oceans through dissolution of the silica valves and the rate depends on the temperature (Treguer et al. 1995).

Due to their broad distribution is important to understand the ecological role of individual diatom species so that the impact of changing environmental parameters e.g. due to climate change as well as bioindicator for water quality and past climates (Evans et al. 2007). As diatoms have existed since the early Mesozoic and preserve well in marine sediments (Medlin et al. 1997), they are considered to be good palaeoindicators of past changes in coastal regions particularly due to their abundance in sediments, their sensitivity to environmental variables and due to their highly silicified valves (Zong 1997, Mann 1999, McQuoid & Nordberg 2003a). Furthermore, specific diatom species, including *Paralia sulcata* have been used as indicator to define stratified or mixed water situations (McQuoid & Nordberg 2003b) as well as for freshwater or marine water influences (Weiss et al. 1978).

Paralia sulcata – environmental aspects and study object

Paralia sulcata is a discoid, chain-forming centric diatom with thick-walled, dissolution resistant siliceous valves (Crawford 1979a, Roelofs 1984, Abrantes 1988a, Zong 1997). As *P. sulcata* is very heavily silicified it preserves well in sediments and can be used as paleoindicator (McQuoid & Nordberg 2003a). It shows the most complex valve structure of the genus *Paralia* (Crawford 1979a, Crawford et al. 1990, Sims & Crawford 2002, Sawai et al. 2005) (Fig. 2).

Several fossil and extant species of *Paralia* have been described but the taxonomic/nomenclatural history of this genus is somewhat difficult. (e.g. Sims & Crawford 2002, Sawai et al. 2005). Crawford (1979a) described *P. sulcata* (Ehrenberg) Cleve as extant species and *P. siberica* was identified as fossil one (Crawford et al. 1990, Sims & Crawford 2002). However, the genus *Paralia* has a long fossil history with *P. crenulata* making the first appearance in the late Cretaceous (Sims & Crawford 2002).

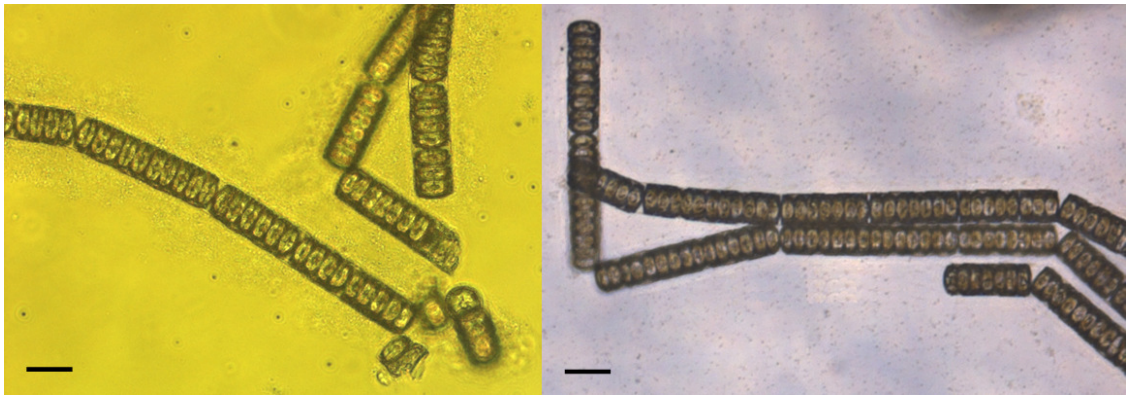

Figure 2: *Paralia sulcata* isolated from Helgoland Roads (scale bar: 10 µm) (Photos: C. Gebühr).

Literature review of the environmental determinants of the distribution of *Paralia sulcata*

The description of *Paralia sulcata* as indicator for past climate poses an interesting question: What are the general ecological optima in which *P. sulcata* occurs and how is the abundance affected in the current environment? Giving a more detail insight into the ecology of *P. sulcata* within its marine habitat and determining the principal environmental parameters influencing its occurrence are the main goals of this thesis. An overview of the ecological description of *P. sulcata* shown in the literature is the focus of the following section.

Paralia sulcata is a cosmopolitan, brackish to marine diatom species found in littoral and sublittoral zones and sediments as well as the phytoplankton and is considered to be a tychopelagic species (Roelofs 1984, Zong 1997, McQuoid & Hobson 1998, McQuoid & Nordberg 2003a). It is often associated with sandy habitats and fine-grained sediments rich in organic material (Zong 1997, McQuoid & Hobson 1998). *P. sulcata* does not typically form large blooms, however it can be found in the water

column during winter probably due to its competitive advantage at low light conditions (Hobson & McQuoid 1997). Furthermore, it is a major species in the phytoplankton in autumn and winter dependent on the re-suspension from the sediment due to strong winds and tidal mixing (Roelofs 1984, Oh & Koh 1995, McQuoid & Hobson 1998, McQuoid & Nordberg 2003b).

Environmental data shows that *P. sulcata* can grow over a wide range of conditions, but that it may favour low temperatures and short day lengths when irradiance is high (Hobson & McQuoid 1997). It is known that *P. sulcata* can occur in a wide salinity range from deep seas where salinity is more than 30 but also in estuarine and coastal sites with lower salinities between 5 and 25 (Zong 1997). Furthermore, Abrantes (1988a) detected higher abundances of *P. sulcata* in coastal regions with high upwelling situations and patchy nutrient concentrations. The influences of different environmental parameters on the occurrence of *P. sulcata* described in the literature can be quite contradictory (Table 1). Temperature can serve as an example. McQuoid and Hobson (1998) and Choudhury and Pal (2010) found that with increasing water temperature the abundance also increased which indicated that *P. sulcata* thrived at warmer water temperatures. In contrast, with decreasing water temperatures the abundance of *P. sulcata* was increasing as well (Hobson & McQuoid 1997). In summary, it becomes obvious that no single environmental factor is exclusively responsible for the change in the relative abundance of *P. sulcata* in a given location. Due to the ubiquitous distribution of this diatom, it is essential to understand more precisely the ecological niche of *P. sulcata* especially assuming further long-term changes in its environment.

The studies summarised above showed the occurrence of *P. sulcata* in the surface waters or sediment surfaces only during a short investigation period. However, to characterise the ecology of single species it is important to understand the habitat and environmental conditions in which this species lives. To achieve this long-term data are absolutely crucial. In this regard the Helgoland Roads long-tem data set is unique and one of the richest data sets available for the marine system (Wiltshire et al. 2010).

Table 1: Summary of the known correlations of environmental parameters with *Paralia sulcata* abundance and valve diameter respectively obtained from the literature. Decreasing environmental parameter "-", increasing means "+", negative correlation: "-", positive correlation "+".

***Paralia sulcata* parameter**	**Environmental parameter**	**Correlation with**	**Sampling location**	**References**
occurrence	strong upwelling, nutrients +	high abundant +	sediment samples, continental shelf of Galicia, Spain	Bao et al. (1997)
	temperature +	abundant, but low	water samples, Bay of Bengal, Eastern India	Choudhury & Pal (2010)
	salinity +	high abundant +	sediment samples, Hudson Estuary	Weiss et al. (1978)
	salinity +, temperature +	high abundant +	sediment samples, southern British Columbia	Roelofs (1984)
	temperature -, day length -, salinity +	high abundant +	water samples, southern British Columbia	Hobson & McQuoid (1997)
	upwelling/ turbulence +	high abundant +	water samples, Bay of Galicia, northwest Spain	Casas et al. (1999)
	temperature +	high abundant +	sediment samples, southern British Columbia	McQuoid & Hobson (1998)
	salinity -, light -nutrients +, temperature +	high abundant +	sediment samples, northwest coast of Scotland	Zong (1997)
large cell diameter	strong upwelling, nutrients +	high abundant +	sediment samples, west coast shelf, Portugal	Abrantes (1988a)
	salinity -, nutrients +, temperature -	high abundant +	sediment samples, Bay of Vigo, northwest Spain	Margalef (1969)
	salinity +	high abundant +	sediment samples, southern British Columbia	Roelofs (1984)
	temperature + , salinity +	high abundant +	sediment samples, southern British Columbia	McQuoid & Hobson (1998)
	upwelling -, nutrients -, salinity -	high abundant +	sediment samples, Koljö Fjord, west Sweden	McQuoid & Nordberg (2003a, 2003b)

Helgoland Roads: long-term data and changing hydrography in the North Sea

Helgoland is situated in the German Bight around 60 km from the main land. The investigations of the ecology of *Paralia sulcata* were conducted at Helgoland Roads, North Sea between the two small islands of Helgoland and Dune (54°11.3'N; 7°54.0'E) (Fig. 3).

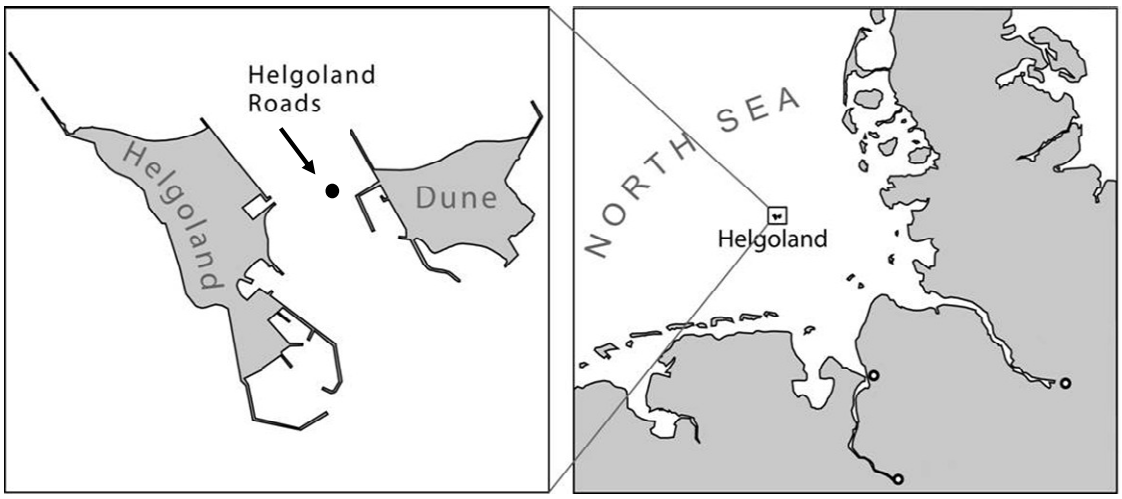

Figure 3: Sampling site of the water samples at Helgoland Roads (54°11.3'N; 7°54.0'E), North Sea (Graphic: modified by A. Neumann).

Helgoland is situated in mixed waters in the German Bight in a hydrographically very dynamic area that can be under the influence of oceanic as well as coastal waters.

The North Sea, including the waters in the vicinity of Helgoland has undergone considerable changes over recent decades. The analysis of long-term data has shown changes in hydrography and concomitant changes in the timing of phyto- and zooplankton (Edwards et al. 2002) in the North Sea. These changes in hydrography in the North Sea have been assumed to be linked to Atlantic inflow (Reid et al. 2003) and to local hydrodynamic parameters (Reid et al. 2001, Beaugrand 2004). Two alternating flow patterns were shown which described the total variability of water transports during 1958 and 2000. Stockmann et al. (2010) have also shown that a change of the predominant flow pattern in the German Bight and thus, in the last 15 years Helgoland was influenced by stronger inflow of waters from the northwest and therefore, leading to a greater influence of oceanic waters. These oceanic waters from the northwest Atlantic are characterised by higher salinities and warmer winter temperatures due to the Gulf Stream whereas the coastal waters are characterised by lower salinities and

higher amounts of nutrients (Wiltshire et al. 2008, Stockmann et al. 2010, Wiltshire et al. 2010).

Furthermore, it was shown that the average water temperature in the North Sea has risen by 1.67°C over the last 50 years (Wiltshire et al. 2010) concurrent with similar warming trends measured in the North Sea (Edwards et al. 2002). Additionally the Secchi depth (proxy for water transparency) significantly increased by almost 1 m and a significant decrease in the phosphate limitation was detected during 1975-2005 (Wiltshire et al. 2008).

Changes in the marine system around Helgoland are therefore visible in the long-term data. More clearer (reflected in higher Secchi depth), marine water with warmer water temperatures (increasing temperature) (Wiltshire & Manly 2004, Wiltshire et al. 2008) which could partly be related to changes in the North Atlantic Oscillation (Beare et al. 2002, Edwards et al. 2002, Reid et al. 2003, Stockmann et al. 2010) will have affected the whole plankton community and the food web at Helgoland Roads.

Our knowledge of how the changing environmental conditions in the North Sea affect the ecology of species and of how phytoplankton adapts to changes in the marine environment is still inadequate (Edwards et al. 2002, Wiltshire & Manly 2004, Wiltshire et al. 2008, Wiltshire et al. 2010). It is therefore important to investigate the ecological niche of phytoplankton species to understand their potential for adaptation to environmental change.

What are the long-term trends and effects of changing environmental conditions on the occurrence and autecology of *Paralia sulcata*?

To answer this question three "methods" were used: 1) a multivariate statistical analysis of the long-term data to determine the generally important environmental parameters influencing *P. sulcata*, 2) a two-year monitoring campaign to examine the occurrence of *P. sulcata* in deeper waters compared with surface water and ambient environmental parameters, and 3) different laboratory experiments to determine the autecological behaviour of this species.

Several statistical methods are used for describing the ecological niche of species, e.g., by measuring the individual distribution of species among environmental parameters (Colwell & Futuyma 1971), by quantifying niche breadth using the proportional similarity index (Feinsinger et al. 1981) or by determining species-environment

relationships using ordination methods (ter Braak 1986, Dolédec & Chessel 1994). In this thesis mainly multivariate statistical analysis was applied with the ordination technique, especially Canonical Correspondence Analysis (CCA). The CCA extracts environmental gradients from ecological data sets and these gradients form the basis for describing habitat preference of a species (ter Braak & Verdonschot 1995, Dolédec et al. 2000). A newly developed approach focuses on the measurements of distances between the average habitat conditions used by a species and the habitat conditions of the sampling area (or period) (Dolédec et al. 2000). This new method determines the niche of a given species and is called outlying mean index (OMI) or niche analysis. This multivariate technique quantifies two niche parameters in consideration to niche position and niche breadth of a given species along several environmental gradients (Dolédec et al. 2000, Thuiller et al. 2005, Lappalainen & Soininen 2006). The niche breadth is a value describing species tolerance associated with the environmental parameters (Dolédec et al. 2000). Applying both methods the ecological niche of *P. sulcata* can be described at Helgoland Roads using environmental variables derived from the Helgoland Roads long-term data set (water surface temperature, Secchi depth, dissolved inorganic nutrients, salinity) (Franke et al. 2004, Wiltshire & Manly 2004, Wiltshire et al. 2008) and weather data (mean and maximal wind speed and sun shine duration) provided by the Deutsche Wetter Dienst (DWD).

To examine the occurrence of *P. sulcata* in the surface and deeper water, water samples from 1 m above the ground, hereafter referred to as 'bottom water samples', were taken during a two year monitoring sampling campaign (October 2007 to October 2009). Furthermore, cell counts of *P. sulcata*, Secchi depth, salinity, temperature and nutrients (dissolved inorganic nitrate, nitrite, ammonia, phosphate and silicate) were measured and determined in the bottom and in the surface water samples as part of the Helgoland Roads long-term monitoring program (Wiltshire & Dürselen 2004, Wiltshire & Manly 2004).

Two different hypotheses were tested in laboratory experiments. The hypothesis that *P. sulcata* is adapted to reach optimal growth rates at low temperatures and also the positive influence of higher silicate and phosphate concentrations was tested in one experimental set-up with three different temperature regimes in combination with eight different nutrient concentrations. Additionally the positive influence of humic substances on the growth of *P. sulcata* as benthic species was tested in a second experimental set-up to investigate different influences of light availability.

Do genetically different populations of *Paralia sulcata* occurs at Helgoland Roads?

The worldwide distribution and the adaptation to a wide range of environmental parameters of *Paralia sulcata* leads to the hypothesis that genetically separated populations exist. Due to the changing hydrography especially in the North Sea, e.g. regime shifts and thus, changing water masses influencing Helgoland Roads (Stockmann et al. 2010) it appears possible that a warmer water adapted species occurring the whole year in the water column was introduced or that two genetically different populations exist, one in summer and one in winter at Helgoland Roads. Thus, is there a difference in the *P. sulcata* population found at Helgoland Roads especially due to the different seasons? This question should be evaluated with a molecular fingerprint technique to determine the genetic population at Helgoland Roads.

Spring bloom development and the role of *Paralia sulcata* in the marine food web

Only a detailed understanding of aquatic food webs will enable us to assess the effects of changing environmental conditions on this food web. It is therefore important to understand the relationships between predator and prey species at different trophic levels and their interactions within the food web. Diatoms provide energy for higher trophic levels influenced by herbivory of the microzooplankton and mesozooplankton (De Laender et al.) and thus, are the main food source for copepods (Miralto et al. 1999) (Fig. 4).

An important aim in the study of the ecological function of diatoms in the food web is to gain an understanding of the seasonal succession and the concurrent influences of different environmental parameters on the occurrence of diatoms. Seasonal phytoplankton succession, especially the spring bloom development, of temperate coastal waters has frequently been investigated *in situ* (e.g. Levasseur et al. 1984, Casas et al. 1999, Gayoso 1999, Rousseau et al. 2002) and in mesocosm experiments (e.g. Graneli & Turner 2002, Aberle et al. 2007, Sommer et al. 2007).

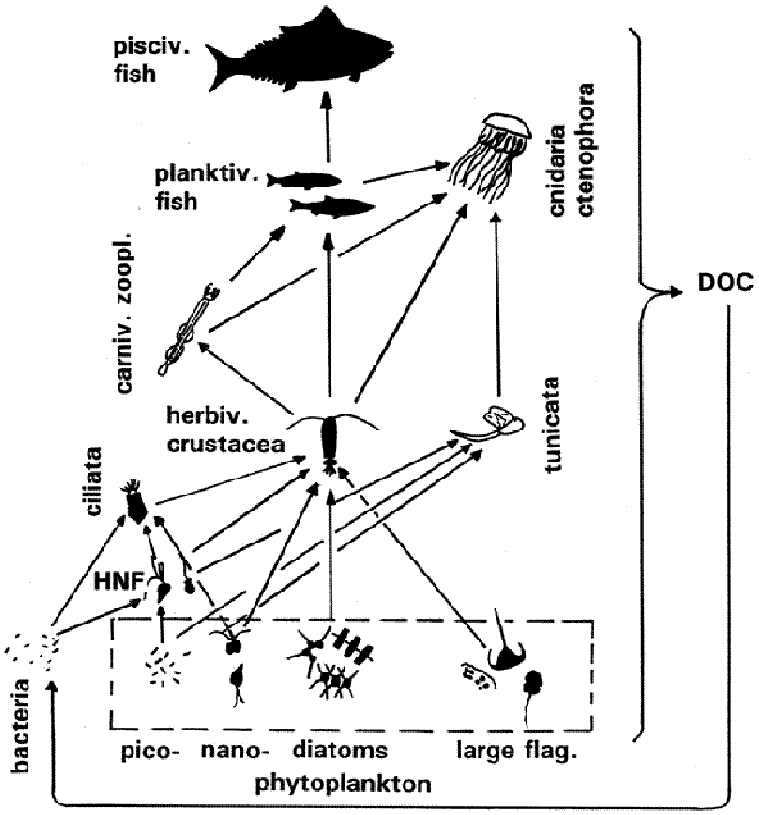

Figure 4: Simplified food web of Helgoland Roads, North Sea (modified after Sommer et al. 2005, De Laender et al. 2010). DOC = dissolved organic carbon, HNF = heterotrophic nanoflagellates.

However, nothing is known about the specific biological interactions (e.g. predator-prey interactions) of *Paralia sulcata* within its environment. But due to its occurrence throughout the year this diatom could also be a food source for micro-and mesozooplankton throughout the whole year, and might act as food in the pre-spring bloom situation. One hypothesis is that *P. sulcata* presents a continuous food source especially during spring bloom events. Thus, one focus of this thesis was the determination of *P. sulcata* as food source for copepods. This investigation was carried out during a mesocosm spring bloom experiment.

FOCUS OF THE PRESENT STUDY

The primary objective of this study was the investigation of the ecological role of the marine centric diatom species *Paralia sulcata* at Helgoland Roads, North Sea. Many previous studies have been concerned with different environmental conditions and their influence on the occurrence of *P. sulcata* in marine and coastal areas, but often these studies provided contrasting results (Table 1). Furthermore, the worldwide distribution and the wide range of adaptations to environmental parameters make it necessary to understand the current ecological characteristics of *P. sulcata*. This study aimed to determine the ecological niche of *P. sulcata* within its marine environment. The focus of the research aims of this thesis will be presented in the following paragraphs.

1) Before the ecological niche of *Paralia sulcata* was investigated a multivariate statistical analysis was performed using the Helgoland Roads long-term data set to answer the following question: How is the ecological niche of *P. sulcata* at Helgoland Roads defined? And which are the most important environmental parameters determining the niche of *P. sulcata* at the population level?
Results of the multivariate statistical analysis of the occurrence of *P. sulcata* and the important environmental parameters influencing the ecological niche were presented in Chapter I. These observations provide the basis for detailed laboratory experiments and serve as a framework for the following chapters

2) To investigate the autecological behaviour the following question should be answered: How is *Paralia sulcata* affected by their abiotic environment under controlled laboratory conditions?
Two hypotheses were tested in laboratory growth experiments. The first hypothesis was that lower temperatures and higher concentrations of silicate and phosphate influence the growth of *P. sulcata* in a positive manner. The second hypothesis that humic acids positively affected the growth of *P. sulcata* due to the absorption of light was tested in another experimental set-up. Furthermore, experimental results were compared with the data obtained form the two year monitoring campaign to draw conclusions on the lifestyle of *P. sulcata* under field and laboratory conditions (Chapter II).

3) The results of the laboratory and especially the field sampling data revealed different behaviours of *Paralia sulcata* during the seasons. As a consequence, a new hypothesis was developed to answer whether genetically different populations of *P. sulcata* occurred at Helgoland Roads. Thus, the genetic diversity of different *P. sulcata* strains isolated over one year at Helgoland Roads was investigated using inter simple sequence repeats (ISSRs) (Chapter III).

4) Additionally, the community structure especially during the spring bloom development was investigated and thus, the role of *Paralia sulcata* as food source in the marine food web in the North Sea was estimated during a mesocosm spring bloom experiment. Due to the annual occurrence of *P. sulcata* in the water column and in the benthos the diatom seemed to be an important food source for benthic as well as pelagic grazers. Therefore, grazing experiments with different food quality of the phytoplankton and microzooplankton were performed as well as selectivity strategies of the copepod were investigated (Chapter IV).

OUTLINE OF THIS THESIS

This study comprises four stand-alone publishable papers (Chapter I to IV) and an overall discussion. Chapter I has already been published in *Aquatic Biology*, Chapter III has been submitted to *European Journal of Phycology* and Chapter II and Chapter IV are being prepared for submission. Additionally, a part of the results was published in a review which I co-authored (Wiltshire et al. 2010).
The titles, authors and the contribution of each author to the manuscripts (Chapters I, II, III and IV) are briefly outlined below:

Chapter I	Influence of nutrients, temperature, light and salinity on the occurrence of *Paralia sulcata* at Helgoland Roads, North Sea
Authors	Christina Gebühr, Karen H. Wiltshire, Nicole Aberle, Justus E. E. van Beusekom & Gunnar Gerdts
Status	published: *Aquatic Biology* 7: 185–197 (2009) All analyses, the text writing and graphical presentation were done by Christina Gebühr under supervision of Prof. Dr. K.H. Wiltshire, Dr. G. Gerdts and Dr. N. Aberle. Dr. J.E.E. van Beusekom provided helpful discussion.

Chapter II	Ecological niche of *Paralia sulcata* determined in the laboratory and with field data
Authors	Gebühr, C., Martin, M.V., Martire, D. & Wiltshire, K.H.
Status	Prepared for submission All experiments, analyses, text writing and graphical presentation were done by Christina Gebühr under supervision of Prof. Dr. K.H. Wiltshire. Dr. N. Aberle provided helpful discussion, S. Peters, K. Carstens and S. B. Moos assisted during the experiment.

Chapter III	Genetic diversity of *Paralia sulcata* (Bacillariophyta) analysed by Inter Simple Sequence Repeats (ISSRs)
Authors	Christina Gebühr, Gunnar Gerdts, Antje Wichels & Karen H. Wiltshire
Status	Re-submission: *European Journal of Phycology*
	All analyses, the text writing and graphical presentation were done by Christina Gebühr under supervision of Dr. G. Gerdts, Dr. A. Wichels and Prof. Dr. K.H. Wiltshire.

Chapter IV	How important is *Paralia sulcata* within its marine food web and as possible food source for copepod grazers?
Authors	Christina Gebühr, Katherina L. Schoo, Martin G.J. Löder, Nicole Aberle, Maarten Boersma & Karen H. Wiltshire
Status	Prepared for submission
	All experimental analyses (grazing experiments), the text writing and graphical presentation were done by Christina Gebühr with careful help of Katherina Schoo under supervision of Dr. N. Aberle, Prof. Dr. M. Boersma and Prof. Dr. K.H. Wiltshire. Martin Löder provided the grazing rates data estimated from dilution experiments during the same mesocosms experiment.

CHAPTER I

Influence of nutrients, temperature, light and salinity on the occurrence of *Paralia sulcata* at Helgoland Roads, North Sea

Influence of nutrients, temperature, light and salinity on the occurrence of *Paralia sulcata* at Helgoland Roads, North Sea

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Running head: Ecological niche of *Paralia sulcata*

ABSTRACT

Paralia sulcata is a tychopelagic centric diatom species common in the North Sea. Due to the fact that *P. sulcata* is found both in the sediment and the water column, it is assumed to be an important food source for both benthic and pelagic grazers. We know a little about the environmental conditions associated with the occurrence of *P. sulcata*, but almost nothing is known of its ecological role. Thus, the aim of this study was to investigate the ecology of *P. sulcata*. The Helgoland Roads long-term data set (North Sea), in which phytoplankton and physicochemical parameters have been sampled since 1962, served as the environmental data set. To detect possible species–environment relationships, multivariate statistical analysis was carried out (canonical correspondence analysis). Annual niche breadth and niche position (outlying mean indices) were calculated for *P. sulcata*. Up to 1996, *P. sulcata* occurred mainly in late autumn and winter, but since 1997 it has been found throughout the year. The niche position and niche breadth for *P. sulcata* varied over the sampling period. There was a change in the ecological niche of *P. sulcata*, with a shift from a more specialised to a more generalised niche and a new occurrence of this diatom in summer during the last 10 yr. Changing temperature, light and nutrient conditions at Helgoland Roads could be responsible for the new occurrence and the shift in the ecological niche of *P. sulcata*.

Key words: Microalgae, *Paralia sulcata*, ecological niche, German Bight, nutrients, temperature, light, multivariate statistical analysis

INTRODUCTION

Paralia sulcata (Ehrenberg) Cleve is a centric diatom with robust, chain-forming valves. It benefits from chain formation in terms of protection and increased nutrient and light availability (Crawford 1979b). This diatom has a wide distribution and is often found in temperate brackish to marine planktonic and benthic waters, in both littoral and sublittoral zones (McQuoid & Nordberg 2003a, 2003b). It is often associated with sandy habitats and fine-grained sediments rich in organic material (Roelofs 1984, Zong 1997, McQuoid & Hobson 1998).

In waters off the southern part of Vancouver Island (British Columbia, Canada), *Paralia sulcata* was found year-round in cell concentrations of 1500 cells l^{-1} reaching maximum numbers in winter blooms of 3000 to 6000 cells l^{-1} (Hobson & McQuoid 1997). As a coldwater alga, *P. sulcata* shows a growth optimum at a temperature of 7 ± 1°C (Hobson & McQuoid 1997, Zong 1997). *P. sulcata* displays a competitive advantage under low light conditions and prefers low temperatures and short day lengths with high irradiance (Roelofs 1984, Hobson & McQuoid 1997, Zong 1997, McQuoid & Nordberg 2003a).

Often found in the benthos, the presence of *Paralia sulcata* in the phytoplankton could be dependent on vertical transport processes and resuspension into the plankton by strong winds and tidal mixing (Roelofs 1984, Hobson & McQuoid 1997). Increased vertical mixing in the water column brings nutrient-rich, saline water to the surface, thus creating conditions which favour the occurrence of *P. sulcata* (McQuoid & Nordberg 2003a). Abrantes (1988a) showed that *P. sulcata* is correlated with higher nutrient concentrations and is typically found in regions with high levels of upwelling. The higher nutrient concentrations due to remineralisation processes in coastal waters and storm activity in winter could explain the occurrence of *P. sulcata* in the water column. Furthermore, salinity may have an impact on the abundance of *P. sulcata*, which has been shown to be negatively correlated with low salinity in British Columbia inlets (Roelofs 1984). Elsewhere, Zong (1997) has shown that *P. sulcata* has a wide salinity tolerance range (5 to > 30).

We know a little about the environmental conditions associated with the occurrence of *Paralia sulcata* but we know almost nothing of its ecological role. Due to its thick siliceous valves, this diatom is often found in late Quaternary sediments (Zong 1997). As diatoms have existed since the early Mesozoic (Medlin et al. 1997), they can serve as good palaeoindicators of past changes in coastal regions due to their abundance in

sediments and can act as proxies for specific environmental conditions (McQuoid & Nordberg 2003b). An understanding of the ecology of *P. sulcata* would therefore be helpful for the reconstruction of past environmental conditions.

To determine the ecological niche of *Paralia sulcata*, an analysis of the response of this species to several environmental parameters is needed. According to the ecological niche concept, each species has its own environmental optimum, and the fundamental niche is defined as a multidimensional space where the environmental conditions could limit the growth of a species (Hutchinson 1957). According to Kearney (2006), a realised niche is defined as the sum of all abiotic and biotic factors which influence the organism's growth and fitness and the interactions between the organism and environmental parameters; the realised niche should be smaller than the fundamental niche (McGill et al. 2006). In order to define optimum conditions for habitats or ecological niches of species with statistical models, it is essential to determine particular niche parameters, i.e. niche breadth and niche position, from the field data.

There are numerous statistical methods for analysing ecological niches. For the present study, canonical correspondence analysis (CCA) and outlying mean index (OMI) analysis were selected to describe the ecological niche of *Paralia sulcata*. CCA extracts environmental gradients from ecological data sets, which are the basis to describe the habitat preference of a species (ter Braak & Verdonschot 1995, Dolédec et al. 2000). A newly developed approach, OMI determines the niche breadth and niche position of a given species (Dolédec et al. 2000).

Since 1962, a daily monitoring program has been maintained at Helgoland Roads which has resulted in one of the most important marine data sets in the world, unique with respect to the length of the time series, sampling frequency and number of parameters measured (Franke et al. 2004, Wiltshire & Manly 2004). These long-term data have been used for modelling ecosystem functions (Wirtz & Wiltshire 2005) as well for investigations into ecological questions concerning bacteria (Gerdts et al. 2004), phytoplankton (Wiltshire & Dürselen 2004, Wiltshire & Manly 2004, Wiltshire et al. 2008), zooplankton (Greve et al. 2004), macroalgae communities (Bartsch & Tittley 2004) and macrozoobenthos (Franke et al. 2004).

From the phytoplankton data set we know that the abundance of *Paralia sulcata* has been changing over the last 40 years at Helgoland Roads (Wiltshire & Dürselen 2004), yet the food quality of this diatom in the marine food web in the North Sea is unknown. It is possible that *P. sulcata* has become a more important food source for

benthic and pelagic grazers due to its increasing abundance in the summer but this requires more detailed investigation.

Here we investigate the influence of nutrients, light, temperature and salinity on the occurrence of *Paralia sulcata* using the long-term data series from Helgoland Roads. We determine the ecological niche of this diatom and how it has adapted to changing environmental conditions. This information will enable a description of the temporal changes of *P. sulcata* at Helgoland Roads, identification of the determining factors of its occurrence and the variability in niche position and breadth of *P. sulcata* over time.

METHODS

Study site

Helgoland is situated in the German Bight about 60 km from the mainland and the estuaries of the rivers Elbe and Weser (54° 11.3' N, 7° 54.0' E). The German Bight is a transition zone between the well-mixed low saline coastal waters and the deeper waters of the south-eastern North Sea (Bauerfeind et al. 1990). Dependent on the meteorological situation, the water around Helgoland is influenced by the lower coastal saline waters or the open North Sea several times in the year due to currents and tidal mixing (Hickel 1998, Wiltshire et al. 2010). The water depth at Helgoland Roads fluctuates between 3 and 8 m over the tidal cycle.

Sampling and data sets

A series of periodic measurements and daily water sampling was initiated by the Biologische Anstalt Helgoland at Helgoland Roads in 1962 (Franke et al. 2004) (54° 11.3'N, 7° 54.0' E). Surface water samples represented the entire water column, which is generally well-mixed as a result of strong tidal currents (Hickel 1998). The surface water samples were taken from the RV 'Aade' with a bucket. Identification and enumeration of the phytoplankton and analyses of physicochemical parameters like salinity, temperature, Secchi depth and dissolved inorganic nutrients (ammonium, nitrate, nitrite, phosphate and silicate) were measured and analysed daily (Wiltshire & Manly 2004, Wiltshire et al. 2010). For quantitative measurement of phytoplankton, the water sample was well mixed, subsampled into a brown glass bottle and fixed with Lugol's iodine solution. Daily counting of the phytoplankton was conducted according

to the method of Lund et al. (1958) in 25 or 50 ml Utermöhl settling chambers with an inverted microscope (Axiovert 135, Zeiss); phytoplankton were identified to species level or separated into size classes by microscopically measuring species size (Wiltshire & Dürselen 2004).

A subsample from the water sample was used to measure the salinity with a Salinometer (Autosal, Gamma Analysen Technik) and for the colorimetric determination of the nutrients after Grasshoff (1976). All data has been reviewed and quality controlled by Raabe & Wiltshire (2009). Radiation data (global net shortwave radiation from 100 to 700 µm) was provided by the GKSS Research Centre (Geesthacht, Germany).

The explanatory variables included in the multivariate analysis are temperature, the temperature difference between 2 consecutive weeks, Secchi depth, salinity, solar radiation and concentrations of ammonium, nitrate, nitrite, phosphate and silicate (Table 1).

Table 1: Explanation and abbreviations of the response variables (algae) and environmental parameters used for the statistical analysis of the algal community at Helgoland Roads, North Sea.

Response variables (algae)	abbreviation	Environmental parameters	abbreviation
Ceratium furca	*C.fur*	Global solar radiation (Wm^{-2})	Rad
Ceratium fusus	*C.fus*	Secchi depth (m)	Secchi
Ceratium horridum	*C.hor*	Temperature (°C)	Temp
Eucampia zodiacus	*E.zod*	Salinity	Sal
Guinardia delicatula	*G.del*	Phosphate (µmol l^{-1})	PO4
Guinardia striata	*G.str*	Nitrite (µmol l^{-1})	NO2
Melosira spp.	*Mel.*spec	Nitrate (µmol l^{-1})	NO3
Navicula spp.	*Nav.*spec	Ammonium (µmol l^{-1})	NH4
Odontella aurita	*O.aur*	Silicate (µmol l^{-1})	SiO4
Odontella mobiliensis	*O.mob*		
Odontella regia	*O.reg*		
Odontella rhombus	*O.rho*		
Odontella sinensis	*O.sin*		
Paralia sulcata	*P.sul*		
Skeletonema costatum	*S.cos*		
Thalassionema nitzschioides	*T.nit*		
Thalassiosira nordenskioeldii	*T.nor*		
Thalassiosira rotula	*T.rot*		

The algal data set used in the multivariate statistical analysis is a subset from the Helgoland Roads algal data. It was composed of 3 species from the class Dinophyceae (*Ceratium furca*, *C. fusus* and *C. horridum*) and 15 from Bacillariophyceae (*Eucampia zodiacus*, *Guinardia delicatula*, *G. striata*, *Melosira* spp., *Navicula* spp., *Odontella aurita*, *O. mobiliensis*, *O. regia*, *O. rhombus*, *O. sinensis*, *Paralia sulcata*, *Skeletonema costatum*, *Thalassionema nitzschioides*, *Thalassiosira nordenskioeldii* and *Thalassiosira rotula*) (Table 1). Some taxa have not been continuously identified to species level (Hoppenrath 2004, Wiltshire & Dürselen 2004), such as *Melosira* spp. and *Navicula* spp., here determined to genus. All algae chosen for analyses regularly occur in the water column at Helgoland Roads and virtually complete records exist from 1962 to the present day (Wiltshire & Dürselen 2004). The phytoplankton data have been quality controlled by Wiltshire and Dürselen (2004). The Bacillariophyceae were used because of their chain-forming properties and centric morphology (as with *Paralia*). *Navicula* spp. and *Thalassionema nitzschioides* were the only pennate exceptions, occurring continuously since 1962 at Helgoland and used here as the counterpart to the centric diatoms. The Dinophyceae were selected because they are representative of summer season algae and there are unbroken records for their occurrence (Wiltshire & Dürselen 2004).

Statistical analysis

Only data from 1968 to 2005 were used for statistical analysis due to the high number of missing data points at the beginning of the monitoring program (e.g. determination of silicate started in 1966 and Secchi depth measurements began in 1968). The weekly mean was calculated for all data. A total of 90 missing weeks were interpolated linearly.

The multivariate statistical analysis of such long-term data is not easy to interpret because these results cannot be verified or refuted by experiments in the laboratory. Statistical analysis is not an analysis of cause or effect, but this multivariate analysis is helpful for describing ecological interactions which could be investigated in experiments on a smaller scale.

Influence of the environmental factors on *Paralia sulcata*

Multivariate ordination techniques were applied to determine the significant environmental factors affecting *Paralia sulcata* and for the investigation of seasonal trends. To estimate whether weighted-averaging or linear techniques should be applied, for each selected data subset (individual years) a detrended correspondence analysis (DCA) was performed using CANOCO for Windows 4.53 (Biometris). The gradient of the first DCA axis describes the extent of the species turnover along the major ecological factors (e.g. the diversity of the community composition along the environmental gradients) and gradients with lengths of more than 4 SD represent a complete species turnover indicating a unimodal ordination technique (Leps & Smilauer 2003, Heino & Soininen 2005). SD values between 3 and 4 did not indicate a clear linear or unimodal relationship (Leps & Smilauer 2003). Thus the selection of redundancy analysis (RDA) or CCA requires the selection of a linear or unimodal model for the species response to the environmental parameters (Dolédec et al. 2000).

Hence, RDA as well as CCA were performed to examine species–environment correlations and to test for the significance of the resulting eigenvalues and species–environment correlations. RDA and CCA were carried out as described by Leps & Smilauer (2003). The marginal and conditional effects quantify the effects of the environmental parameters on the response variables and were selected according to their ranking or significance level ($p < 0.05$) as determined by Monte Carlo permutation (499 permutations). Marginal effects represent the influence of each environmental parameter on the algal community. Higher values indicate a greater influence on the algal community. Conditional effects demonstrate the combined effects of environmental variables on the algal community (Leps & Smilauer 2003). Bi-plot scaling was used for the community ordination analysis (Leps & Smilauer 2003). An overall CCA for the total time period (1968 to 2005) was performed to determine the general pattern of environmental parameters and algal community. An automated forward selection with a restricted permutation test for temporal structure of the time-series was used with 499 permutations.

To support the results of the CCA and to show correlations between *Paralia sulcata* and environmental parameters, the non-parametric Spearman rank correlation was calculated using STATISTICA 7.1 (StatSoft) with a significance level of $p < 0.05$.

Ecological niche of *Paralia sulcata*

The OMI of *Paralia sulcata* was calculated using R version 2.6.0 and the software package ADE-4 (R Development Core Team 2007) (Thioulouse et al. 1997). This multivariate technique quantifies the niche parameters with consideration of niche position and niche breadth for the diatom along several environmental gradients (Dolédec et al. 2000, Lappalainen & Soininen 2006). In this analysis, the realised niche was calculated using the measured and sampled field data. Niche position is a measure of the distance of average habitat conditions (the measured environmental parameters) used by this species from the average habitat conditions of the sampling site. Species tolerance represents the niche breadth of this species associated with the environmental parameters (Dolédec et al. 2000). If the values of species tolerance are lower, the species is considered a specialist. In contrast, generalists are assumed to live in a habitat with widely varying environmental conditions and thus they show higher values of species tolerance (Dolédec et al. 2000, Heino & Soininen 2006, Tsiftsis et al. 2008).

To determine similarities between different years from the given environmental parameters an analysis of similarities (ANOSIM) was performed using PRIMER software (version 6.1.6, PRIMER-E). ANOSIM is a nonparametric method which allows statistical comparisons for multivariate data in a similar way to univariate techniques (Clarke & Warwick 2001). All environmental parameters for the years 1968 to 2005 were normalised before analysis and the Euclidean distance was calculated. Hierarchical cluster analysis (pairwise tests, group averages) was carried out on the basis of ANOSIM rho-values. The result of the hierarchical cluster analysis is the differentiation of 4 groups of year clusters based on the environmental parameters.

Niche position and niche breadth of *Paralia sulcata* were correlated with the most important environmental parameters, as extracted from the CCA, to test for the effects of these parameters on niche position and niche breadth. The Spearman rank and Pearson correlation coefficients were calculated with annual mean environmental parameters and their standard deviation for niche position and niche breadth for the 4 different clustered year groups.

RESULTS

Figure 1 gives a data plot of the important environmental parameters influencing the occurrence of *Paralia sulcata* at Helgoland Roads and shows significant changes in temperature, Secchi depth and phosphate concentrations from 1968 to 2005. From 1968 to 1996, *Paralia sulcata* occurred at Helgoland Roads exclusively in autumn, winter and early spring with a mean abundance of around 3000 cells l^{-1} (Fig. 2). Since 1997, this diatom has also been detected during summer in the water column at around 1000 cells l^{-1}. There is a high intra- and inter-annual variability in the abundances of *P. sulcata*. This may be due to the overall patchiness in the water column at Helgoland Roads and upwelling processes in the North Sea, as well as strong tidal mixing and storm-induced mixing in autumn and winter.

Influence of the environmental factors on *Paralia sulcata*

The results of the DCA showed that the length of gradients varied from 2.63 to 6.77, so CCA or RDA was subsequently performed (Table 2). A high variability in the algal community and the species–environment correlation was observed, which is explained by the first axis of the CCA and RDA.

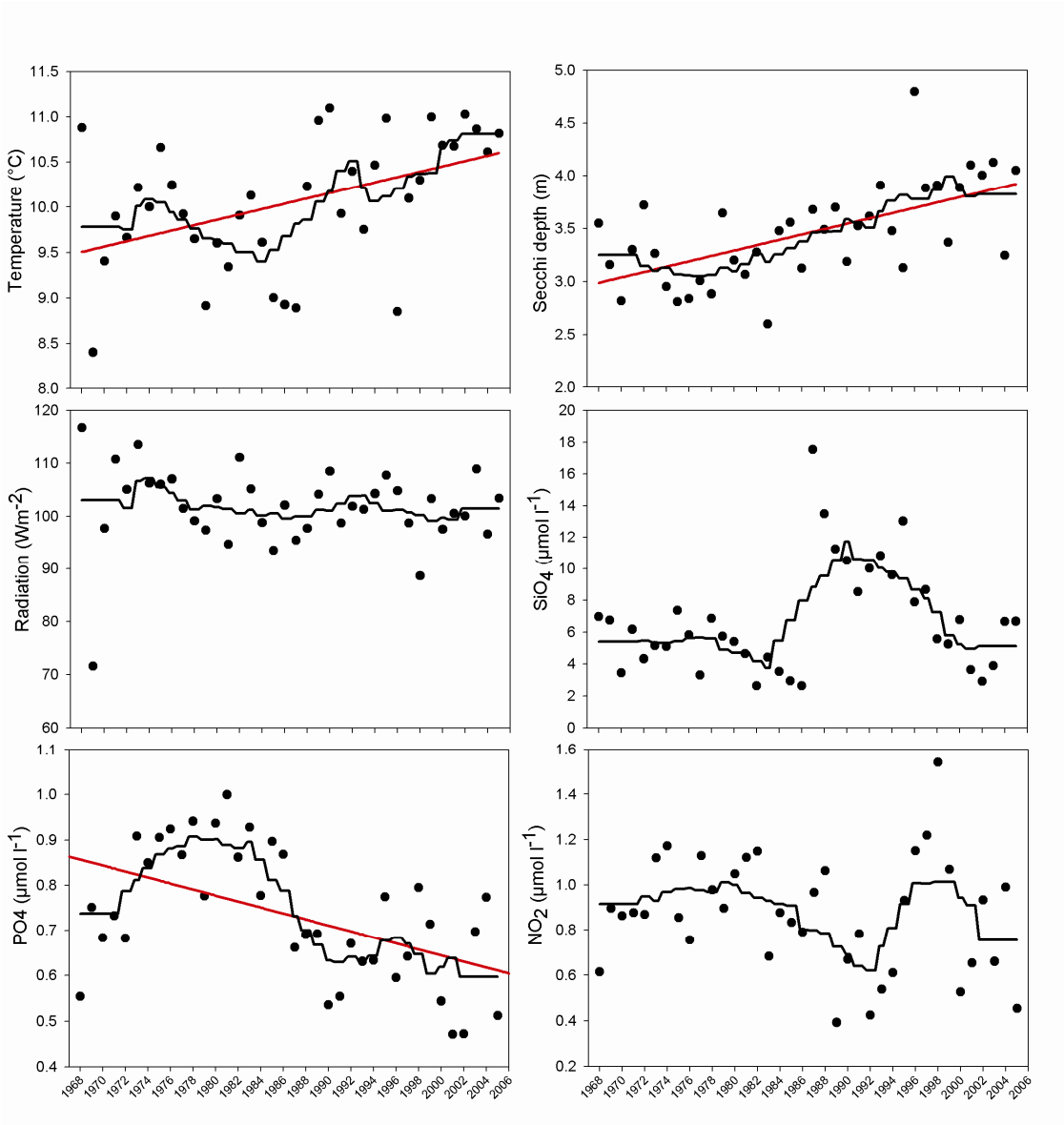

Figure 1: Time-series plots of the annual mean of the environmental parameters measured at Helgoland Roads. Running means indicate the trend of these parameters. Significant changes are shown for temperature ($R^2 = 0.2055$, $p < 0.05$), Secchi depth ($R^2 = 0.3732$, $p < 0.05$) and phosphate ($R^2 = 0.2566$, $p < 0.05$).

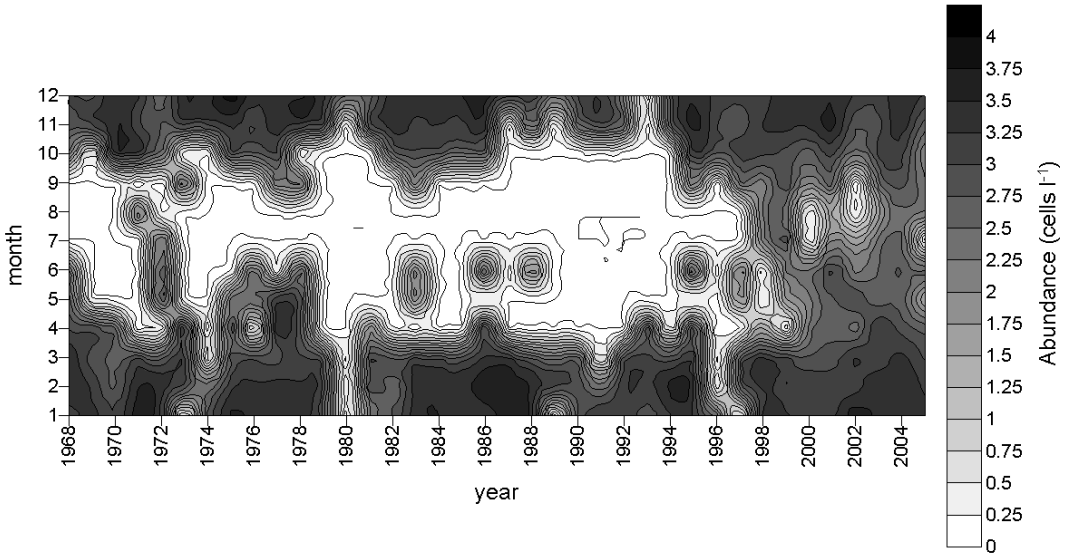

Figure 2: *Paralia sulcata* log-transformed abundance (cells l^{-1}) from 1968 to 2005.

A CCA covering all years from 1968 to 2005 was carried out for the impact of environmental parameters on the algal community. The position of the algal species on the first 2 canonical axes gave a qualitative indication of their environmental optima (Fig. 3). The algal community is influenced by 6 major parameters: water temperature, Secchi depth, global solar radiation and phosphate, nitrite and silicate concentrations extracted from the conditional effects (Table A1 in Appendix 1). High water temperatures, Secchi depth and radiation are positively correlated, as are silicate, phosphate, nitrite and nitrate concentrations. It was shown that light, Secchi depth and temperature were mostly negatively correlated with nutrients. This pattern reflects the typical conditions found for different seasons, where spring to summer (April to September) is characterised by higher light levels and warmer water temperature and autumn to winter (October to March) by higher nutrient concentrations. This reflects a common pattern for temperate regions where, for example, the winter period is characterised by lower water temperatures and light conditions but higher concentrations of nutrients due to recycling processes in the water column like we observed at Helgoland Roads. In late spring and summer, the concentrations of nutrients are much lower, limiting the algal growth. The monthly pairwise test of the ANOSIM with all environmental parameters showed the same pattern for the seasons (data not shown).

Table 2: Length of gradients, variability of algae community and species–environment correlations of the first axis of the canonical correspondence (CCA) and redundancy analyses (RDA). Significant axes are labelled with an asterisk ($p < 0.05$).

year	lengths of gradient	CCA/RDA	variability of algae community	species- environment correlations
1968	3.419	CCA	17.5	0.876 *
1969	4.945	CCA	49.5	0.962 *
1970	4.201	CCA	22.0	0.963 *
1971	4.235	CCA	21.6	0.988 *
1972	4.13	CCA	18.5	0.980 *
1973	3.416	CCA	24.0	0.964 *
1974	4.611	CCA	22.0	0.983 *
1975	3.788	CCA	27.0	0.961 *
1976	4.012	CCA	23.8	0.938 *
1977	4.882	CCA	23.6	0.953 *
1978	3.758	CCA	26.6	0.941 *
1979	4.207	CCA	26.4	0.977 *
1980	0	RDA	36.4	0.842 *
1981	4.65	CCA	23.1	0.918 *
1982	3.368	RDA	21.6	0.707 *
1983	3.946	CCA	20.5	0.830 *
1984	6.768	CCA	19.2	0.943 *
1985	4.278	CCA	25.9	0.968 *
1986	3.829	CCA	34.4	0.992 *
1987	5.336	CCA	19.5	0.969 *
1988	5.209	CCA	26.0	0.982 *
1989	4.913	CCA	27.0	0.996 *
1990	4.027	CCA	29.0	0.957 *
1991	4.411	CCA	20.4	0.977 *
1992	5.913	CCA	24.2	0.983 *
1993	3.775	CCA	34.5	0.996 *
1994	3.051	RDA	25.9	0.540
1995	3.793	CCA	30.7	0.940 *
1996	3.149	RDA	38.3	0.621 *
1997	4.022	CCA	29.7	0.985 *
1998	3.052	RDA	25.3	0.547
1999	2.825	RDA	19.8	0.450
2000	2.814	RDA	38.9	0.674 *
2001	2.63	RDA	23.3	0.489
2002	3.69	CCA	26.8	0.922 *
2003	2.91	RDA	26.3	0.615
2004	3.121	RDA	22.8	0.516
2005	4.162	CCA	16.3	0.928 *
1968-2005	4.581	CCA	5.8	0.697 *

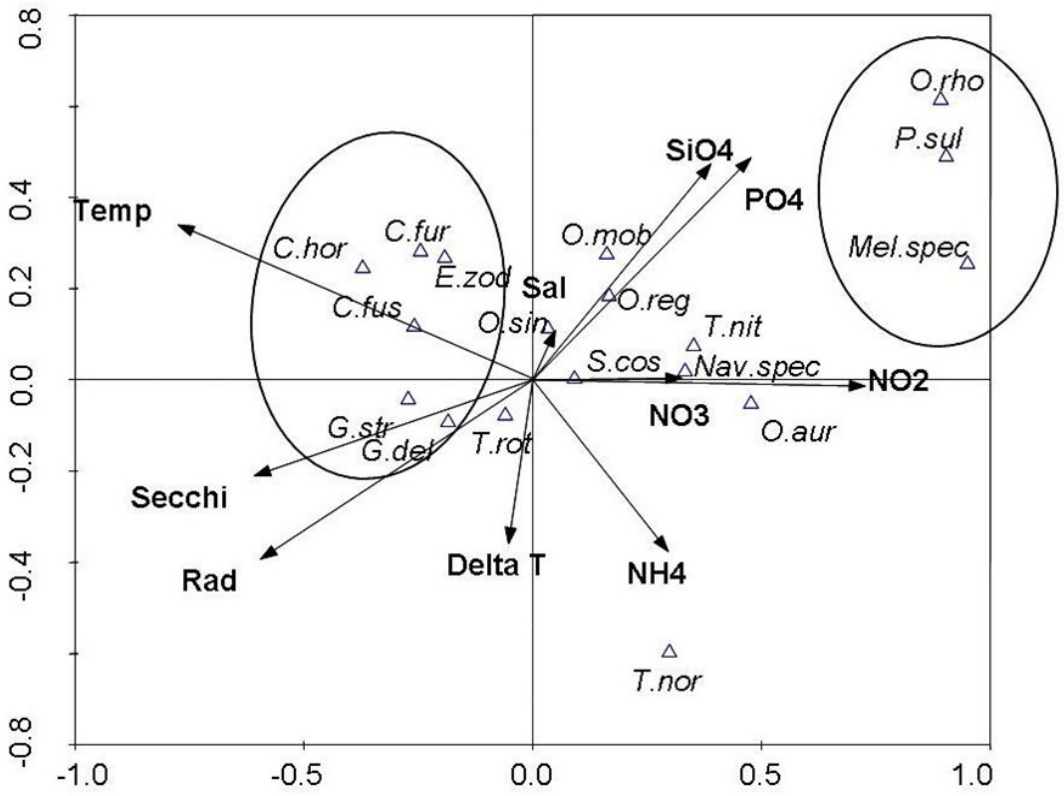

Figure 3: Biplot diagram showing the 1st (horizontal) and 2nd (vertical) canonical correspondence analysis (CCA) axes of inter-species distance from 1968 to 2005 of phytoplankton composition and environmental parameters at Helgoland Roads, North Sea. Length and direction of environmental parameter arrows indicate their importance (in terms of the influence on) the phytoplankton community. Algae data are shown as triangles, using the calculated weighted averaging method, indicating the species optima in the environment. Algae grouped together are mostly pooled together in the CCA, and indicate seasonal assemblages (winter: *Paralia sulcata*, *Odontella rhombus* and *Melosira* spp.; summer: *Ceratium* spp., *Guinardia* spp. and *Eucampia zodiacus*). Abbreviations of algae and environmental parameters were shown in Table 1.

This CCA showed a typical pattern for the algae species. Along with *Paralia sulcata*, a group of algae was associated with comparable environmental conditions such as very low light and Secchi depth, low water temperature and high nutrient concentrations, which is representative of winter and early spring conditions (Fig. 3). This group consisted of *Melosira* spp., *Odontella rhombus*, *O. aurita*, *O. mobiliensis*, *O. regia* and *Thalassionema nitzschioides*. Another species group, *Ceratium furca*, *C. fusus*, *C. horridum*, *Eucampia zodiacus*, *Guinardia delicatula*, *G. striata* and *Odontella sinensis*,

was mostly abundant at warmer water temperatures, high light conditions and higher Secchi depth, indicating typical summer conditions. The growth of *Ceratium* spp. was mostly independent of silicate concentrations, and negatively correlated to nutrients, reflecting typical summer conditions.

To show the correlation of *Paralia sulcata* with the environmental parameters, the Spearman rank correlation coefficient was calculated for the entire time period (1968 to 2005). The Spearman rank correlation for *P. sulcata* was negative with radiation ($R = –0.658$, $p < 0.05$), Secchi depth ($R = –0.587$, $p < 0.05$) and temperature ($R = – 0.411$, $p < 0.05$) and positive with phosphate ($R = 0.483$, $p < 0.05$), nitrite ($R = 0.411$, $p < 0.05$), silicate ($R = 0.303$, $p < 0.05$) and salinity ($R = 0.281$, $p < 0.05$). The occurrence of *P. sulcata* blooms in winter is reflected by these correlations, as this alga shows an adaptation to low light conditions and low water temperatures. A positive relationship between the occurrence of *P. sulcata* and the silicate concentrations in the water column was found, which might fuel the high silicate demand of *P. sulcata*. Silicate is a nutrient of pivotal importance as it is required for the production of *P. sulcata*'s strongly silicified valves.

Summarizing the results shown by the CCA, the most important environmental parameters influencing *Paralia sulcata* abundance were temperature, light conditions and phosphate and silicate concentrations.

Ecological niche of *Paralia sulcata*

With the exception of 1980 (where *Paralia sulcata* appeared only once), the yearly niche position and niche breadth was calculated for all years of the time series. The niche position and niche breadth of *P. sulcata* showed large interannual differences from 1968 to 2005 and could be grouped into 4 clusters (Fig. 4). At the beginning of time period (1968 to 1978), niche position fluctuated, while niche breadth did not change a great deal. From 1979 to 1986, niche position decreased continuously, indicating a switch from a specialised to a more generalised niche position. The narrow niche breadth indicated a specialised niche for *P. sulcata*. In the following years until 1995, niche position fluctuated and the narrow niche breadth again indicated a more specialised ecological niche for *P. sulcata*. From 1996 to 2005, niche position decreased and niche breadth became wide, reflecting a more generalised ecological niche of *P. sulcata* (Fig. 4).

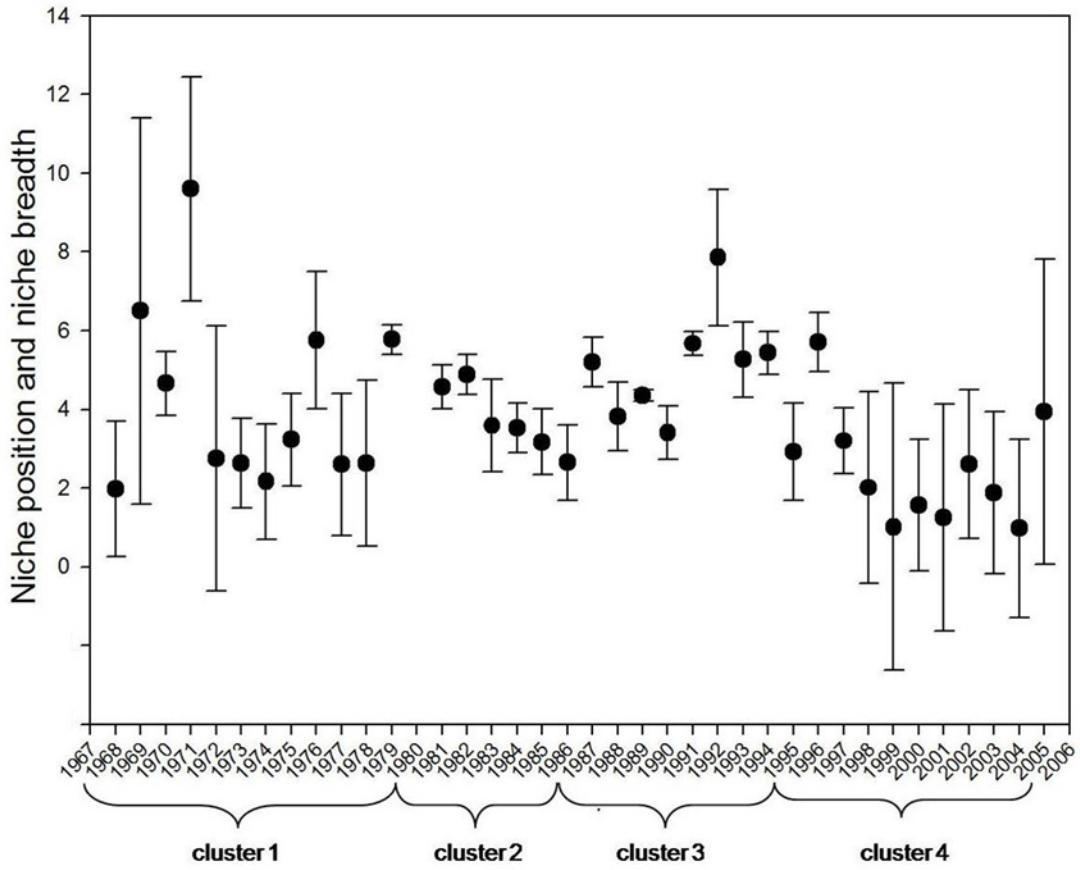

Figure 4: *Paralia sulcata*. Niche position (filled circle) and niche breadth (species tolerance, bars) from 1968 to 2005. The years which are grouped together are the same clusters as those found in the ANOSIM cluster analysis of the environmental parameters for the years 1968 to 2005 (see Fig. 5).

Interestingly, the similar years which grouped together in the ANOSIM cluster analysis presented the same year cluster in niche position and niche breadth of *Paralia sulcata* (Figs. 4 & 5). Based on the ANOSIM analysis, similar environmental conditions were found for Cluster 1 from 1968 to 1980, Cluster 2 from 1981 to 1986, Cluster 3 from 1987 to 1995 and Cluster 4 from 1996 to 2005. Only the transition years such as 1978 to 1981 showed a bigger change in niche position and niche breadth. A clear change in niche position of *P. sulcata* was found from 1986 to 1987, indicating the transition between Clusters 2 and 3. The niche breadths of Clusters 2 and 3 were significantly smaller than those of Clusters 1 and 4 (ANOVA, LSD-test, $p < 0.05$).

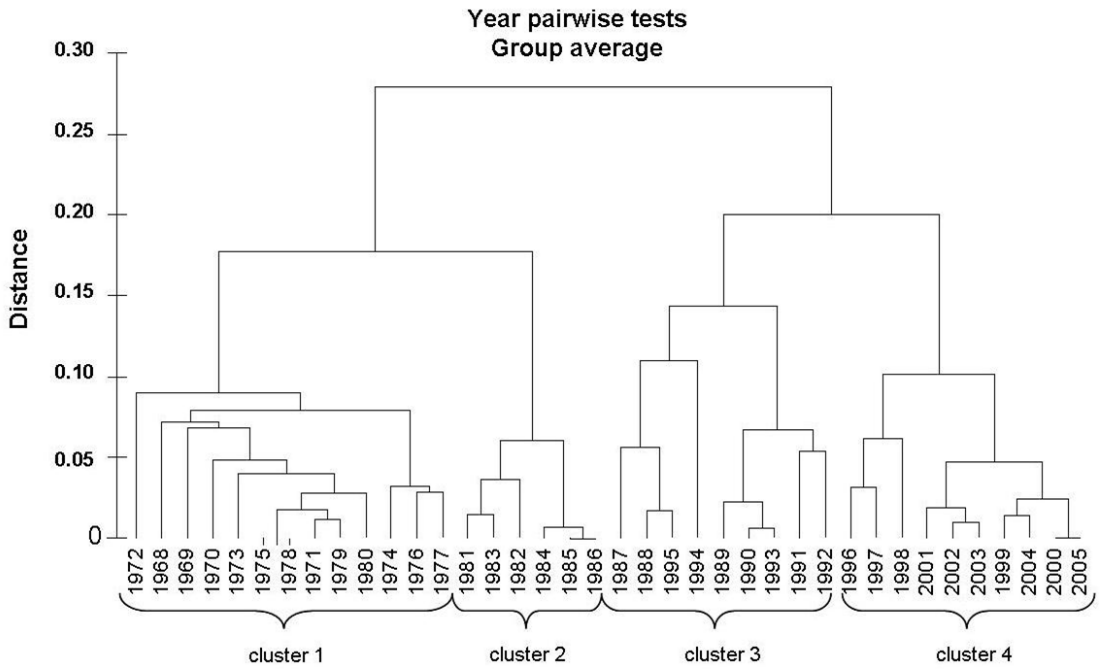

Figure 5: Dendrogram of the ANOSIM cluster analysis of environmental parameters for the years 1968 to 2005. Four clusters are shown; years in each cluster indicate similar environmental conditions.

The Spearman rank and Pearson correlation coefficients were also calculated for niche position and niche breadth of *Paralia sulcata* with the annual mean environmental parameters and their standard deviations (Table 3). Cluster 1 is influenced by the negative correlation for niche breadth of *P. sulcata* with standard deviations of temperature and salinity explaining 33 and 38% of the interannual variability, respectively. Cluster 2 clearly shows a smaller niche breadth of *P. sulcata* indicating a specialised niche. The main factors influencing the ecological niche are nitrite concentrations and salinity. Cluster 2 is affected by the standard deviation of salinity, which explains 87% of the variability in niche position, and the annual mean of nitrite, which explains 70% of the variability in niche position and 84% of the variability in niche breadth. The last cluster (1996 to 2005) exhibits a clear generalised ecological niche of *P. sulcata*. Cluster 4 is influenced by annual mean temperature, explaining 55% (niche position) and 43% (niche breadth) of the variability, and Secchi depth, explaining 62% of the variability in niche position (Table 3). There is a clear trend of *P. sulcata* switching to a more specialised niche position with increasing Secchi depth. The correlations of niche position and niche breadth with annual mean temperature are

interesting, indicating a clear trend from a specialised to a generalised niche with increasing temperature.

Table 3: Spearman rank and Pearson correlation coefficients for the annual mean ("mean) environmental parameters and their standard deviation (SD) and niche parameters of *Paralia sulcata* in the 4 clusters (significance level, $p < 0.05$). Abbreviations were shown in Table 1.

Environmental parameters	Niche parameters	Spearman rank correlation
cluster 1		
Temp SD	niche breadth	-0.587
Sal SD	niche breadth	-0.643
cluster 2		
NH4 SD	niche breadth	0.886
Sal SD	niche position	0.943
Sal SD	niche breadth	-0.829
cluster 4		
Secchi SD	niche position	0.661

Environmental parameter	Niche parameter	Pearson correlation
cluster 1		
Sal SD	niche breadth	-0.616
cluster 2		
NO2 mean	niche position	0.839
NO2 mean	niche breadth	-0.917
NO2 SD	niche position	0.990
Sal SD	niche position	0.934
Sal SD	niche breadth	-0.860
cluster 4		
Temp mean	niche position	-0.742
Temp mean	niche breadth	0.654
Temp SD	niche position	0.645
Secchi mean	niche position	0.787

The results of the Spearman rank correlation analysis of niche position and niche breadth showed that only the niche position of *Paralia sulcata* was highly negatively correlated with temperature ($R = –0.493$, $p = 0.002$), whereas the other parameters showed no significant influence on niche position or niche breadth. This indicates a strong influence of temperature on *P. sulcata*; increasing temperatures in the North Sea could have led to a change in niche position to a more generalised niche. But no environmental factor alone is responsible for the shift in the ecological niche of *P. sulcata*; therefore, other parameters such as Secchi depth, nitrite and salinity could have also influenced this shift, albeit to a lesser degree.

DISCUSSION

Influence of the environmental parameters on *Paralia sulcata*

At Helgoland Roads, high abundances of *Paralia sulcata* do not occur relative to the total diatom counts, due to the fact that *P. sulcata* does not form typical blooms (Roelofs 1984). The occurrence of *P. sulcata* at Helgoland Roads has changed over the last 40 years (Wiltshire & Dürselen 2004, Wiltshire et al. 2010). Up to 1996, *P. sulcata* occurred only in autumn and winter; however, since 1997 this diatom has been abundant year-round. Changing environmental conditions at Helgoland Roads could be an explanation for the new occurrence in summer.

The CCA extracted a typical seasonal pattern for *Paralia sulcata*, which occurs mostly in winter with low light and temperature conditions and higher nutrient concentrations, but does not show a shift in the ecological niche of *P. sulcata*. *P. sulcata* abundance is positively influenced by higher silicate, phosphate and nitrite concentrations and negatively influenced by higher light and temperature conditions.

A negative correlation of the abundance of *Paralia sulcata* and temperature was described by Hobson & McQuoid (1997). Although temperatures in the southern part of Vancouver Island were slightly higher in winter (mean of 8.1°C) and cooler in summer (mean of 13.7°C) (Hobson & McQuoid 1997), the abundance pattern of *P. sulcata* shows similar seasonal patterns to those found at Helgoland Roads. Another study has shown that temperature has positive effects on the abundance of *P. sulcata* in the water column in British Columbia inlets (Roelofs 1984, Zong 1997). In the present study, we detected a clearly negative correlation between temperature and *P. sulcata*. The new occurrence of *P. sulcata* in the last 15 yr in summer could be explained by the adaptation of *P. sulcata* to a recent warming trend of the North Sea of 1.13 to 1.33°C over the last 40 to 50 years (Wiltshire & Manly 2004, Wiltshire et al. 2008) or hidden species diversity, i.e. the introduction of *P. sulcata* from warmer waters.

Hobson & McQuoid (1997) found that the abundance of *Paralia sulcata* increased under low light conditions. This fact is supported by the present study, where growth of *P. sulcata* was negatively correlated with radiation and Secchi depth. Interestingly, annual mean Secchi depth has increased by 1 m in the North Sea over the last 30 years (Wiltshire et al. 2008). However, no significant correlation with the Secchi depth and total algal densities in winter (January to March) has been found (Wiltshire et al. 2008), which could explain the increasing Secchi depth. The total algal density has increased in winter since the late 1980s in spite of decreasing phosphate and ammonia

concentrations (Wiltshire et al. 2008). As a tychopelagic diatom species, *P. sulcata* could be indirectly influenced by the higher light availability in winter and summer due to an adaptation to higher light conditions. This could result in a change in the life cycle and ecological niche, which in turn could be a possible explanation for the new occurrence of this diatom in summer. Nevertheless, nothing is known about the exact life cycle of *P. sulcata* due to the slow growth rates of this diatom.

High nutrient concentrations in the water column in winter as a result of remineralisation processes are typical for temperate coastal waters (Wafar et al. 1983). This could be an explanation for the positive correlation between nutrient concentration and abundance of *Paralia sulcata*. Some studies have shown comparable positive correlations with abundance and higher nutrient concentrations, indicating that *P. sulcata* is common in nutrient-rich waters (Abrantes 1988a, Zong 1997). The absence of the fast-growing spring bloom species in winter may support the occurrence of *P. sulcata* in the water column (Roelofs 1984). The significantly positive relationship between the abundance of *P. sulcata* and silicate concentrations indicate a high silicate demand of *P. sulcata* which could be attributed to its strongly silicified valves. The highest silicate concentrations in the present study were measured in winter and spring, after which the concentration decreased to half of those measured until autumn. In the spring bloom, silicate concentrations decrease rapidly due to the fast-growing spring bloom phytoplankton species which utilize the silicate very quickly (Wafar et al. 1983).

The present study showed no strong correlations between salinity and abundance of *Paralia sulcata*, suggesting that this diatom can live in a wide range of salinities, as previously described in the literature (Zong 1997). In comparison, the distribution of *P. sulcata* in the phytoplankton in the British Columbia inlets appears to be strongly correlated to salinity, and *P. sulcata* abundance was negatively correlated with low salinity (Roelofs 1984).

The study site at the southern part of Vancouver Island is characterised by storm activity in the winter months, which could explain the higher abundance of *Paralia sulcata* in the phytoplankton as algal cells are transferred from the sediments into the plankton by wave activity (McQuoid & Hobson 1998). Storm activity was not included in the present study. However, there are strong tidal mixing and storm activities at Helgoland Roads in summer and winter, which could explain the occurrence of *P. sulcata*, caused by the resuspension of chains into the water column.

The higher nutrient concentrations and lower temperature and light conditions in the water column during winter could be of advantage to the slow-growing *Paralia sulcata*. During spring, when water temperatures and light conditions are increasing, the fast growing spring bloom species may outcompete *P. sulcata* (Roelofs 1984), which could explain the absence or lower abundances of *P. sulcata* in summer.

Long-term trends and ecological niche of *Paralia sulcata*

This is the first time that niche parameters such as niche breadth and niche position were calculated for an alga found in a long-term data set, and a long-term trend can be shown for *Paralia sulcata*. This is also the first study to examine the ecological niche of *P. sulcata* in detail. Several studies with other organisms use the new method of OMI analysis of Dolédec et al. (2000). Heino & Soininen (2006) used this method to characterise the stream diatom communities in 47 streams in northern Finland. The determinants of fish distribution in 97 lakes in southern and central Finland were also investigated with this method (Lappalainen & Soininen 2006).

In the present study we investigated the ecological niche of *Paralia sulcata* over a time period of 38 yr. Changes from a more specialized niche in the 1980s to a more generalized niche in the late 1990s were exemplified by changes in the occurrence of *P. sulcata* over the last decade. Since 1996–1997, *P. sulcata* has occurred in the water column during summer at Helgoland Roads, resulting in a wider niche breadth and a more generalised ecological niche. These results indicate that the niche position of *P. sulcata* can change considerably within a time period of several decades.

The correlations between niche parameters of *Paralia sulcata* and environmental parameters grouped into the 4 clusters were inconsistent. The annual mean SD of the environmental parameters describes the variation of the parameters in an individual year. Significant correlations within the annual mean SD of individual environmental parameters and niche parameters of *P. sulcata* in the clusters indicate variation in the marine system at Helgoland Roads. If the marine system is variable (i.e. in terms of temperature, salinity, nitrite and Secchi depth), the ecological niche of *P. sulcata* becomes more specialised. This means that the change in variability of an environmental parameter could lead to a change in the tolerance of this environmental parameter by *P. sulcata*, which leads to a more specialised niche. Therefore, the tolerated range of the individual parameter for *P. sulcata* could decrease when the

range of the variability of this parameter converges to the upper or lower limit tolerated by *P. sulcata*.

Changes in the ecological niche of *Paralia sulcata* are mainly influenced by temperature and Secchi depth. This may be explained by the fact that regime shifts have occurred in the North Sea over the last 3 decades, which has resulted in a change in the fundamental niche of the North Sea. Weijerman et al. (2005) examined evidence for the regime shift in the North Sea using existing long-term data series on a wide range of physical and biological parameters from 1960 to 2002. Their results indicate that substantial regime shifts occurred in the North Sea in 1979 (salinity and changing weather conditions), 1988 (temperature and changing North Atlantic Oscillation Index) and 1998 (temperature). These regime shifts are most evident among biological parameters, e.g. the changes in the abundance of copepods (Reid et al. 2001, Weijerman et al. 2005, Martens & van Beusekom 2008, Schlüter et al. 2008). The 1998 regime shift comprised an increase in storm activity and wind speed in the North Sea (Alexandersson et al. 2000, Weijerman et al. 2005, Wiltshire et al. 2008). Because of this storm-induced mixing of the water column, *P. sulcata* may increasingly be transferred from the benthos into the pelagial in summer at Helgoland Roads. Therefore, the realised ecological niche of *P. sulcata* could lead to a shift from a specialised to a more generalised niche.

Another possible explanation for the new occurrence of *Paralia sulcata* is a shift in the algal population that occurs in summer and winter at Helgoland Roads to include those species which are more adapted to warmer conditions. The next step of our research therefore will be to investigate genetic isolates from different times of the year.

Conclusion

The present study found a change in the ecological niche of *Paralia sulcata* indicating a shift from a specialised (1980s) to a more generalised niche (since 1996) and a new occurrence of this diatom in summer over the last 10 yr. CCA extracted the most important factors influencing the abundance of *P. sulcata* in the water column: temperature, Secchi depth, light conditions and silicate, phosphate and nitrite concentrations. A seasonal pattern has shown that *P. sulcata* is a typical winter alga adapted to low light conditions under colder water temperatures but high concentrations of nutrients. However, the CCA does not show the shift in the

occurrence of *P. sulcata* in the summer months since 1997 and cannot explain the niche displacement. Changing environmental conditions in the North Sea, such as increasing temperature and Secchi depth and decreasing phosphate and ammonia concentrations, may influence the occurrence of *P. sulcata* and result in the clear shift from a specialised to a generalised ecological niche. Another possibility is the introduction of *P. sulcata* strains from warmer waters which are adapted to the temperature conditions resulting from the recent warming trend of the North Sea. However, these factors alone do not explain the niche displacement of *P. sulcata*. Adaptation to the higher light regime and decreasing nutrient concentrations in the North Sea could be an advantage for the slow-growing *P. sulcata* in contrast to fast-growing phytoplankton species, as it may have resulted in a changing occurrence and a shift in the ecological niche of *P. sulcata*.

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APPENDIX 1

Table A1: Conditional effects of the environmental parameters of the canonical correspondence redundancy analyses for the years 1968 to 2005. Significant values are indicated by asterisks: *p < 0.05; **p < 0.01; ns = not significant.

year	Temp	Delta T	Secchi	Sal	Rad	PO_4	NO_2	NO_3	NH_4	SiO_4
1968	*	*	*	n.s.	**	*	*	n.s.	**	*
1969	**	*	n.s.	**	n.s.	n.s.	**	n.s.	n.s.	**
1970	n.s.	n.s.	*	**	*	*	*	n.s.	**	**
1971	**	*	n.s.	n.s.	**	n.s.	**	n.s.	n.s.	n.s.
1972	**	n.s.	*	**	**	*	n.s.	n.s.	*	**
1973	**	n.s.	n.s.	*	**	n.s.	n.s.	**	n.s.	n.s.
1974	n.s.	**	**	n.s.	**	**	n.s.	**	n.s.	*
1975	**	*	**	**	*	**	**	n.s.	**	**
1976	n.s.	*	**	*	n.s.	n.s.	**	**	n.s.	*
1977	n.s.	n.s.	**	n.s.	n.s.	**	*	n.s.	**	**
1978	n.s.	n.s.	**	n.s.	n.s.	*	*	**	n.s.	**
1979	**	n.s.	**	n.s.	**	**	n.s.	n.s.	n.s.	**
1980	n.s.	n.s.	n.s.	**	*	n.s.	n.s.	**	n.s.	n.s.
1981	n.s.	n.s.	**	*	*	**	*	**	n.s.	**
1982	n.s.	n.s.	**	*	**	n.s.	n.s.	n.s.	n.s.	*
1983	**	n.s.	**	n.s.	n.s.	**	**	*	n.s.	*
1984	**	n.s.	*	n.s.	**	n.s.	n.s.	*	n.s.	n.s.
1985	n.s.	n.s.	**	n.s.	n.s.	**	*	**	n.s.	n.s.
1986	n.s.	**	**	**	**	n.s.	n.s.	**	*	n.s.
1987	**	**	**	n.s.	n.s.	**	*	n.s.	**	n.s.
1988	**	*	n.s.	n.s.	n.s.	*	**	*	*	**
1989	**	*	n.s.	*	**	n.s.	n.s.	n.s.	*	**
1990	**	n.s.	*	n.s.	**	n.s.	**	**	**	n.s.
1991	n.s.	*	*	*	n.s.	*	**	**	n.s.	**
1992	**	*	*	**	**	n.s.	n.s.	n.s.	**	**
1993	**	n.s.	**	**	*	n.s.	**	n.s.	n.s.	**
1994	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.
1995	**	n.s.	*	**	**	**	**	n.s.	n.s.	**
1996	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.
1997	n.s.	**	**	**	*	n.s.	**	**	*	**
1998	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.
1999	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.
2000	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	*	n.s.	n.s.
2001	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
2002	**	n.s.	**	n.s.	**	*	**	n.s.	*	*
2003	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.
2004	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.
2005	**	n.s.	*	*	**	n.s.	n.s.	n.s.	**	n.s.
1968-2005	**	**	**	**	**	**	**	*	**	*

CHAPTER II

Ecological niche of *Paralia sulcata* determined in the laboratory and with field data

Ecological niche of *Paralia sulcata* determined in the laboratory and with field data

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Running head: Autecology of *Paralia sulcata*: comparison between experiment and field data

ABSTRACT

Paralia sulcata is a tychopelagic centric, marine diatom species common in the North Sea, occurring in high numbers in the water column mainly in the winter months. In recent years we observed a trend towards a less seasonal appearance of *P. sulcata*. Changing temperatures, light and nutrient conditions at Helgoland Roads are one reason for the shift in the ecological niche of *P. sulcata* over a period of 40 years. We hypothesised that the growth of *P. sulcata* is positively influenced by higher silicate and phosphate concentrations and lower temperatures. We tested the second hypothesis that humic acids positively influenced the growth of *P. sulcata* due to reduced light conditions. We compared the experimental results with data obtained from field sampling (two year monitoring program) in order to detail the occurrence of *P. sulcata* and to estimate the main factors affecting the life at different water depths. Laboratory studies showed the best growth conditions at higher temperatures with high available silicate concentrations. At 10°C the growth was significantly higher under all phosphate limiting conditions compared to the silicate limiting conditions indicating a higher demand of silicate for *P. sulcata*. Furthermore, the addition of humic acids favoured the growth of *P. sulcata* and coincided well with the adaptation of this diatom species to lower light conditions. Results obtained from the field sampling showed significant correlations of the abundance with Secchi depth and sunshine duration (negative), silicate and phosphate concentrations, mean and maximal wind speed (positive) and were well separated by the seasons displaying a slightly differing influence of the parameters on the abundance of *P. sulcata*. The niche position and niche breadth for *P. sulcata* was found to vary over the seasons. The ecological niche was different in spring and summer between the depth and surface water samples which were influenced by different environmental parameters.

Key words: ecological niche, Helgoland Roads, humic acids, long term data, marine diatom, North Sea, nutrients, *Paralia sulcata*, temperature

INTRODUCTION

Only a detailed knowledge regarding the physiology and ecology of marine phytoplankton might allow us a true understanding of the changes in the marine environment and their effects on the phytoplankton community. Most of the understanding of phytoplankton ecology has largely resulted from closer analysis of the environmental parameters influencing the occurrence of the phytoplankton in marine habitats. To gain a clearer view of the influence of different environmental parameters on the growth of marine phytoplankton species laboratory experiments are an important tool, particularly in association with field data.

The concept of the ecological niche according to Hutchinson (1957) assumed the niche to be a multidimensional "hypervolume" containing all environmental parameters influencing the existence of the species. This definition of the ecological niche of one species under isolated abiotic and biotic conditions is referred to as a "fundamental niche" in which a species can exist (Hutchinson 1957, McGill et al. 2006). Under field conditions, as a result of competition and interaction (abiotic and biotic) of a species with its environment, a species occupies an ecological niche which is narrower than the fundamental one and thus, mostly highly adapted to its "realised niche" (Hutchinson 1957, Kearney 2006). Therefore a species can have a broad (generalist) or narrow (specialist) niche breadth and this is referred to as species tolerance (Dolédec et al. 2000). There are two possibilities for investigating niche in experimental ecology, in laboratory experiments and with field data. In the laboratory all but a few environmental parameters are kept constant and usually biotic factors are excluded (depending on the question of the research) (McGill et al. 2006), thereby describing more or less the fundamental niche of this species. When using field data, which naturally were influenced by biotic and abiotic factors and their interactions (e. g. competition and predator-prey interactions), the realised niche can be described and analysed. Thus, any niche reconstructed from field data is necessarily a realised niche and may vastly differ from the fundamental niche reconstructed from laboratory experiments.

The autecology of a single species can be described using experimental set-ups. Investigations of the interactions of a species with the relevant parameters from its environment, such as light and nutrient availability, can be considered to be an effort to understand the life history and living behaviour of this species. To answer ecological questions, for modelling changes in ecological niches and to describe the interactions

of a species with its environment, long-term data sets are useful. Since 1962, a work-daily phytoplankton monitoring program has been maintained at Helgoland Roads, North Sea, which has resulted in one of the most important marine data sets in the world, unique with respect to the length of the time series, sampling frequency and number of parameters measured (Franke et al. 2004, Wiltshire & Manly 2004). Quality and quantity of the phytoplankton and physicochemical parameters like salinity, temperature, Secchi depth, and nutrients (dissolved inorganic nutrients such as nitrate, nitrite, ammonia, phosphate and silicate) were measured work-daily (Wiltshire & Dürselen 2004, Raabe & Wiltshire 2009).These long-term data have been used for modelling ecosystem function related to questions on climate changes in the Southern North Sea as well as for important ecological questions (Franke et al. 2004, Wirtz & Wiltshire 2005, Wiltshire et al. 2008).

Paralia sulcata (Ehrenberg) Cleve (1873) is a marine chain forming centric diatom with thick-walled, highly silicified valves (Crawford 1979a, Roelofs 1984). Due to these silicified valves *P. sulcata* is preserved in sediments and is often used as a paleoindicator species (McQuoid & Nordberg 2003b). *P. sulcata* is found in littoral and sublittoral zones and fine-grained sediments (Zong 1997), but it occurs also in the pelagic phytoplankton (Roelofs 1984, Zong 1997, McQuoid & Hobson 1998). Several studies have investigated the influence of environmental parameters (e.g. temperature, salinity, upwelling processes and nutrient distribution) on *P. sulcata* (Margalef 1969, Roelofs 1984, Abrantes 1988a, Zong 1997, McQuoid & Nordberg 2003a). These data show that *P. sulcata* is capable of growing in a wide range of environmental conditions over an annual cycle, but it may favour low temperatures and short day lengths when irradiance is high (Hobson & McQuoid 1997), as well as low transparency of the water column (Zong 1997).

The analysis of the Helgoland long-term data showed that *P. sulcata* not only occurred in winter but it was detected throughout the year since the mid of the 1990s. The results of niche analysis displayed a niche shift of *P. sulcata* over the last 38 years from a more specialised niche in the 1980s to a generalised niche in the middle of the 1990s (Gebühr et al. 2009). The main factors influencing the ecological niche were the changing temperature, Secchi depth, as well as silicate and phosphate concentrations at Helgoland Roads. This alteration in the ecological behaviour and the ubiquitous distribution of *P. sulcata* make it an interesting study object.

In general, the main limiting factors on the growth of marine diatoms at the sediment are the availability of nutrients and light (Wolf 1979, MacIntyre et al. 1996). Dissolved organic matter in coastal waters mainly derives from humic substances from land-runoff and can accumulate in the sediment-intersurface layer on the sea bottom. Humic substances are therefore present in the same layer as benthic diatoms and thus there is an increasing interest regarding the influence of humic substances on the microphytobenthic diatom community within the marine habitat. Humic substances are naturally polyelectrolyte high molecular weight compounds which are the main components of organic matter in soils and waters. Furthermore, humic substances are known to affect the water quality due to their dark colour. They can also act as complexing agent for inorganic ions (Aiken et al. 1985). The need for knowledge on the role of humic substances in aquatic ecosystems is indeed underpinned by this complexing ability of humic acids (Prakash & Rashid 1968, Lund 1990). Humic substances also form both soluble and insoluble complexes with metal ions and therefore affect the transport of the ions to plant roots and potentially to microalgae. This cation-humic acid interactions affect the bioavailability of the nutrient ions in the marine sediment (Lund 1990) and thereby potentially their availability as micronutrients to algae.

This study focused on the investigation of the autecology of *Paralia sulcata* as determined with laboratory experiments and field observations, consequently the characterisation of the marine environment of *P. sulcata*. The general hypothesis was that the variability of nutrients, especially silicate and phosphate, as well as the temperature and the dissolved organic matters (investigated here as humic acids) would influence the abundance of *P. sulcata* and could determine the ecological niche of this species. The motivation for the first experimental set-up was to test the influence of silicate and phosphate concentrations in combination with temperature on the growth of *P. sulcata*. We hypothesised that *P. sulcata* would grow better at lower temperatures. Additionally, due to the tychopelagic life style of *P. sulcata*, we hypothesised that the humic acids positively influence the growth of *P. sulcata* due to the better availability of nutrients from the sediment, as well as the protection against higher light intensities. This was investigated in a second experimental set-up. We aimed to describe the fundamental niche of *P. sulcata* using laboratory experiments and comparing them with field data. We also wished to evaluate these niche investigations against the field situation. Therefore, the abundance of *P. sulcata* with

respect to the specific influence of the environmental parameters at Helgoland Roads at two different depths of the water column, the surface and the bottom water, were studied. We wanted to investigate the ecological niche as it related to seasonal trends and to compare these results with laboratory results to obtain a more detailed picture of the life-cycle and ecological behaviour of *P. sulcata* within its marine habitat at Helgoland Roads.

MATERIAL & METHODS

1. Determination of the autecology of *Paralia sulcata* in laboratory experiments

1.1. Sampling site and isolation of *Paralia sulcata*

Helgoland is situated in the German Bight around 60 km from the main land. Water samples were taken from Helgoland Roads (54°11.3'N; 7°54.0'E), the long-term sampling station since 1962. For the growth experiments *Paralia sulcata* was isolated on February 2007 using an 80 µm plankton net from the surface water with the research vessel Aade. In the laboratory single *P. sulcata* chains were isolated with the help of a dissecting microscope and transferred with a micropipette into 6-well-plates containing f/2 medium (Guillard & Ryther 1962, Guillard 1975). After two weeks *P. sulcata* chains were washed in f/2 medium and transferred into culture flasks (73.5 ml) for cultivation. *P. sulcata* was cultured under controlled conditions at 12:12 hours light:dark photoperiod at 14 - 15°C with approximately 50 µE s^{-1} m^{-2}.

Sediment surface samples for the extraction of humic acids (HA) for the second growth experiment were taken at the beach of Helgoland (54º11'N; 7º53'E) in March 2008. These sediments were sterilised and stored at -20°C before use.

1.1. Influence of temperatures and nutrients on the growth of *Paralia sulcata*

Experimental set-up and treatments

In order to evaluate the influence of nutrient limitation in combination with temperature on the growth of *Paralia sulcata* an experiment with eight nutrient conditions and three temperatures was conducted in batch cultures. The seawater for the preparation of the different media for the growth experiments was sterile filtered (0.2 µm). As control treatments seawater (control sea) with low concentrations of

nutrients and f/2 medium (control f/2) (Guillard & Ryther 1962, Guillard 1975) with high amounts of nutrients were used. To test the hypothesis that silicate and phosphate have a positive influence on the growth of *P. sulcata* f/2 medium was limited with regard to silicate or phosphate, respectively, as follows:

a) silicate limitation of the full medium: f/2-7/8 SiO_2 (12.5% of silicate concentration of the f/2 media), f/2-3/4 SiO_2 (25% of silicate concentration) and f/2-1/2 SiO_2 (50% of silicate concentration) and

b) phosphate limitation of the full medium: f/2-7/8 PO_4 (12.5% of phosphate concentration of the f/2 media), f/2-3/4 PO_4 (25% of phosphate concentration) and f/2-1/2 PO_4 (50% of phosphate concentration).

Three different temperature conditions were chosen according to the mean temperatures in the North Sea in winter (4°C), spring and autumn (10°C) and summer (16°C) in order to test for the influence of temperatures and nutrient conditions of the seasons on the growth of *P. sulcata*. Each temperature was held constant (± 0.3°C) during the experiment. The experiment was conducted for all nutrient conditions in three separate runs at the three different temperatures.

For each growth experiment four replicates were used. The growth of *P. sulcata* was monitored in 500 ml glass flasks (Erlenmeyer). The starting volume of the medium was 300 ml for each nutrient treatment and starting biovolume for *P. sulcata* was 10,000,000 μm^3 $cells^{-1}$. The biovolume was determined according to Hillebrand et al. (1999) by measuring the cell size of *P. sulcata* in the culture. The growth experiments were carried out in a culture room (RUMED Rubarth Apparate GmbH, Laatzen, Germany) at the different constant temperatures, a 12:12 light:dark photoperiod with 40 to 50 $\mu E\ s^{-1}\ m^{-2}$ light conditions depending on the position of the flasks. The position of each flask was randomly assigned every day in order to avoid different light influences on the culture flasks in the culture room.

1.2. Influence of humic substance concentrations on the growth of *Paralia sulcata*

Extraction of the humic acids

The extraction of the humic acids from the sediment at Helgoland was carried out according to the method of Moreda-Piñeiro et al. (2004, 2006) with the following modifications: 200 ml of 0.1 M HCl were added to 400 g of marine sediment, shaken for 4 h at room temperature and settled overnight. Subsequently, the supernatant was

decanted and the residue was neutralised with 200 ml of 0.1 M NaOH under an O_2-free atmosphere. For the alkaline extraction the mixture was shaken for 8 h at room temperature and settled overnight. After sedimentation, the supernatant was acidified with 6 M HCl to pH = 1 for 24 h following a centrifugation (10 min at 7500 rpm). The solid phase was dissolved by adding 4.4 g KCl and 200 ml 0.1 M KOH. The solution was shaken for a few minutes under an O_2- free atmosphere and centrifuged at high speed (10 min at 7500 rpm) to remove suspended solids. Thereafter, the humic acids in the supernatant were precipitated by addition of 6.0 M HCl and the suspension was stored at -20°C for 24 h. Following a further centrifugation step, the precipitated humic acids were filtered through 0.45 µm membrane filters (Whatman, Maidstone UK), washed several times with sterile Millipore water and kept in a drying oven at 36ºC for five days to allow elimination of the residual water. The detailed description and characterisation of the humic acids extracted from the marine sediment at Helgoland will be carried out in cooperation with M. Martin and D. Martire from the National University of La Plata, Argentina, in the near future. With this procedure 7.98 g of a sediment extract with a high content of humic acids were obtained from 400 g of marine sediment, while these extracts were not completely pure, we will refer to the extract as humic acid. This value was taken as a natural value of humic acid concentration in the marine sediment from Helgoland. Thus, the concentration of the extractable humic acid in the natural marine sediment was given as C_{HA} = 0.02 g of extract per gram of sediment.

Experimental set-up and treatments

To test our hypothesis that the humic acids positively influence the growth of *P. sulcata* due to the supply of nutrients and reduction of light intensity four different nutrient treatments were chosen: 1) control = a mix of 2/3 seawater and 1/3 f/2 medium which reflected low nutrient conditions, 2) low HA = low concentration of humic acids with 2/3 seawater and 1/3 f/2 medium, 3) high HA = high concentration of humic acids with 2/3 seawater and 1/3 f/2 medium and 4) f/2 = full medium with high nutrient concentration.

Each treatment was carried out in four replicates (300 ml medium and 10 g quartz sand) and the growth of *P. sulcata* was monitored. The seawater was sterile filtered (0.2 µm) and the f/2 medium was prepared according to Guillard & Ryther (1962) and Guillard (1975). The concentration of humic acids (HA) extracted from sediments on

Helgoland was 0.02 g HA per gram of marine sediment. The lower concentration of humic acids (low HA) was 50% less of the concentration isolated from the natural sediment (0.03 g HA per 300 ml medium, corresponding a concentration of 0.1 g l^{-1}) whereas the highest concentration (high HA) was 50% more than the concentration isolated from the natural sediment (0.09 g HA per 300 ml medium corresponding to a concentration of 0.3 g l^{-1}).

The medium starting volume and biovolume was the same as described for the other growth experiment with 12:12 hours light:dark photoperiod at 12°C. The position of each flask relative to the light source was randomly assigned every day in order to avoid different light influences on the culture flasks in the culture room.

1.4. Sampling and data analysis of both growth experiments

To investigate the growth of *P. sulcata* sampling took place every second day under the clean bench (sterile conditions). The flasks were gently mixed before sampling and 1.5 ml samples for cell counts were taken from each flask and fixed with 50 µl of Lugol's solution. The cells were enumerated using Sedgwick rafter's counting chambers (Graticules Limited, Tonbridge, UK) under a light microscope (Axioskope, Carl Zeiss, Germany) with 100 fold magnification. For each sample the whole chamber or a minimum of 400 chains (cells were counted as single units) were counted to estimate the cells ml^{-1}.

The chlorophyll *a* concentration (µg l^{-1}) was measured daily in all replicates to observe the development of the growth *in situ*. A culture sample (25 ml) was taken from each flask and the chlorophyll *a* concentration was determined via *in situ* fluorescence in the laboratory using a multialgal fluorometer (BBE Moldaenke, Kiel, Germany). After the measurement the sample was decanted back into its original flask. To avoid a contamination between the treatments the glass cuvette was washed with ethanol (90%) and repeatedly with Millipore water.

In the middle of the exponential growth phase and in the stationary phase (end of the growth experiment) 60 ml culture samples of each replicate and treatment were filtered through 0.45 µm nylon membrane filters (Whatman, Maidstone UK) under dimmed light conditions to avoid the loss of pigments during the filtration process. The filters were subsequently fixed with 2 ml acetone (100%) for chemical extraction of the chlorophyll and frozen at -70°C for the determination of pigments via high

performance liquid chromatography (HPLC). The preparation and extraction of the pigments by HPLC was performed according to the method of Wiltshire et al. (1998) and Knefelkamp et al. (2007). Pigments were separated and identified using the retention time and the integrated area in combination with a commercial standard for each pigment (Wiltshire et al. 2000).

Furthermore, in the stationary phase the residual culture was filtered through 0.45 µm membrane filters (Whatman, Maidstone UK) and the filtrate was frozen at -20°C for the analysis of the nutrients. The colorimetric determination of nutrients (silicate, nitrate, nitrite, ammonia and phosphate concentrations) at the beginning and the end of the experiments were analysed according to the method of Grasshoff (1976) using a spectrophotometer (Hitachi U-1100). Additionally, the pH was measured in all flasks at the end of the experiment.

To test the hypothesis that the best growth occured at lower temperatures, and that the growth was positively influenced by higher silicate and phosphate concentrations, the growth rate of *P. sulcata* was compared with the factors *temperature* and *nutrients* in a two-way Analysis of Variance (ANOVA) with the Fisher's least significant difference (LSD) post-hoc test and a significance level of $p < 0.05$. Furthermore, to test for significant differences in the chlorophyll *a* and fucoxanthin concentration determined via HPLC in the stationary phase as well as in the silicate and phosphate concentrations between the start and the end conditions a two-way ANOVA with the Fisher's LSD post-hoc test ($p < 0.05$) between the factors *temperature* and *pigments* and *nutrients*, respectively, was performed. The Pearson correlation coefficient was used to investigate the relationship between the *in situ* fluorescence (chlorophyll *a* concentration) and the abundance of *P. sulcata* (cells ml^{-1}) in each nutrient treatment and temperature.

To test the hypothesis that the humic acids had a positive influence on the growth of *P. sulcata* abundance (cells ml^{-1}), chlorophyll *a* and fucoxanthin concentrations in the stationary phase was compared with the factor *treatments* in a ANOVA with the Fisher's LSD post-hoc test (significance level: $p < 0.05$). Correlations between the abundance and the *in situ* fluorescence were calculated with the Pearson correlation coefficient. All statistical analysis for the growth experiments were performed with STATISTICA (STATISTICA 7.1, StatSoft Inc, USA).

2. Determination of the seasonal variations in the ecological niche of *Paralia sulcata* at Helgoland Roads

2.1. Sampling and preparation of the water samples

A two year water sampling monitoring program (once per week from October 2007 to October 2009) was carried out at Helgoland Roads in order to investigate the occurrence of *Paralia sulcata* in association with the physico-chemical water parameters (dissolved inorganic nutrients, salinity, temperature, pigment concentration, *in situ* fluorescence and pH) in the water from the bottom of the water column ("bottom water sample") at the long-term sampling station Helgoland Roads (54°11.3'N; 7°54.0'E). To enable a comparison between the data from these bottom water samples and those from surface water samples (same biological and physico-chemical parameters) both of the samples were taken simultaneously. Surface water samples were taken with a bucket from the water surface (Wiltshire & Dürselen 2004) and the bottom water samples at 1 m above the ground with a 5 l Niskin bottle (HydroBios, Kiel, Germany) from the RV Aade. Water temperatures in both samples were measured directly after the sampling. Both water samples were treated in the same way for the best comparability of the samples.

A subsample of 100 ml from the bottom water sample was fixed with 500 µl Lugol's solutions. According to the method of Lund et al. (1958) 50 ml were settled for 24 h and the whole surface of the sedimentation chamber was counted with an inverted microscope (Axiovert 135, Carl Zeiss, Germany) at 100 fold magnification to determine the abundance of *P. sulcata*. Algae composition was examined via *in situ* fluorescence using an algae analyser (BBE FluoroProbe, Moldaenke, Kiel, Germany). The determination of the pigments was carried out with high-performance liquid chromatography (HPLC). For this purpose 2 x 1000 ml of the bottom water sample were filtered through 0.45 µm nylon membrane filters (Whatman, Maidstone UK) under low light conditions to avoid the destruction of the pigments. Afterwards the filters were fixed with 2 ml acetone (100%) and frozen at -70°C for further extraction of the pigments (as described above). The preparation and extraction of the pigments by HPLC was carried out according to the method of Wiltshire et al. (1998) and Knefelkamp et al. (2007). The filtrate was used for the colorimetric determination of the inorganic nutrients (phosphate, silicate, nitrate, nitrite and ammonia) according to the method of Grasshoff (1976). Measurement of pH of the bottom water sample was carried out with a pH Meter (pH 526, WTW, Weilheim, Germany) and the

determination of salinity was carried out with a Salinometer (Autosal, Gamma Analysen Technik GmbH) after the method of Grasshoff (1976).

Further environmental data such as mean wind speed (Bft; Beaufort scale), maximal wind speed (m sec^{-1}), sunshine duration (hours) and mean cloud cover were provided by Germany's National Meteorological Service (Deutscher Wetterdienst, DWD) and included in the statistical analyses.

2.2. Data analysis

To test for significant correlations between the environmental parameters as independent variable and the abundance of *Paralia sulcata* as a dependent variable for the bottom and surface water samples the Pearson correlation coefficient and multiple regression analysis was carried out. In order to test for significant differences within the environmental parameters and the abundance of *P. sulcata* in the bottom and surface water samples a one-way analysis of variance (ANOVA) with the Fisher's least significant difference (LSD) post-hoc test ($p < 0.05$) was carried out. To answer the question as to how the abundance of *P. sulcata* was influenced by the different environmental parameters in a seasonal aspect, and therefore the ecological niche, the data set was separated according to the four seasons: winter (December to February), spring (March to May), summer (June to August) and autumn (September to November). For significant interactions of the factors *seasons* (spring, summer, autumn and winter) and *water sample* (surface and bottom) a two-way ANOVA was used for the environmental parameters and the abundance of *P. sulcata*. The Fisher's LSD post-hoc test was used for equal sample sizes. All statistical analyses were performed with STATISTICA (STATISTICA 7.1, StatSoft Inc, USA).

To determine the ecological niche (niche position and niche breadth) of *P. sulcata* in the surface and bottom water samples the outlying mean index analysis (OMI) using R 2.6.0. (2007) and the software package ADE-4 (Thioulouse et al. 1997) was performed. This multivariate technique quantifies the niche parameters along several environmental gradients (Dolédec et al. 2000, Lappalainen & Soininen 2006) and is based on the abundance of species. Niche position is a measure of the distance between the mean habitat conditions used by this species and the mean habitat conditions of the sampling site. Therefore, niche position describes the location of the realised niche in the n-dimensional hypervolume of the environment in which this

species can exist. Niche breadth describes the tolerance of a species associated with the environmental parameters, i.e. the expansion of the niche in the hypervolume (Dolédec et al. 2000). If the values for species tolerance are low, the species is considered a specialist which lives in a narrow range of the environmental conditions. Generalists are assumed to live in a range of widely varying environmental conditions and thus show higher values of species tolerance (Dolédec et al. 2000, Heino & Soininen 2006, Tsiftsis et al. 2008). The ecological niche for *P. sulcata* in the bottom and surface water samples were calculated for the four seasons (spring, summer, autumn and winter) and compared with each other.

RESULTS

1. Determination of the autecology of *Paralia sulcata* in laboratory experiments

1.1. Influence of temperatures and nutrients on the growth of *Paralia sulcata*

The general growth of phytoplankton followed a typical sigmoid curve with well defined growth phases in batch cultures: a short lag phase, the exponential or growth phase and the stationary phase with cell death at the end. The growth rate is important for investigations of the ecological success of a species in adapting to its environmental conditions.

The growth of *Paralia sulcata* at different temperatures and nutrient conditions was investigated during three independent growth experiments to analyse the growth in relation to the seasons. In general, *P. sulcata* required between 49 and 53 days to reach the stationary phase in the treatments and all replicates. The exponential phase started between day 7 and 11 and ended between day 39 and 43, depending on the nutrient condition and temperature (Fig. 1). *P. sulcata* exhibited a good growth pattern in all nutrient conditions at 10°C and 16°C and significant differences in each nutrient treatment between the temperatures (two-way ANOVA, LSD post-hoc test; $F_{(2,48)}$ = 91.746, $p < 0.0001$), nutrient treatments ($F_{(7,48)}$ = 12.009, $p < 0.0001$) and interactions between temperature and nutrients ($F_{(14,48)}$ = 6.2003, $p < 0.0001$) were detected. No growth at all was visible at 4°C. Growth rates at 4°C were significantly lower for all nutrient concentrations, except for the seawater at 10°C and 16°C (ANOVA, LSD post-hoc test, $p < 0.05$). In addition, no growth was observed for any temperatures within the control seawater condition (Fig. 1). The highest abundances of *P. sulcata* were reached at 16°C. Only one exception in the f/2-7/8 PO_4 treatment at 10°C showed the same growth rates as seen in the 16°C treatment. The best nutrient concentrations for the growth of *P. sulcata* were observed in the control f/2 and the f/2-1/2 PO_4 with significantly higher abundances. Lower abundances were detected in the nutrient treatments with all limitations of silicate and two of phosphate (12.5% and 25%) (Fig. 1).

The correlations between the *in situ* fluorescence (chlorophyll *a* concentrations) and the *P. sulcata* abundance (cells ml^{-1}) displayed a significant relationship, especially for all nutrient treatments at 10°C (Table 1). The correlations at 16°C were only significant in the f/2-3/4 SiO_2, f/2-1/2 SiO_2, f/2-1/2 PO_4 and control f/2 nutrient

treatments. Only the results for 10°C and 16°C are shown due to the low concentration of chlorophyll *a* in 4°C (mostly not detectable).

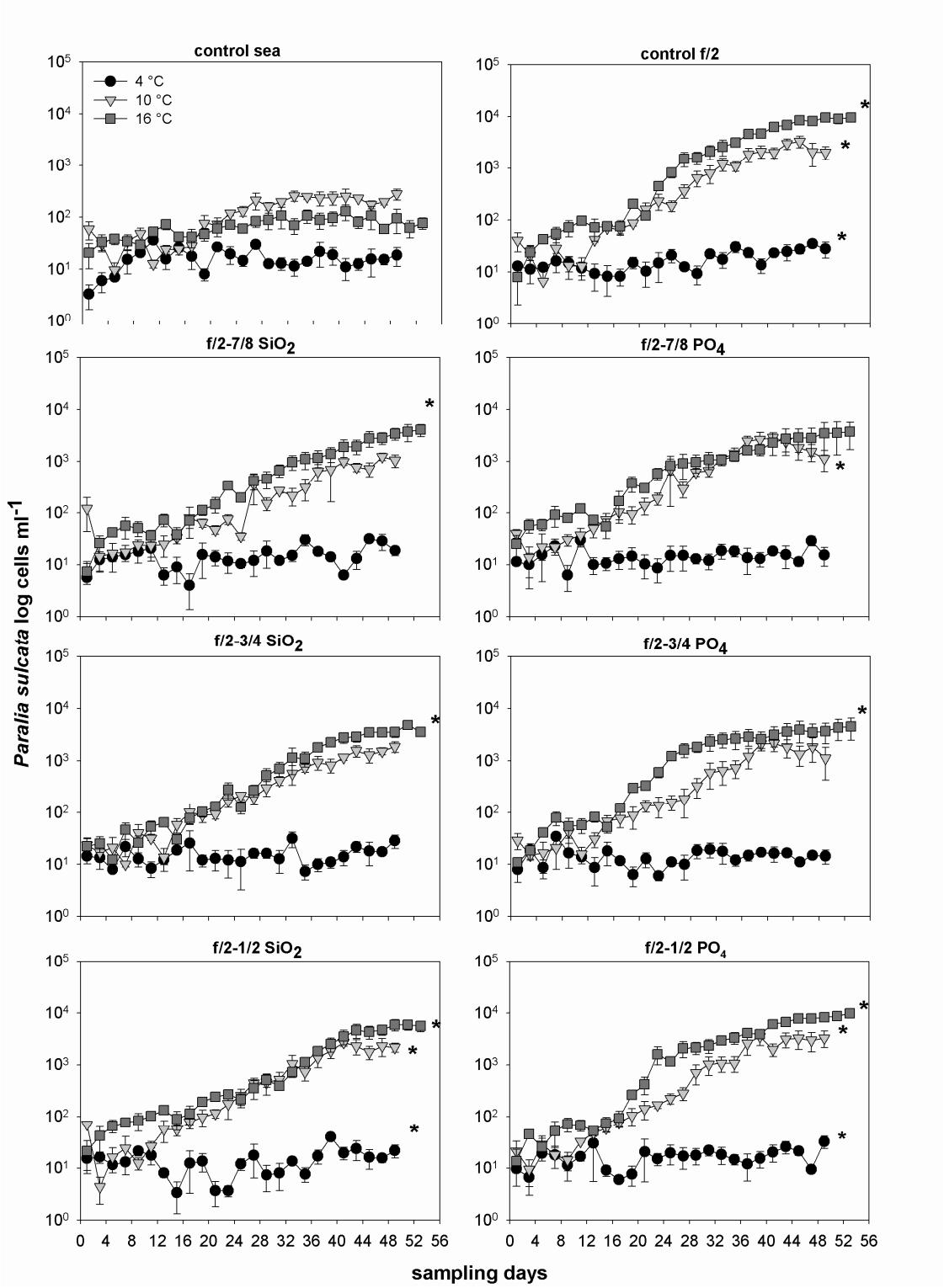

Figure 1: Monitoring of the growth of the logarithmic transformed abundance (cells ml^{-1}) (mean ± SE) of *Paralia sulcata* at three different temperatures and eight different nutrient conditions. The asterisk * indicates significant differences in each nutrient treatment (two-way ANOVA, LSD post-hoc test, $p < 0.001$).

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Table 1: Pearson correlation coefficients of the *in situ* fluorescence (chlorophyll *a* concentrations (µg l^{-1})) and the *Paralia sulcata* abundance (cells ml^{-1}) at 10°C and 16°C within each nutrient treatment. Significant correlations are labelled with an asterisk *, $p < 0.05$.

Nutrients	Pearson correlation at 10°C	p-value	Pearson correlation at 16°C	p-value
Control seawater	0.33*	0.0041	-0.17	0.1132
f/2-7/8 SiO_2	0.90*	<0.0001	-0.09	0.3676
f/2-3/4 SiO_2	0.92*	<0.0001	0.60*	<0.0001
f/2-1/2 SiO_2	0.88*	<0.0001	0.86*	<0.0001
f/2-7/8 PO_4	0.91*	<0.0001	-0.20	0.0788
f/2-3/4 PO_4	0.91*	<0.0001	-0.07	0.7135
f/2-1/2 PO_4	0.85*	<0.0001	0.36*	0.0108
Control f/2	0.89*	<0.0001	0.39*	<0.0001

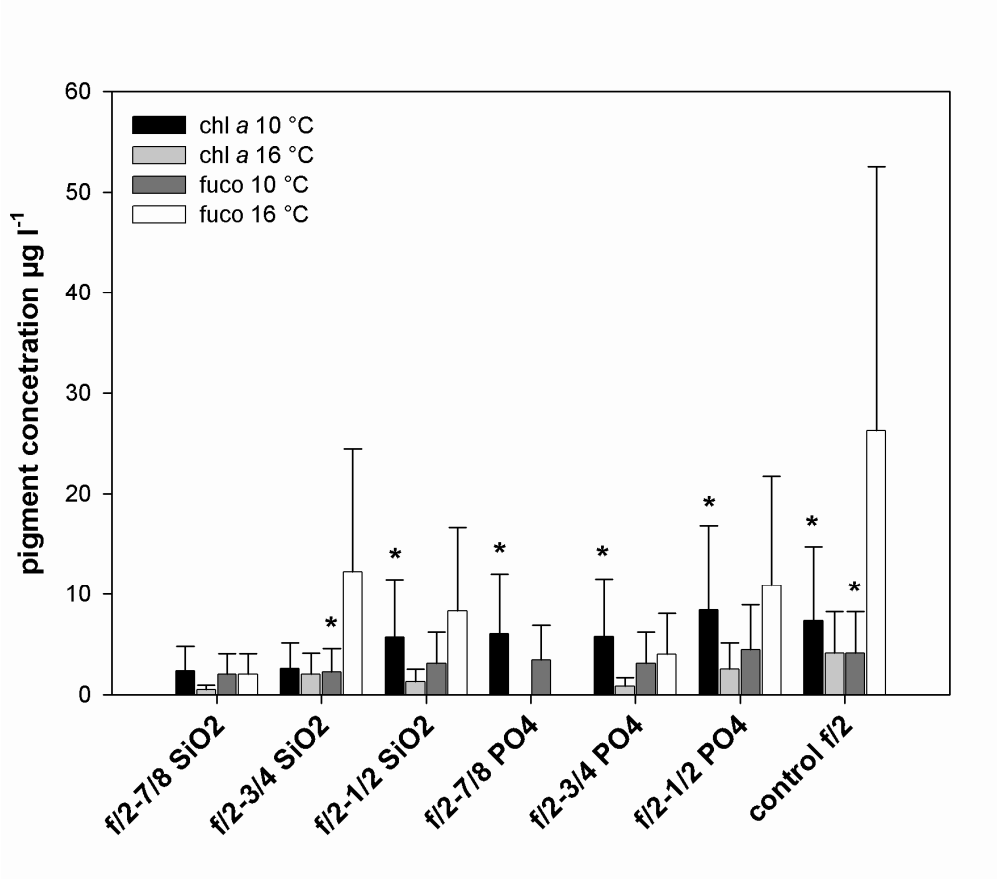

Figure 2: Concentrations of chlorophyll *a* (chl *a*) and fucoxanthin (fuco) (µg l^{-1}) (mean ± SE) of *Paralia sulcata* in the stationary phase at 10 and 16 °C. The statistical evaluation was done for each pigment among all nutrient treatments. The asterisk * indicates significant differences in the treatment within the pigment concentration (two-way ANOVA, LSD post-hoc test, $p < 0.03$).

The pigment concentrations at 4°C in all nutrient concentrations and for the control seawater at 10°C and 16°C were under the detection limit determined using the HPLC. Thus, only the results for 10°C and 16°C are shown and these results were plotted for the stationary phase. The main pigment involved in photosynthesis, chlorophyll *a*, displayed significantly higher concentrations at 10°C in the f/2-1/2 SiO_2, all phosphate limitations and control f/2 compared to the chlorophyll *a* concentrations at 16°C (Fig. 2). In contrast, fucoxanthin, an important antenna pigment, exhibited significantly higher concentrations at 16°C within the f/2-3/4 SiO_2 and control f/2 compared to the 10°C treatment (two-way ANOVA, LSD post-hoc test, $p < 0.03$) (Fig. 2). Additionally, pigments such as diadinoxanthin, diatoxanthin and *β*-carotene displayed higher concentrations at 16°C, especially in the phosphate limitations and f/2 media. The concentrations of these pigments were not detectable in any treatments at 10°C and 16°C.

The nutrient concentrations in all treatments in the start medium were significantly higher than the concentrations at the end after the growth of *P. sulcata* (two-way ANOVA, LSD post-hoc test, $p < 0.01$). The highest silicate and phosphate concentrations were detected at 4°C ($p < 0.001$). Silicate concentrations were significantly reduced at 10°C and 16°C in all treatments with an exception for f/2-7/8 PO_4 and f/2-3/4 PO_4 (16°C). Phosphate concentrations were only significantly reduced in all three phosphate limitations at 10°C and 16°C (Fig. 3a, b). For silicate significant effects between the temperature ($F_{(2,103)} = 825.94$, $p < 0.0001$), the nutrient treatments ($F_{(7,103)} = 54.765$, $p < 0.0001$) and interactions between temperature and nutrients ($F_{(14,103)} = 55.247$, $p < 0.0001$) were detected. For phosphate significant effects between the temperature ($F_{(2,103)} = 46.665$, $p < 0.0001$), nutrient treatments ($F_{(7,103)} = 97.265$, $p < 0.0001$) and interactions between temperature and nutrients ($F_{(14,103)} = 2.877$, $p < 0.01$) existed.

The pH measured at the end of the growth experiment showed significant differences with lowest pH values at 4°C ranging from 7.89 to 7.94, intermediate pH values at 10°C varying between 8.03 to 8.10 and highest values at 16°C ranging from 8.18 to 8.30 in all nutrient treatments (two-way ANOVA, LSD post-hoc test, $p < 0.05$).

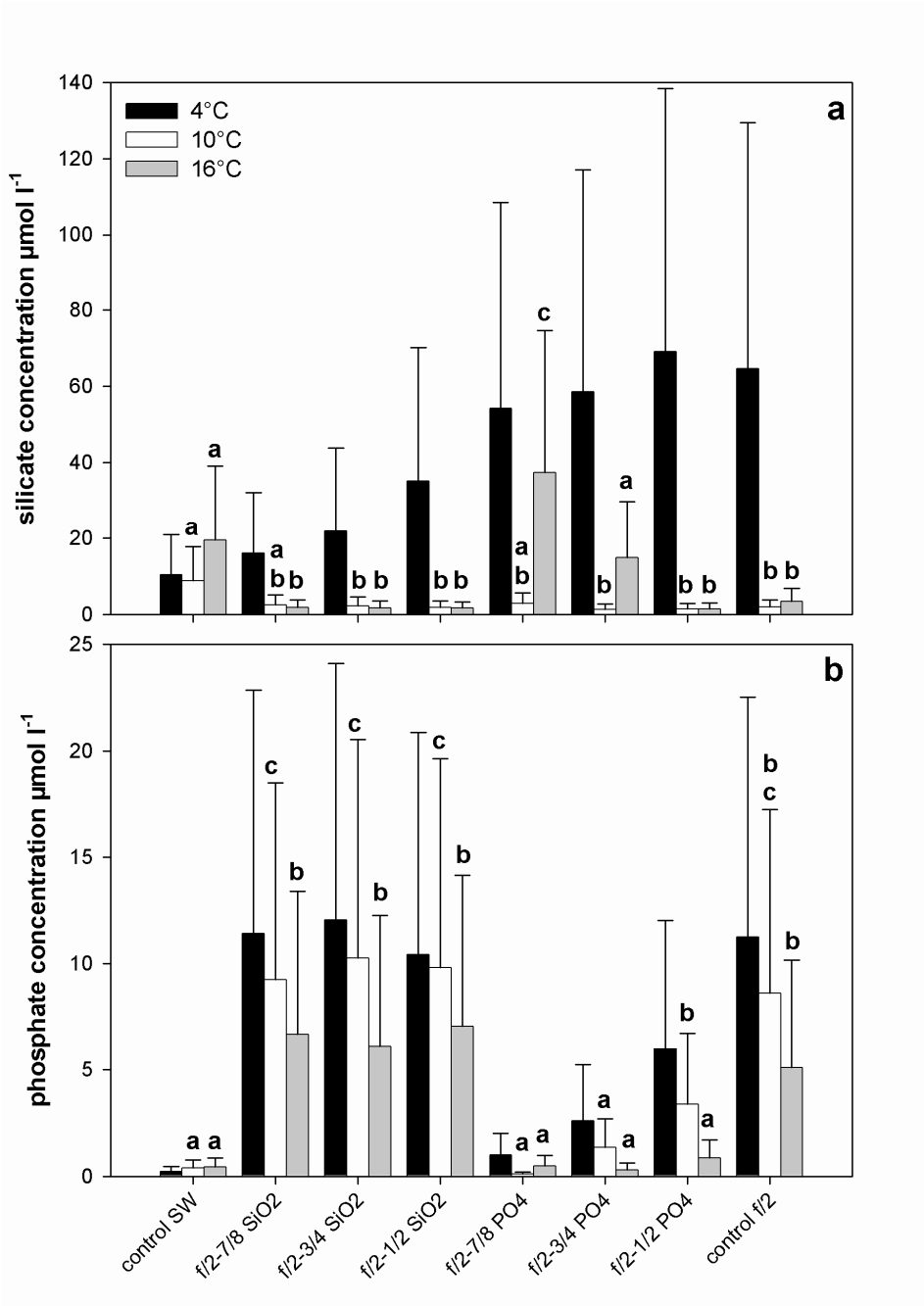

Figure 3: Concentrations of a) silicate and b) phosphate (µmol l^{-1}) (mean ± SE) in the stationary phase for 4°C, 10°C and 16°C. The statistical evaluation was done for each temperature within the treatments for silicate and phosphate, respectively. Different letters indicate significant differences, whereas the same letters display no significant differences between the treatments of each temperature ($p < 0.01$).

1.2. Influence of humic substance concentrations on the growth of *Paralia sulcata*

Paralia sulcata required 29 days to reach the stationary phase with different humic acid concentrations and this period was much shorter than with different nutrient treatments. The exponential growth phase started on day 5 and ended on day 25 to 27. Higher but not significantly growth rates of *P. sulcata* were observed in the treatments with humic acids (low HA and high HA) (Fig. 4). A focus on the *in situ* fluorescence (chlorophyll *a* concentration µg l^{-1}) and cells ml^{-1} displayed positive correlation in all treatments excepted for f/2 (Pearson Correlation, $p < 0.05$) (Table 2).

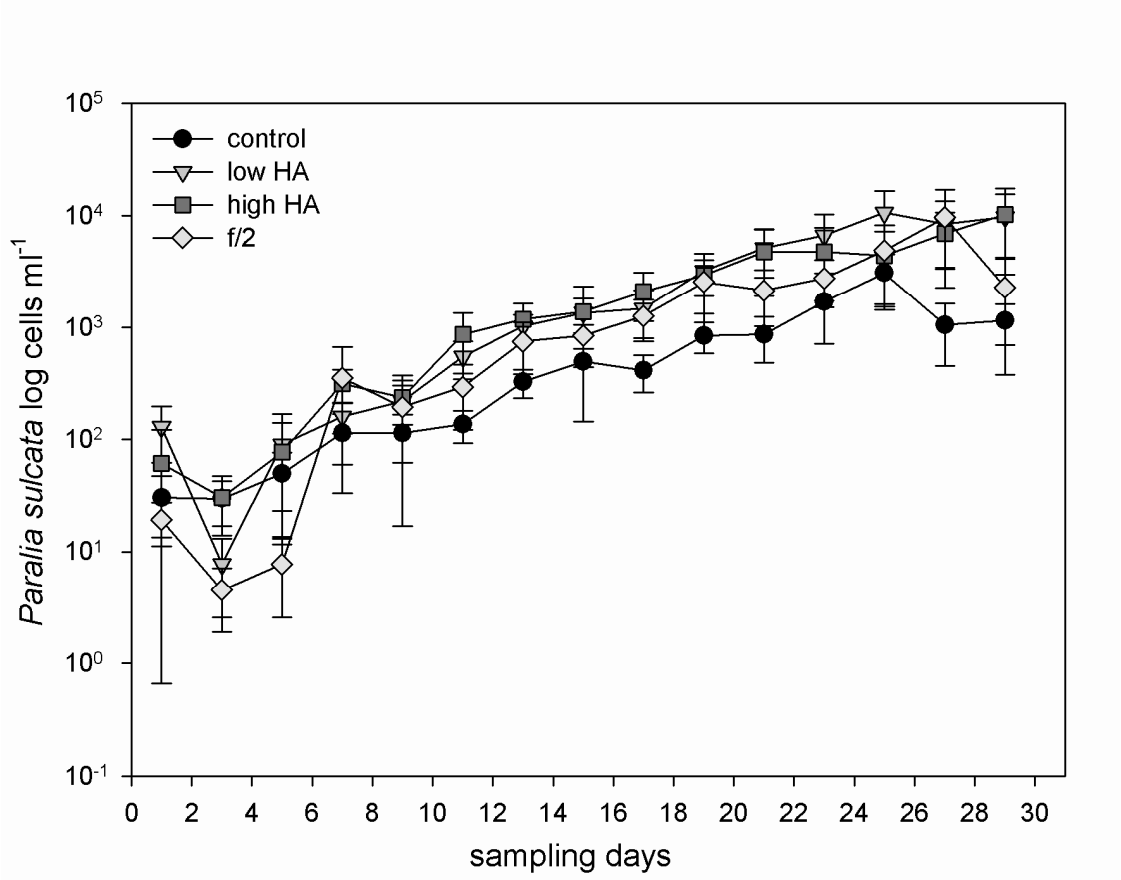

Figure 4: Monitoring of the growth of the logarithmic transformed abundance (cells ml^{-1}) (mean ± SE) of *Paralia sulcata* from four different treatments with and without humic acids (HA).

Table 2: Pearson correlation coefficients of the *in situ* fluorescence (chlorophyll *a* concentrations (µg l^{-1})) and the *Paralia sulcata* abundance (cells ml^{-1}) within the treatments. Significant correlations are labelled with an asterisk *, $p < 0.05$.

Treatment	Pearson correlation	p-value
Control	0.29*	0.0238
Low HA	0.72*	<0.0001
High HA	0.81*	<0.0001
f/2	0.16	0.2118

As shown by the abundances of *P. sulcata*, highest concentrations of chlorophyll *a* and fucoxanthin were reached in the low HA treatment in the stationary phase (ANOVA, LSD post-hoc test, $p < 0.05$). The lowest pigment concentrations were observed for the diadinoxanthin and *β*-carotene (Table 3).

Table 3: Concentrations of the main pigments (µg l^{-1}) (mean ± SE) in *Paralia sulcata* determined in the stationary phase of the growth experiment. The statistical evaluation was done for each pigment in comparison with the treatment, the asterisk* indicates significant differences between the chl *a* concentration in all treatments (ANOVA, LSD post-hoc test, $p < 0.05$).

Pigments	control	low HA	high HA	f/2
fucoxanthin	28.83 ± 4.33	61.43 ± 15.56	34.01 ± 3.96	23.89 ± 10.91
diadinoxanthin	1.79 ± 0.52	3.23 ± 0.80	2.39 ± 0.68	2.68 ± 0.07
chlorophyll *a*	22.64 ± 7.16	54.60 ± 22.63*	6.18 ± 4.81	18.75 ± 11.63
β-carotene	2.54 ± 0.38		2.25 ± 0.01	

Additionally, the nutrient concentrations differed significantly between the treatments for the *P. sulcata* growth (Fig 5). Taking into account that the medium enriched with the two different humic acid concentrations contained a lower nutrient concentration compared with the f/2 medium an interesting result was detected. Silicate concentration was significantly higher in the high HA treatment (ANOVA, LSD post-hoc test, $p < 0.05$), whereas the phosphate concentrations were significantly higher in both the high HA and f/2 treatment ($p < 0.02$) compared to the other treatments. Furthermore, the total dissolved inorganic nitrogen (DIN) concentrations were significantly higher in the control and f/2 treatment ($p < 0.05$) (Fig 5).

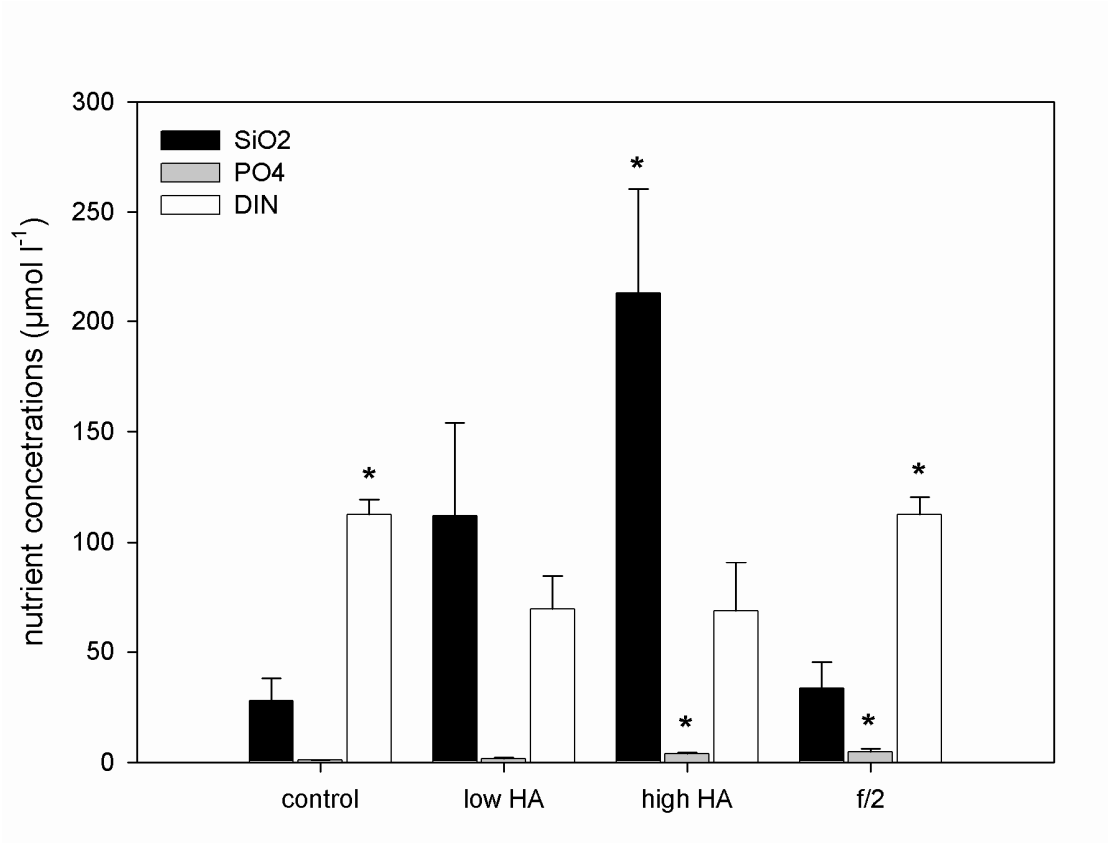

Figure 5: Concentrations of the nutrients (silicate [SiO_2], phosphate [PO_4] and total dissolved inorganic nitrogen [DIN] (µmol l^{-1}) as mean ± SE in the stationary phase at the treatments. The statistical evaluation was done for each nutrient in comparison within the treatments. The asterisk * indicates significant differences in the treatments within the nutrient concentration (ANOVA, LSD post-hoc test, $p < 0.05$).

Summarising the results of both different growth experiments it was shown that the best growth of *P. sulcata* was at higher temperatures (16°C) with high nutrients; especially silicate and humic acid addition resulted in higher growth.

2. Determination of the autecology of *Paralia sulcata* from field observations at Helgoland Roads

With regard to the results from the laboratory experiments we wanted to use the monitoring program to answer the following questions: Which environmental conditions affect the abundance of *Paralia sulcata* in different water depths at Helgoland Roads? How was the abundance influenced by the environmental parameters during the seasons and can we draw conclusions for the ecological niche for *P. sulcata* from these observations?

The abundance (cells l^{-1}) of *P. sulcata* in the bottom and surface water samples varied over the two years, with generally higher abundances recorded in autumn to winter times (bottom, mean with standard deviation: 1728 ± 1372 cells l^{-1} and 1695 ±1263 cells l^{-1} respectively; surface: 451 ± 550 cells l^{-1} and 562 ± 442 cells l^{-1} respectively) and lower abundances in spring to summer times (bottom: 545 ± 438 cells l^{-1} and 454 ± 432 cells l^{-1} respectively; surface: 213 ± 409 cells l^{-1} and 133 ± 340 cells l^{-1} respectively) (Fig. 6). Significantly higher abundances of *P. sulcata* were detected in the bottom water sample compared to the surface water sample (ANOVA, $p < 0.0001$). Despite this difference significantly positive correlations between the abundances in the bottom and surface water sample were found (Pearson correlation coefficient $R = 0.62$) (Table 4).

The temperature showed the typical amplitude for temperate coastal waters according to the seasons with high temperatures in summer times (both: 16.1 ± 2.1 °C) and lowest in winter (bottom: 5.8 ± 1.5°C; surface: 3.8 ± 2.2°C) (Fig. 6). No significant differences between the bottom and surface water temperatures could be detected and a significant high correlation was shown (Pearson correlation coefficient $R = 1.0$) indicating a strong mixing of the water column at Helgoland Roads without stratification (Table 6). Both abundances of *P. sulcata* were not significantly correlated with their corresponding temperature in the bottom and surface water sample (Table 4, 5) although a slight negative tendency was shown. However, the abundances of *P. sulcata* in the bottom water sample were strongly correlated to some of the environmental parameters: significantly negative correlations were observed with Secchi depth, sunshine duration and pH, while positive correlations were detected with phosphate and silicate concentrations, mean and maximal wind speed as well as mean cloud cover (Fig. 7, Table 4). The abundances of *P. sulcata* in the surface water sample were significantly negatively correlated with Secchi depth and sunshine

duration and positively with salinity and mean cloud cover (Fig. 7, Table 5) during the investigated time period of two years.

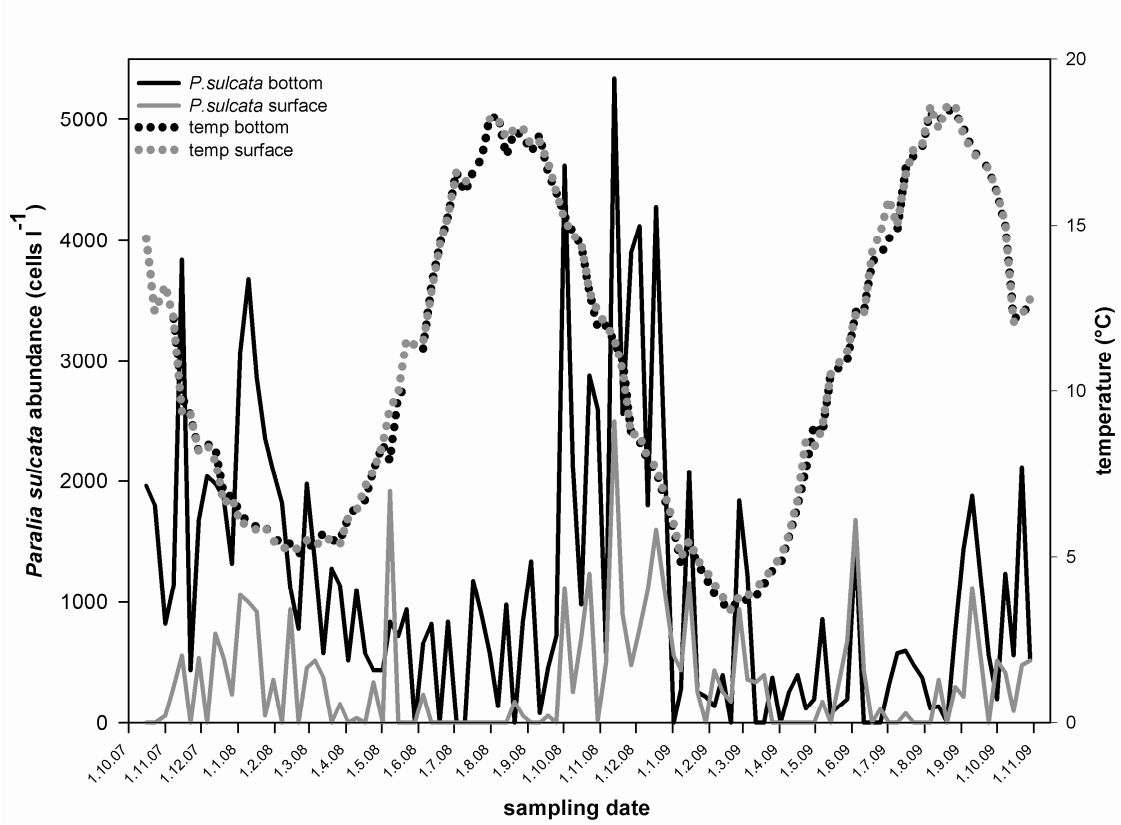

Figure 6: Abundance of *Paralia sulcata* (cells l^{-1}) and the temperature (°C) of the bottom and surface waters during the sampling period from 16.10.2007 to 29.10.2009 at Helgoland Roads. Analysis of variance (ANOVA) showed significant differences between the abundance of *P. sulcata* in bottom and surface waters ($p < 0.001$) but no significant differences within the temperatures ($p = 0.871$) over the sampling period.

Table 4: Results of the Pearson correlation coefficients and regression analysis (R^2, F and p value) for the *Paralia sulcata* abundance versus the environmental parameters from the bottom water samples. Significant correlations are labelled with an asterisk * ($p < 0.05$).

***Paralia sulcata* abundance bottom**	**Pearson correlation**	**Regression analysis (R^2)**	**Regression analysis (F-value)**	**Regression analysis (p-value)**
P. sulcata surface (cells l^{-1})	0.62*	0.382	62.891	<0.0001*
Diatoms (BBE) (µg l^{-1})	-0.28*	0.081	8.711	0.004*
Total chl *a* (BBE) (µg l^{-1})	-0.26*	0.067	7.062	0.009*
pH	-0.30*	0.089	7.817	0.006*
Temperature (°C)	-0.14	0.020	2.017	0.159
Salinity	-0.03	0.001	0.119	0.731
Secchi (m)	-0.58*	0.339	51.695	<0.0001*
Silicate (µmol l^{-1})	0.32*	0.102	11.422	0.001*
Phosphate (µmol l^{-1})	0.44*	0.198	24.689	<0.0001*
Dissolved inorganic nitrogen (µmol l^{-1})	0.12	0.014	1.403	0.239
Mean wind speed (Bft)	0.36*	0.131	15.414	0.0002*
Max. wind speed (m sec^{-1})	0.33*	0.112	12.849	0.001*
Sunshine duration (h)	-0.40*	0.158	19.076	<0.0001*
Mean cloud cover	0.27*	0.074	8.171	0.005*

Table 5: Results of the Pearson correlation coefficients and regression analysis (R^2, F and p value) for the *Paralia sulcata* abundance versus the environmental parameters from the surface water samples. Significant correlations are labelled with an asterisk * ($p < 0.05$).

***Paralia sulcata* abundance surface**	**Pearson correlation**	**Regression analysis (R2)**	**Regression analysis (F-value)**	**Regression analysis (p-value)**
Diatoms (BBE) (µg l-1)	-0.24*	0.059	6.306	0.014*
Total chl a (BBE) (µg l-1)	-0.21*	0.044	4.607	0.034*
Temperature (°C)	-0.19	0.035	3.677	0.058
Salinity	0.23*	0.053	5.667	0.019*
Secchi (m)	-0.27*	0.075	8.188	0.005*
Silicate (µmol l-1)	0.06	0.003	0.345	0.558
Phosphate (µmol l-1)	0.05	0.002	0.219	0.641
Dissolved inorganic nitrogen (µmol l-1)	-0.02	0.0004	0.044	0.834
Mean wind speed (Bft)	0.18	0.031	3.274	0.073
Max. wind speed (m sec-1)	0.15	0.023	2.364	0.127
Sunshine duration (h)	-0.26*	0.070	7.684	0.007*
Mean cloud cover	0.19*	0.038	4.005	0.048*

Table 6: Results of the Pearson correlation coefficients, regression analysis and analysis of variance (ANOVA) for the *Paralia sulcata* abundance and the environmental parameters compared between surface and bottom water samples. Significant correlations are labelled with an asterisk * ($p < 0.05$).

Parameters surface vs. bottom	Pearson correlation	Regression analysis (R^2)	Regression analysis (F-value)	Regression analysis (p-value)	ANOVA (F-value)	ANOVA (p-value)
Paralia sulcata (cells l^{-1})	0.62*	0.382	62.891	<0.0001*	40.454	<0.0001*
Diatoms (BBE) (µg l^{-1})	0.87*	0.758	306.987	<0.0001*	0.742	0.390
Total chl *a* (BBE) (µg l^{-1})	0.88*	0.782	351.681	<0.0001*	1.283	0.259
Temperature (°C)	1.0*	0.996	21704.706	<0.0001*	0.026	0.871
Salinity	0.95*	0.909	1007.169	<0.0001*	0.366	0.546
Silicate (µmol l^{-1})	0.96*	0.895	1252.744	<0.0001*	0.052	0.820
Phosphate (µmol l^{-1})	0.81*	0.633	188.397	<0.0001*	0.019	0.892
Dissolved inorganic nitrogen (µmol l^{-1})	0.84*	0.702	240.502	<0.0001*	1.017	0.344

That the water column at Helgoland Roads is well mixed could be explained by the good correlation of the environmental parameters between the bottom and surface water sample (Table 6), all showing a highly significant correlation within the *in situ* fluorescence (diatoms and chlorophyll *a*), temperature, salinity and nutrients within the two years.

In a next step the influence of the environmental parameters on the abundance of *P. sulcata* based on the seasons at Helgoland Roads was analysed to gain a better insight into the ecological niche of this diatom species at this particular sampling site. To achieve this the seasons were defined based on the meteorological seasons: spring (March to May), summer (June to August), autumn (September to November) and winter (December to February). All further analyses took this into account.

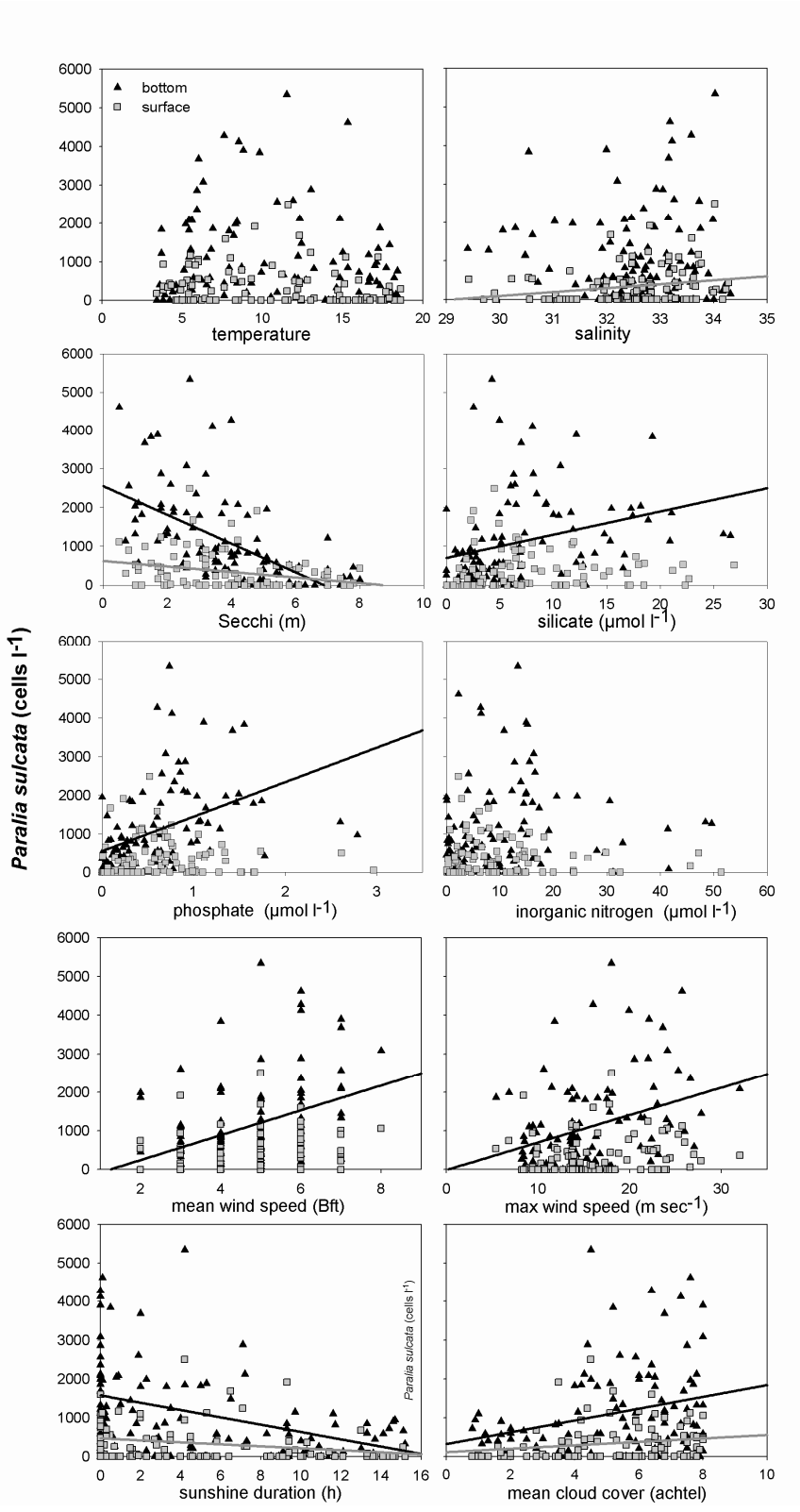

Figure 7: Correlations of the abundance of *Paralia sulcata* (cells l^{-1}) and the environmental parameters in the bottom (black triangle) and surface (gray squares) water samples during the sampling period from 16.10.2007 to 29.10.2009 at Helgoland Roads (regression lines indicate significant correlations, $p < 0.05$). Statistics for the correlation coefficient and regression analysis are shown in Table 4 for the bottom and in Table 5 for the surface water samples.

Box-Whisker plots (Fig. 8 and Table 7) show the distribution pattern of *P. sulcata* abundance, temperature, salinity and nutrients over the seasons in the bottom and surface water samples. Secchi depth, mean and maximal wind speed, sunshine duration and cloud cover were also analysed at the sampling station. The seasonal pattern typical for temperate coastal waters due to the increase of temperature in summer times, nutrient depletions (spring-summer) and nutrient increase (autumn) were reflected at the sampling site within both water depths. No significant differences between both water samples were found with season as comparison factor. Despite the thorough mixing of the water column during the seasons at Helgoland Roads, it was shown that especially in autumn and winter the abundance of *P. sulcata* in the bottom water sample was significantly higher compared to the surface water sample (Fig. 8).

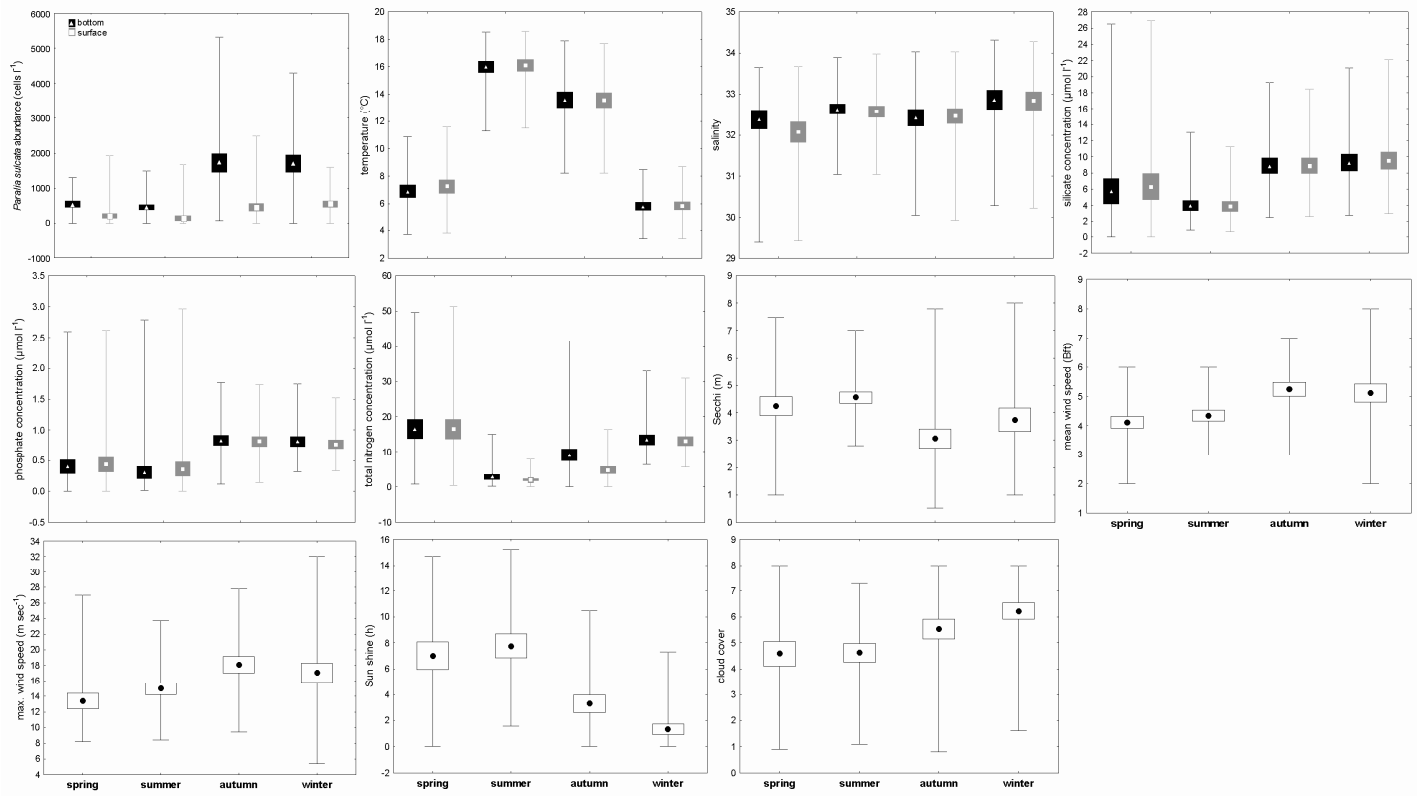

Figure 8: Box-Whisker plots with the dots as mean, the box represents mean ± standard error and the whiskers the minimum and maximum range of the abundance of *Paralia sulcata* (cells l^{-1}), temperature (°C), salinity and nutrients (µmol l^{-1}) in the bottom (black boxes) and surface (grey boxes) water samples and of Secchi depth (m), mean wind speed (Beaufourt scale), maximal wind speed (m sec^{-1}), sunshine duration (hours) and mean cloud cover (white boxes) calculated for the different seasons: spring (N = 25), summer (N = 26), autumn (N = 28) and winter (N = 25) during the sampling period from 16.10.2007 to 29.10.2009 at Helgoland Roads. Statistics for the analysis of variance are shown in Table 7. The asterisk * indicates the significant difference of the *P. sulcata* abundance between bottom and surface water sample in autumn and winter time (ANOVA, LSD post-hoc test, $p < 0.0001$).

Table 7: Results of the analysis of variance (ANOVA, F and p value) for the *Paralia sulcata* abundance, temperature, salinity and nutrients in the bottom and surface water samples as well as Secchi depth, mean and maximal wind speed, sunshine duration and mean cloud cover in comparison due to the seasons at Helgoland Roads. Significant differences between the seasons and the water sample for each parameter were labelled with an asterisk * ($p < 0.05$). Box-Whisker plots of these parameters are shown in Figure 9.

Parameter	Bottom water ANOVA		Surface water ANOVA	
Paralia sulcata (cells l^{-1})	$F_{(3,100)} = 13.026$	$p < 0.0001$*	$F_{(3,100)} = 5.212$	$p < 0.01$*
Temperature (°C)	$F_{(3,96)} = 124.815$	$p < 0.0001$*	$F_{(3,99)} = 117.438$	$p < 0.0001$*
Salinity	$F_{(3,99)} = 1.216$	$p = 0.308$	$F_{(3,100)} = 2.373$	$p = 0.075$
Silicate (µmol l^{-1})	$F_{(3,99)} = 5.423$	$p < 0.01$*	$F_{(3,100)} = 5.319$	$p < 0.01$*
Phosphate (µmol l^{-1})	$F_{(3,98)} = 8.240$	$p < 0.0001$*	$F_{(3,100)} = 5.609$	$p < 0.01$*
Dissolved inorganic nitrogen (µmol l^{-1})	$F_{(3,99)} = 11.036$	$p < 0.0001$*	$F_{(3,100)} = 17.662$	$p < 0.0001$*
Other environmental parameters	**ANOVA**			
Secchi (m)	$F_{(3,99)} = 3.745$	$p = 0.014$*		
Mean wind speed (Bft)	$F_{(3,100)} = 5.502$	$p = 0.001$*		
Maximal wind speed (m sec-1)	$F_{(3,100)} = 4.255$	$p = 0.007$*		
Sunshine (h)	$F_{(3,100)} = 13.981$	$p < 0.001$*		
Mean cloud cover	$F_{(3,100)} = 4.017$	$p = 0.01$*		

A closer look at the correlations of the *P. sulcata* abundances in the bottom and surface water sample with their corresponding environmental parameters exposed an interesting pattern for the seasons. At first the abundances of *P. sulcata* in the bottom and surface water samples were significantly correlated in summer, autumn and winter times, but not in spring. Furthermore, the abundance of *P. sulcata* in the surface water showed only significant correlations with temperature (negative) and total dissolved inorganic nitrogen (positive) in the summer period (Fig. 10, Table 8). The most significant correlations in the bottom water sample were recorded for the abundance of *P. sulcata* with salinity and Secchi depth (negatively) and silicate, phosphate as well as nitrogen concentrations (positively) in spring (Fig. 9, Table 8), whereas only Secchi depth in summer and autumn and Secchi depth and temperature in winter had a negatively significant influence (Fig. 11, Table 8).

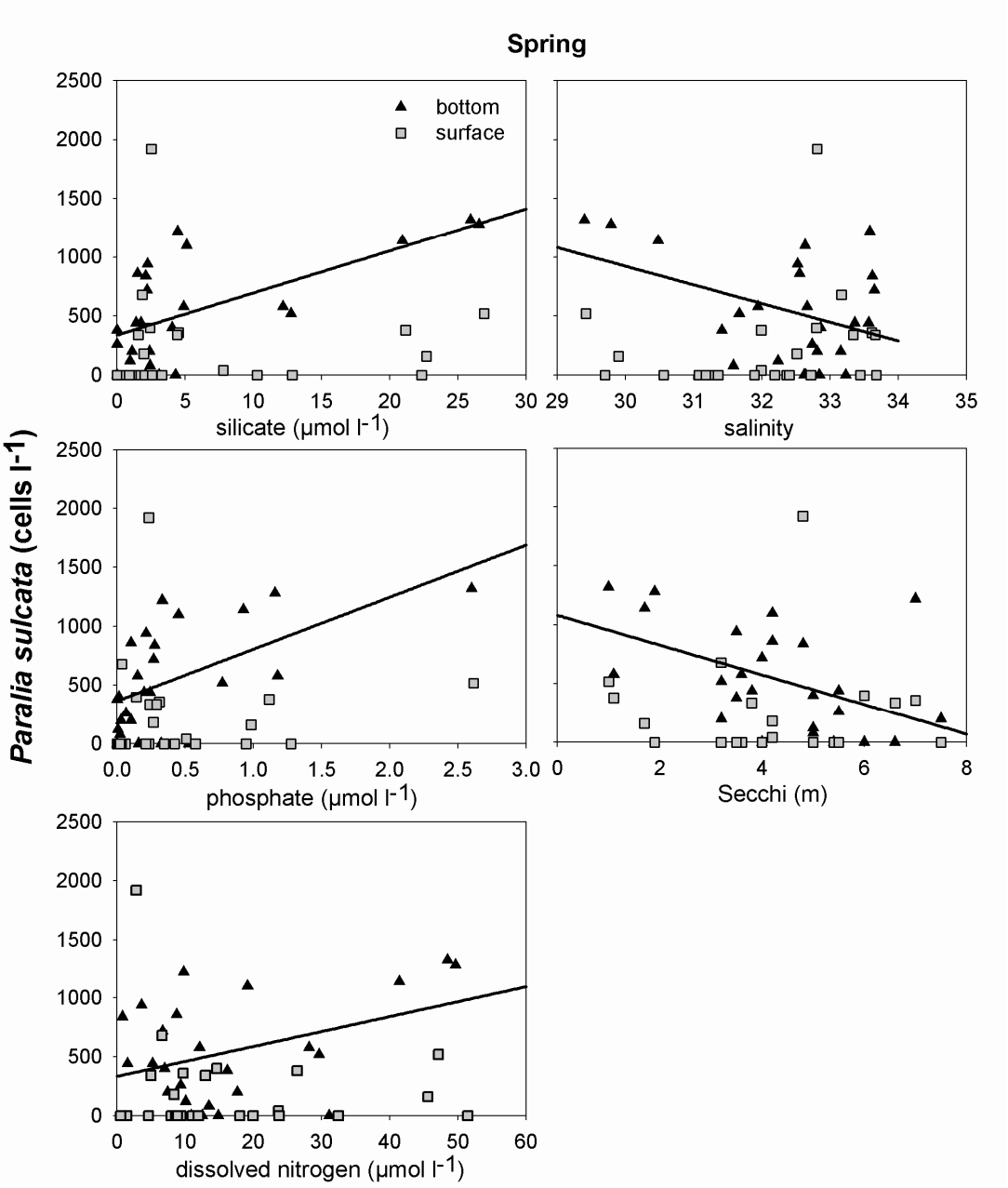

Figure 9: Significant correlations of the environmental parameters with the abundance of *Paralia sulcata* are shown for spring in the bottom (black triangle) and surface (gray squares) water samples during the sampling period from 16.10.2007 to 29.10.2009 at Helgoland Roads (regression lines indicated significant correlations, $p < 0.05$). Statistics for the correlation coefficient and regression analysis are shown in Table 8.

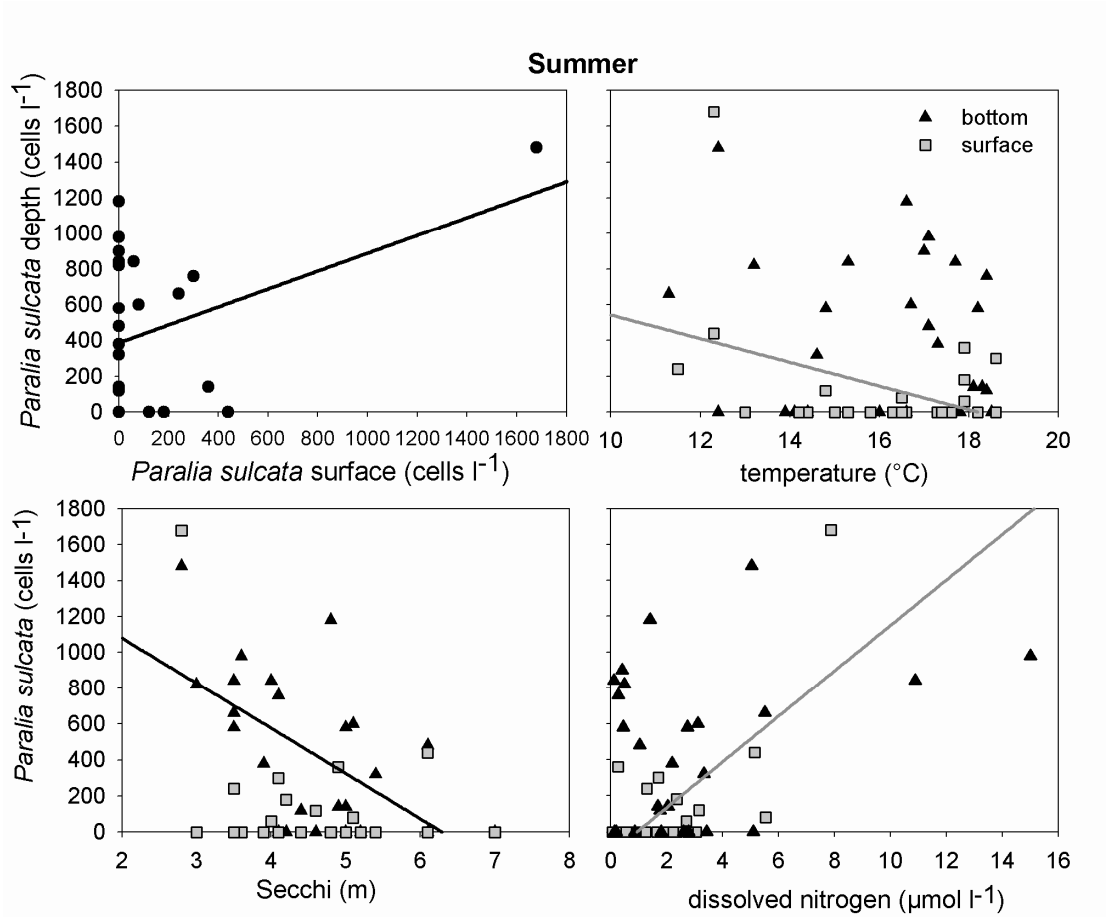

Figure 10: Significant correlations of the environmental parameters with the abundance of *Paralia sulcata* are shown for summer in the bottom (black triangle) and surface (gray squares) water samples during the sampling period from 16.10.2007 to 29.10.2009 at Helgoland Roads (regression lines indicated significant correlations, $p < 0.05$). Statistics for the correlation coefficient and regression analysis are shown in Table 8.

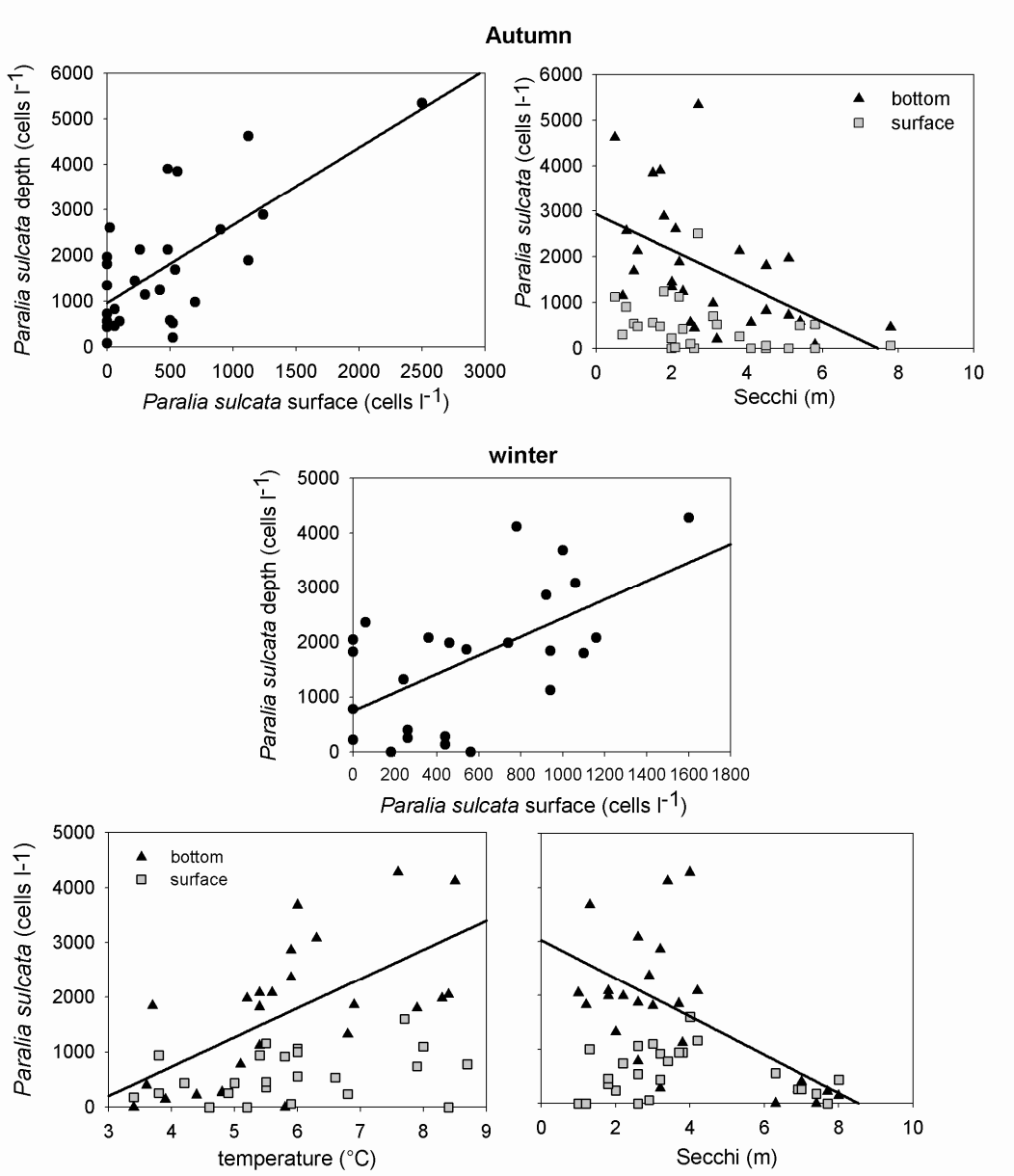

Figure 11: Significant correlations of the environmental parameters with the abundance of *Paralia sulcata* are shown for autumn and winter in the bottom (black triangle) and surface (gray squares) water samples during the sampling period from 16.10.2007 to 29.10.2009 at Helgoland Roads (regression lines indicated significant correlations, $p < 0.05$). Statistics for the correlation coefficient and regression analysis are shown in Table 8.

Table 8: Results of the Pearson correlation coefficients and regression analysis of the *Paralia sulcata* abundance versus the environmental parameters of the bottom and surface water samples respectively. Significant correlations are labelled with an asterisk * ($p < 0.05$).

***Paralia sulcata* abundance vs. environmental parameters**	**Pearson correlation**	**Regression analysis (R^2)**	**Regression analysis (F-value)**	**Regression analysis (p-value)**	**Pearson correlation**	**Regression analysis (R^2)**	**Regression analysis (F-value)**	**Regression analysis (p-value)**
spring		**bottom**				**surface**		
Paralia sulcata (cells l^{-1})	0.17	0.029	0.677	0.419				
Temperature (°C)	-0.09	0.008	0.174	0.681	0.08	0.006	0.145	0.707
Salinity	-0.41*	0.169	4.660	0.042*	0.19	0.036	0.855	0.365
Secchi (m)	-0.49*	0.242	7.325	0.013*	-0.04	0.001	0.031	0.861
Silicate (µmol l^{-1})	0.63*	0.402	15.459	< 0.001*	0.04	0.002	0.042	0.839
Phosphate (µmol l^{-1})	0.58*	0.333	11.505	0.003*	0.09	0.008	0.184	0.672
Dissolved inorganic nitrogen (µmol l^{-1})	0.40*	0.161	4.399	0.047*	-0.13	0.017	0.392	0.538
Mean wind speed (Bft scale)	0.02	0.0004	0.010	0.92	-0.07	0.005	0.108	0.746
Maximal wind speed (m sec^{-1})	0.21	0.046	1.097	0.306	-0.03	0.001	0.019	0.893
Sunshine duration (h)	-0.21	0.044	1.069	0.312	-0.02	0.0004	0.010	0.921
Mean cloud cover	0.17	0.028	0.671	0.421	0.04	0.002	0.034	0.856
summer		**bottom**				**surface**		
Paralia sulcata (cells l^{-1})	0.40*	0.157	4.452	0.046*				
Temperature (°C)	-0.13	0.017	0.407	0.530	-0.42*	0.172	4.793	0.039*
Salinity	0.16	0.027	0.667	0.422	-0.11	0.011	0.274	0.605
Secchi (m)	-0.59*	0.347	12.213	0.002*	-0.31	0.096	2.453	0.131
Silicate (µmol l^{-1})	0.02	0.001	0.013	0.910	-0.01	0.0001	0.002	0.962
Phosphate (µmol l^{-1})	0.21	0.045	1.140	0.296	-0.09	0.008	0.201	0.658
Dissolved inorganic nitrogen (µmol l^{-1})	0.29	0.086	2.246	0.147	0.69*	0.479	22.075	< 0.001*
Mean wind speed (Bft scale)	0.07	0.005	0.121	0.731	0.20	0.039	0.964	0.336
Maximal wind speed (m sec^{-1})	0.08	0.007	0.166	0.688	0.14	0.020	0.493	0.489

Sunshine duration (h)	0.07	0.004	0.107	0.747	-0.09	0.009	0.213	0.648
Mean cloud cover	-0.12	0.014	0.332	0.570	0.09	0.009	0.205	0.655
autumn		**bottom**				**surface**		
Paralia sulcata (cells l^{-1})	0.68*	0.467	22.749	< 0.001*				
Temperature (°C)	-0.37	0.139	3.719	0.066	-0.20	0.039	1.068	0.311
Salinity	0.13	0.016	0.408	0.529	0.35	0.122	3.603	0.069
Secchi (m)	-0.53*	0.283	10.287	0.004*	-0.36	0.131	3.916	0.059
Silicate (µmol l^{-1})	0.05	0.003	0.064	0.803	-0.18	0.032	0.860	0.362
Phosphate (µmol l^{-1})	0.25	0.063	1.604	0.218	-0.23	0.051	1.402	0.247
Dissolved inorganic nitrogen (µmol l^{-1})	0.05	0.003	0.077	0.783	-0.09	0.007	0.195	0.663
Mean wind speed (Bft scale)	0.20	0.039	1.047	0.316	0.16	0.027	0.707	0.408
Maximal wind speed (m sec^{-1})	0.21	0.045	1.236	0.276	0.29	0.082	2.320	0.140
Sunshine duration (h)	-0.34	0.115	3.365	0.078	-0.15	0.023	0.603	0.443
Mean cloud cover	0.22	0.047	1.290	0.266	0.16	0.025	0.657	0.425
winter		**bottom**				**surface**		
Paralia sulcata (cells l^{-1})	0.59*	0.353	12.551	0.002*				
Temperature (°C)	0.64*	0.404	15.603	< 0.001*	0.32	0.104	2.662	0.116
Salinity	-0.25	0.062	1.516	0.231	0.19	0.035	0.835	0.370
Secchi (m)	-0.61*	0.373	13.656	0.001*	-0.12	0.015	0.355	0.557
Silicate (µmol l^{-1})	0.17	0.027	0.647	0.429	-0.20	0.040	0.954	0.339
Phosphate (µmol l^{-1})	0.37	0.135	3.582	0.071	-0.22	0.051	1.226	0.280
Dissolved inorganic nitrogen (µmol l^{-1})	-0.02	0.000	0.007	0.936	-0.28	0.077	1.905	0.181
Mean wind speed (Bft scale)	0.33	0.109	2.810	0.107	-0.03	0.001	0.026	0.874
Maximal wind speed (m sec^{-1})	0.28	0.080	1.990	0.172	-0.19	0.035	0.831	0.372
Sunshine duration (h)	-0.26	0.068	1.673	0.209	-0.11	0.012	0.282	0.601
Mean cloud cover	0.20	0.039	0.940	0.342	0.04	0.002	0.045	0.835

The niche analysis (OMI) showed an interesting pattern for the ecological niche of *P. sulcata* in the bottom and surface water samples at Helgoland Roads defined by the seasons. The niche in the bottom water sample displayed a broad species tolerance, especially in spring when compared to the rest of the year. However, the niche position did not change during the seasons, indicating a more general niche in the bottom water sample (Fig. 12). In contrast to the bottom water sample the ecological niche of *P. sulcata* from the surface water sample showed a more or less inverse pattern for the spring and summer period. In spring the ecological niche was narrow, indicating a really specialised ecological niche, whereas during summer a shift to a wider adapted niche occurred due to the broad species tolerance (niche breadth) (Fig. 12). Additionally, the ecological niche of *P. sulcata* in autumn and winter was more or less the same for both water samples (bottom and surface).

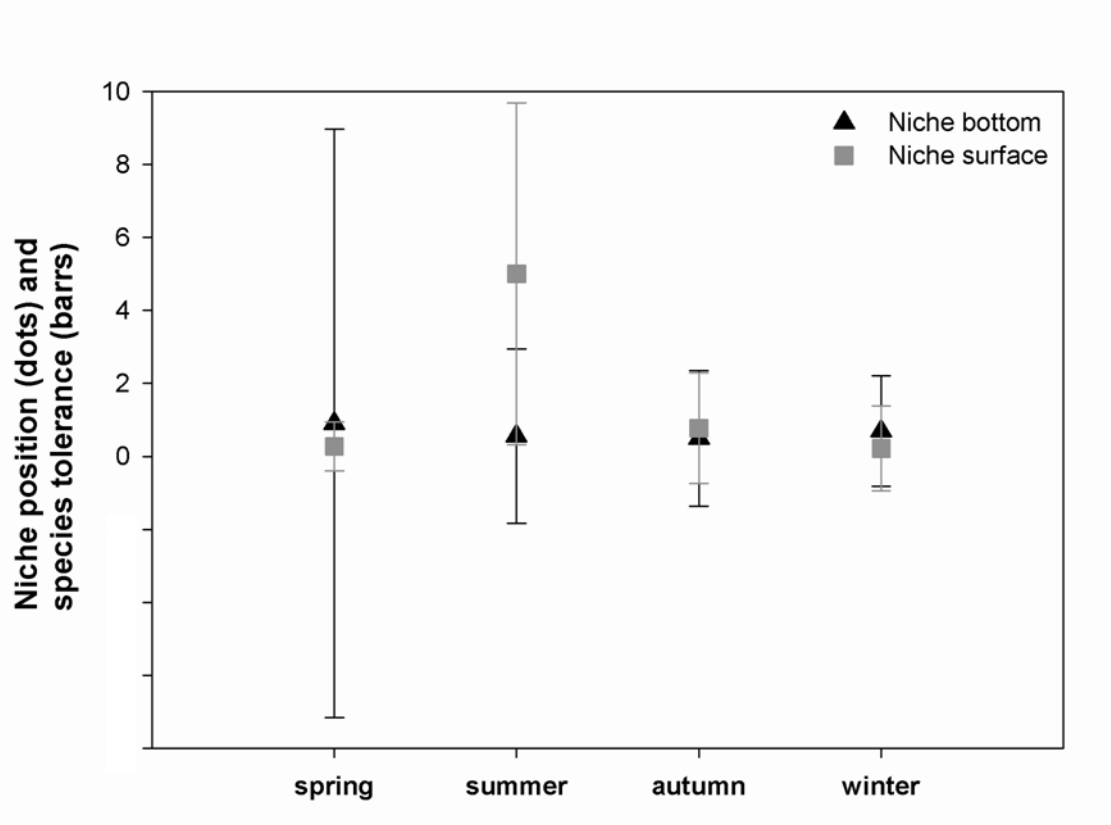

Figure 12: Ecological niche (niche position: dots) and species tolerance (niche breadth: bars) of *Paralia sulcata* in the bottom (black triangle) and surface (grey squares) water samples calculated for the different seasons (spring, summer, autumn and winter) during the sampling period from 16.10.2007 to 29.10.2009 at Helgoland Roads.

Summarising the results for the two years monitoring program it was shown that the water column at Helgoland was generally well mixed resulting in similar temperatures, nutrient concentrations and salinity in the bottom and surface water samples. Furthermore, the most important environmental parameters influencing the abundance of *P. sulcata* in the water (bottom and to a lesser degree in the surface water) were Secchi depth, silicate and phosphate concentrations, mean and maximal wind speed, sunshine duration and mean cloud cover. In comparison to that the correlations of the abundance of *P. sulcata* with the environmental parameters tested separately by seasons displayed a slightly different importance of the parameters. The ecological niche was different in spring and summer between the bottom and surface water samples which were influenced by different environmental parameters.

DISCUSSION

Autecology of *Paralia sulcata*: comparison between laboratory experiments and field observations

The general goal of ecological studies is to define the autecology of single species and to identify the interactions with the environmental parameters that affected its growth (Leibold 1995). Therefore, the autecology is primarily of experimental nature to study and to measure the influence of e.g. nutrient effects, light availability or temperature influences on a single species in order to understand the ecological role. Although laboratory conditions are never a complete reflection of the natural environment they can to a certain degree answer important ecological questions on the growth response of diatoms when exposed to different environmental parameters. In order to answer the question as to how the abundance of *Paralia sulcata* is influenced and which are the most important factors determining the autecology, different growth experiments and a field sampling campaign were conducted at Helgoland Roads. All but a few (temperature and nutrient concentrations) parameters were kept constant within the batch cultures of the growth experiments and all biotic interactions were excluded. In the following section individual tested environmental parameters will be discussed.

Influence of temperature: comparison between experiment and field observations with the literature

Phytoplankton communities and biomass have often been documented with respect to the physico-chemical parameters of their marine habitat. Thus, vertical phytoplankton patterns, which were highly dependent on the availability of light, temperature, salinity and nutrients, were detected in coastal areas (Lunven et al. 2005). The observations on the abundance of *Paralia sulcata* from the long-term sampling site at Helgoland Roads indicated an interesting pattern for the bottom and surface water samples. Particularly in autumn and winter the abundance of *P. sulcata* was significantly higher in the bottom water sample compared with the surface water sample, but a strong correlation between both water samples existed. *P. sulcata* as a tychopelagic diatom species occurs in both the benthos and the pelagic (Roelofs 1984, McQuoid & Nordberg 2003a, 2003b) and is well adapted to lower winter temperatures as shown by other studies (e.g.Roelofs 1984, Sancetta 1989, Hobson & McQuoid 1997). Despite this fact the temperature had no influence on the abundance of *P. sulcata* in the bottom water sample over the investigated time period. As reported in the literature and further from the results of the niche analysis on the occurrence of *P. sulcata* at Helgoland Roads (Gebühr et al. 2009), the abundance is strongly dependent on the temperature and *P. sulcata* is mostly described as a winter diatom. However, the mean annual winter temperature (4°C) did not lead to a growth of *P. sulcata* as e.g. shown for the long-term analysis of the abundance of *P. sulcata* at Helgoland Roads where the temperature was negatively correlated with the abundance (Gebühr et al. 2009). In the laboratory experiments no growth of *P. sulcata* at colder temperature (4°C) was observed. This could indicate that the limiting temperature for the growth in the laboratory was 4°C where *P. sulcata* can exist, but could not reproduce independently from the nutrient concentrations. Moreover, it was shown that this low temperature seemed to be the lower limit where *P. sulcata* can survive, thereby limiting the species tolerance in the ecological niche.

Interestingly, the growth of *P. sulcata* in the batch cultures was highest for higher temperatures, especially when nutrients were freely available. This positive correlation of the temperature with the abundance of *P. sulcata* is in contrast with what we observed outside in the field, where a clear negative correlation was found (especially in summer times (see also Gebühr et al. 2009). Especially in contrast to the long-term analysis (Gebühr et al. 2009), Choudhury & Pal (2010) found completely different

conditions, where *P. sulcata* occurred at the coast in the Bay of Bengal (Eastern India). At this site *P. sulcata* occurred only in warmer summer months (April to July) with temperatures between 28°C and 32°C and salinity ranging from 32 to 35 in the water column.

However, the influence of temperature on the growth of *P. sulcata* is not easy to explain. A closer look at the seasonal influence of temperature on the occurrence of *P. sulcata* revealed a positive correlation in winter times. This means that with increasing water temperatures in winter the abundance, and therefore the growth of *P. sulcata*, will be enhanced. The strong negative influence of temperature on the occurrence of *P. sulcata* in summer showed the total range of the optimal temperatures at which *P. sulcata* can grow successfully. Taking into account that the long-term trend of the North Sea indicates increasing water temperatures of 1.7°C over the last 50 years (Wiltshire et al. 2010), this tolerance for the temperature variations will benefit the growth of *P. sulcata* within the next decades which could lead to higher abundances in winter as well as in summer times.

Thus, we conclude that the temperature for the optimal growth of *P. sulcata* at Helgoland Roads is between 10°C to 20°C. Lower and higher temperatures can be tolerated and due to this temperature range it is possible for *P. sulcata* to survive in the winter period where the most phytoplankton species could not survive. But also other factors in combination with the temperature (e.g. light and nutrient availability as well as biotic interactions) are important for surviving in winter times in the water column.

Influence of nutrient concentrations: comparison between experiment and field observations with the literature

The optimal condition for the growth of *Paralia sulcata* in the laboratory was at higher temperatures (ranging from 10°C to 16°C) when high concentrations of nutrients were available (especially silicate). This result coincided with my field observations, where the abundance was strongly correlated with higher silicate and phosphate concentrations, and also with the literature where higher abundances at higher concentrations of nutrients were reported (Margalef 1969). Furthermore, it was shown that *P. sulcata* was strongly dependent on silicate concentrations. This was reflected by the best growth observed under silicate-limited conditions, independently from the temperature (10°C or 16°C). This fact can be explained by the high silicate demand

due to the highly silicified valves of this diatom species (Crawford 1979a, Abrantes 1988a, 1988b, McQuoid & Nordberg 2003a) and the requirement of silicate for growth (Lewin 1962, Egge & Aksnes 1992, Bidle & Azam 1999). Del Amo et al. (1997) and Ragueneau et al. (2002) pointed out that there is an enrichment of dissolved silicate concentrations on the sediment due to an intense re-dissolution of silica at the benthic interface in deep stratified waters, whereas in well mixed ecosystems the silicate concentrations can be recovered in a faster way and are available again for the summer bloom development with diatoms. This recycling process of silicate from the sediment during the summer months could be a good explanation as to why the abundance of *P. sulcata* at the bottom of the sea was higher compared with the surface water samples. Furthermore, Del Amo et al. (1997) investigated the role of biogenic silicate on the dominance of phytoplankton in the Bay of Brest (France) and showed that despite a well-mixed water column the total biogenic silicate concentrations in bottom waters were slightly higher than those of the surface waters. However, this observation was in contrast to our results, which showed a strong correlation and no significant differences between the surface and bottom water silicate concentrations.

During spring blooms the nutrient concentrations in the surface layers were reduced due to the uptake by the diatoms during the development of the bloom (Lunven et al. 2005). Phosphate concentrations in particular limited the growth of the phytoplankton (Labry et al. 2005, Lunven et al. 2005). The fact that phosphate was an important nutrient for the development and growth of *P. sulcata* was shown in this study especially by the significant correlation of the algal abundances with phosphate concentration during the spring period. On the other hand the laboratory growth experiments showed that *P. sulcata* can cope to a slight degree with limiting phosphate concentrations. It can therefore be concluded that phosphate concentrations did not play that important role during the growth of *P. sulcata* in the laboratory, although phosphate has an essential role in the metabolism, especially in energy transformation processes of diatoms (Kuhl 1962).

Therefore, it is the combination of nutrients which influence the growth and the abundance of *P. sulcata* in the field and the laboratory. Higher concentrations of silicate and phosphate favour the growth of *P. sulcata*, but this diatom is also able to tolerate to a slight degree limited concentrations of both nutrients. The distribution of nutrients in the annual cycle favours a phase shift of *P. sulcata* from a pelagic diatom species during autumn-winter to spring to a benthic life in summer times.

Influence of light availability: comparison between experiment and field observations with the literature

Carbon sources, as one of the regulatory factors for the growth of diatoms, and the role of humic acids in turbid marine systems for diatoms are almost unknown. The manner in which the humic acids affect the growth of marine diatoms could be as follows: On one hand humic acids can act as nutrients enhancing the carbon content of the water. Due to their complexing chemistry they can act as metal-HA complexes and influence the bioavailability of metal ions in the marine ecosystem, which facilitates the uptake of metal ions by the microalgae (Lund 1990). Humic acids can, however, prevent the growth of diatoms by deactivating toxic oxygen species and can negatively affect the light regime in the water by increasing turbidity. Thus, little is known about how the humic acids affect the growth of benthic diatoms. Therefore the second hypothesis that humic acids positively affect the abundance of *P. sulcata* due to the absorption of light and increasing nutrient supply was tested in an experimental set-up.

The addition of humic acids to the growth media resulted in a significantly higher growth of *P. sulcata* in these treatments, although the nutrient concentration in these media was lower compared to that of the full nutrient medium. The humic substances can inhibit the growth of phytoplankton (dinoflagellates) at higher concentrations (over 0.035 g l^{-1}) due to the increased amount of yellow substances absorbing the light in coastal waters (Prakash & Rashid 1968). The higher cell abundances of *P. sulcata* in the treatments with humic acid addition observed in our growth experiments indicate an adaptation to low light conditions. This was confirmed by the long-term data analysis which exhibited a negative correlation with high light intensities (Gebühr et al. 2009). In contrast to our results with the good growth of *P. sulcata* on different humic acid concentration were the results from a study of Prakash et al. (1973). The authors used pelagic diatom species (*Skeletonema costatum*, *Thalassiosira nordenskioeldii* and *Phaeodactylum tricornutum*) and showed the best growth at concentrations of 0.03g l^{-1} humic substances. This was about 10 times lower than our concentrations (low HA = 0.1 g l^{-1} and high HA = 0.3 g l^{-1}). Testing the effect of different concentrations of humic acids on the growth of *S. costatum* resulted in findings similar to ours, with an increase in the growth at higher concentrations (0.003 g l^{-1} to 0.018 g l^{-1}) compared to the f/2 medium (Prakash et al. 1973). In contrast to this, *P. sulcata* was not inhibited in the growth by higher concentrations of humic substances. Taking into account that *P. sulcata* as benthic species is highly adapted to

live on the sediment at higher humic acid concentrations and therefore the good growth can be explained with a wide tolerance to different humic acid concentrations. Interestingly, low molecular weight humic acids influenced the growth of dinoflagellates in a positive manner, whereas high molecular weight humic acids could inhibit the growth (Prakash et al. 1973). A detailed characterisation of the humic acids extracted from the sediment at Helgoland will be carried out in cooperation with Marcela Martin (National University of La Plata, Argentina). One important characteristic feature is the absorption ratio of humic acids at special wavelengths. The absorption at 465 nm divided by that at 665 nm (E_4/E_6) is used to characterise the dissolved organic matter and to compare humic acids of different origin. The absorption at higher wavelengths (E_6) is indicative of the presence of larger molecules. Higher values of the E_4/E_6 are obtained for fractions with low molecular weights (negative correlation). Generally, the E_4/E_6 ratio is expected to decrease with increasing molecular weight and content of aromatic rings (Lguirati et al. 2005). The E_4/E_6 ratio for the sediment extract with a high content of humic acids was 7.82 indicating that the humic acids from the sediment extract is mainly composed of low molecular weight molecules. Thus, the low molecular weight humic acids seemed to be a good explanation for the good growth of *P. sulcata* on the sediment at Helgoland Roads.

However, the ecological significance of humic acids in coastal waters is not yet fully understood. Humic acids can enhance the growth due to stimulation processes within the phytoplankton cells and their chelating ability for e.g. iron (metal-complexing capacity) could influence the growth in a positive manner (Prakash & Rashid 1968). Thus, further investigations are necessary to better understand the positive influence of humic acids on the growth on benthic diatoms including *P. sulcata*.

Additionally, the influence of light was examined during the field sampling campaign by measuring the Secchi depth (an indication for water transparency) and the sunshine duration. Our study showed that higher abundances of *P. sulcata* in the bottom and surface water samples were negatively correlated with light (Secchi depth and sunshine duration) indicating the adaptation to low light conditions. This coincided with the results from a study by Hobson & McQuoid (1997) who observed a higher correlation with nutrients and short day lengths. Bernardez et al. (2008) showed higher abundances of *P. sulcata* in February correlated with reduced light conditions and

water mixing. These results showed in a very clear way the adaptation of *P. sulcata* in its marine environment independently of the water depth.

Influence of storm activity: comparison between field observation and the literature

McQuoid & Hobson (1998) described that *Paralia sulcata* can easily be sloughed off the sediment during storm activities, leading to a re-dispersal of the population in the water column. Tidal mixing is another mechanisms for the transport of cells into the plankton (Oh & Koh 1995). As a predominantly benthic species (Sancetta 1989), the presence of *P. sulcata* in the phytoplankton is dependent upon some form of vertical transport. Furthermore, Casas et al. (1999) found *P. sulcata* in the water column throughout the whole year with higher occurrence in winter due to the resuspension into the water column.

The hydrography at Helgoland Roads is highly variable in the winter and late summer periods with strong mixing (Wiltshire et al. 2008, Wiltshire et al. 2010) which would positively influence the *P. sulcata* population in the water column especially near the sediment. Over the two years observation period a positive correlation of the abundance of *P. sulcata* in the bottom water sample with the mean and maximal wind speed was detected. Our results showed a generally higher abundance in the bottom water sample throughout the year. Therefore, the sediment provided more or less a "stock" of *P. sulcata* cells which can be resuspended in the water column due to storm activity. Due to the wind and therefore a mixing of the water column, *P. sulcata* seemed to be dispersed into the water column from the sediment. The analysis of the wind data showed an increase in the mean and maximal wind speed in autumn and winter times (Fig. 8). Furthermore, it has been reported that Helgoland Roads are influenced by the oceanic waters from the Atlantic with higher salinities and high winter temperatures due to the warm Gulf Stream (Stockmann et al. 2010, Wiltshire et al. 2010), leading to a mixing of the water column as shown by our analysis. Thus, as shown by McQuoid & Nordberg (2003b) along the Swedish west coast, the abundance of *P. sulcata* at Helgoland Roads is influenced in a comparable manner: the strong wind activities especially in late summer and winter lead to water mixing and to higher winter temperatures due to the Gulf Stream. This resulted in higher upwelling of

nutrients during the year, leading to higher occurrence of *P. sulcata* throughout the year at Helgoland Roads.

Implications for the ecological niche of *Paralia sulcata* at Helgoland Roads

The results of the niche analysis by Gebühr et al. (2009) illustrated a changing ecological niche of *Paralia sulcata* over the last 38 years from a more specialised niche in the 1980s to a generalised niche since the middle of the 1990s. The main factors influencing this niche were temperature and light regime in the North Sea. Temperatures at Helgoland Roads increased by about 1.67°C over the last 40-50 years (Wiltshire et al. 2008, Wiltshire et al. 2010). The canonical correspondence analysis applied by Gebühr et al. (2009) showed that *P. sulcata* is highly adapted to low temperatures and light conditions. However, higher abundances were detected during the summer months, which is in contrast with the increasing warming trend in the North Sea. Therefore, not the absolute temperature, but rather the temperature range is important for the determination of the ecological niche of *P. sulcata*. This study showed clearly that the tolerance range of temperature is important for the abundance of *P. sulcata* in the North Sea. It could be possible that warmer winter and summer temperatures lead to the optimal growth temperatures of *P. sulcata* at Helgoland Roads.

Furthermore, the ecological niche of *P. sulcata* is positively influenced by high concentrations of silicate and phosphate (Gebühr et al. 2009). This coincides with the results found in this study, where higher nutrient concentrations enhanced the growth of *P. sulcata* in the laboratory experiments as well as for the observations of the positive correlations of high abundances and nutrient concentrations in the field, especially in spring. The long-term data set, however, showed a significantly decreasing trend for the phosphate concentrations in the North Sea and it was shown that phosphate concentrations seemed to reach limiting concentrations rapidly (Wiltshire et al. 2008, Gebühr et al. 2009). The effects of the silicate and phosphate concentrations as well as light conditions (Secchi depth and sunshine duration) reflected the same influences on the ecological niche of *P. sulcata* within the two year investigation in comparison with the long-term analysis (Gebühr et al. 2009).

Two observed niche ranges for *P. sulcata* within the water column (bottom and surface waters) were detected at Helgoland Roads in the North Sea. The broad niche of *P. sulcata* in the bottom water sample in spring could be explained by the strong influence of salinity, Secchi depth and high concentrations of dissolved inorganic nutrients showing an adaptation of *P. sulcata* to low light conditions and high nutrient supply in the bottom water. The species tolerance of *P. sulcata* for the present environmental parameters from summer to winter was more or less the same with a tendency to a more generalised niche. It is important to notice that the niches from summer to winter were affected only by the Secchi depth. The fact that only a few environmental parameters affected the ecological niche might be an explanation for the generalised niche of *P. sulcata* with a good adaptation to its marine habitat. In contrast to the bottom water niche, the ecological niche in the surface water in spring was different, showing a really narrow species tolerance which could not be explained with the measured parameters, as none of them influenced the niche. One explanation for this narrow species tolerance may be the development of the spring bloom and therefore the intensive biotic interactions (competition) along with the grazing of the micro- and mesozooplankton on the phytoplankton as a whole (own data and e.g. Smetacek 1981, Sommer et al. 1986, Sommer 1996, Löder 2010b). The changing species tolerance and niche position of *P. sulcata* in summer times could be accompanied by the decrease of the phytoplankton spring bloom species. Another aspect could be the negative influence of the temperature and the positive influence of high nitrogen concentrations on the abundance of *P. sulcata*, which means that with lower summer temperatures and higher nitrogen concentrations the abundance was increasing. This fact was supported by the growth experiments displaying a good growth at 10°C and 16°C even when silicate availability is limited.

To summarise, the results from the laboratory experiment showed that the autecological study on *P. sulcata* revealed an optimal growth at higher temperatures (ranging from 10 to 16°C), especially when silicate concentrations were not limiting. No growth occurred at 4°C. This is in contrast to the temperature range in field observations where highest abundances of *P. sulcata* were found in autumn and winter, demonstrating that they can tolerate lower temperatures very well. *P. sulcata* as a benthic-pelagic species is well adapted to survive on the sediment, a trait which is supported by the adaptation to the low light conditions and higher nutrient availability (provided e.g. by the humic acids). This is in agreement with observations from both

the laboratory and the field. Furthermore, the seasonal observations at Helgoland Roads indicated a wide range of different environmental parameters that can be tolerated and therefore also possible changes in the marine environment.

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CHAPTER III

Genetic diversity of *Paralia sulcata* (Bacillariophyta) analysed by Inter Simple Sequence Repeats (ISSRs)

Genetic diversity of *Paralia sulcata* (Bacillariophyta) analysed by Inter Simple Sequence Repeats (ISSRs)

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Running head: Genetic diversity of *Paralia sulcata*

ABSTRACT

Paralia sulcata is a centric, marine diatom and is abundant in the water column at Helgoland Roads (North Sea, Germany) mainly in the winter months. In recent years we observed a trend towards a less seasonal appearance of *P. sulcata* and not only restricted to low water temperatures. We hypothesised that this change in the ecological niche might be reflected in a genetically different population of *P. sulcata*. Therefore, we evaluated the genetic diversity of *P. sulcata* at Helgoland Roads based on strains isolated over one year using an inter simple sequence repeat (ISSR) fingerprinting method. Surprisingly, the ISSR approach unveiled large intraspecific diversity. On the other hand analysis of similarity (ANOSIM) and non-metric multidimensional scaling (nMDS) based on Jaccard's similarities of combined ISSR patterns revealed well separated *P. sulcata* populations. Strains isolated in January were clearly different to those isolated in July, September, October and December. Significant correlations of the ISSR pattern with environmental parameters were detected. Specifically, the phosphate concentration, Secchi depth and temperature were shown to structure the *P. sulcata* population at Helgoland Roads. Furthermore, the high genetic diversity indicates that *P. sulcata* is well adapted to its frequently changing environment suggesting a more general ecological niche within its marine habitat.

Key words: 18S rDNA, ecological niche, Helgoland Roads, inter simple sequence repeat (ISSR), long-term data, marine diatoms, *Paralia sulcata*

INTRODUCTION

Intraspecific diversity in terrestrial plant communities has been studied for a long time. It is supposed that the success of ecological communities is partly dependent on the genetic diversity of their populations which enables the adaptation of the species to a dynamic environment (Whitlock et al. 2007). Thus, the physiological response of species combined with environmental parameters is strongly dependent on the genetic diversity of the population and enables a high adaptation rate to new ecological species niches. In this context fitness can be considered as a product of genotype and environmental interactions (Aarssen 1989, Whitlock et al. 2007). To investigate the community structure of species and the genetic diversity within plant populations molecular markers are an important tool (Serra et al. 2007).

Phytoplankton, in particular the diatoms, are at the basis of the marine food web. The identification and monitoring of the phytoplankton species is currently mostly based on microscopic investigations of taxonomic characteristics. Due to optical limitations the differentiation of single species is sometimes difficult and also time intensive (Evans et al. 2007). Moreover, morphological features can often be very similar and thus, differentiation next to impossible. Genetic markers can provide a fast and specific means for identifying differences in the genetic composition and species dynamics of phytoplankton both on a spatial and a temporal scale (Medlin et al. 1988, Medlin 1990). Different molecular techniques, such as the analysis of ribosomal or functional genes and other molecular markers as well as random amplified polymorphic DNA (RAPD), simple sequence repeats (microsatellites, SSR) or amplified fragment length polymorphisms (AFLP), are increasingly important in investigations on intraspecific interactions in plant communities and also marine phytoplankton (Bornet & Branchard 2001, Serra et al. 2007). However, for the use of these molecular markers basic knowledge of specific genome sequences is necessary and the costs can be higher for developing initial primers (Zietkiewicz et al. 1994, Jarne & Lagoda 1996, McGregor et al. 2000, Serra et al. 2007). An advantage of microsatellite (SSR) markers is the fine-scale recognition of species on population levels allowing detection of intraspecies variations on both temporal and spatial scales (Evans et al. 2005, Alverson 2008). Rynearson & Armbrust (2004) evaluated the high genetic diversity of *Ditylum brightwellii* populations using microsatellites. The authors identified three distinct *D. brightwellii* populations related to different sampling locations.

Over the past decades the use of inter simple sequence repeats (ISSRs) as molecular markers is becoming more common. Within genomes ubiquitously distributed short tandem repeated sequence pattern exist (Zietkiewicz et al. 1994). Furthermore, each band amplified with ISSR-PCR corresponds to a DNA sequence with an individual fragment length and is enclosed by two inverted ISSR which leads to highly polymorphic band patterns (Bornet & Branchard 2001). In addition, ISSR markers require no genomic information, are very abundant and easy to use (Zietkiewicz et al. 1994, McGregor et al. 2000, Bornet & Branchard 2001, Bornet et al. 2004).

ISSR-PCR are well established in the identification of plant populations or plant cultivars via to differences in the mean number of fragments and polymorphic bands (Barth et al. 2002, Galvan et al. 2003, Serra et al. 2007) as well as for genetic characterisation and diversity studies on phytoplankton species (Bornet & Branchard 2001, Bornet et al. 2004). Bornet et al. (2004) showed that comparisons of ISSR pattern of all their tested species were discriminated between toxic and nontoxic species of *Alexandrium* and *Pseudo-nitzschia* as well on a local scale.

In this study we use ISSR fingerprints for investigation of intraspecific diversity of *Paralia sulcata* (Ehrenberg; Cleve, 1873). *P. sulcata* is a tychopelagic, centric marine diatom species (McQuoid & Nordberg 2003a) occurring in the water column at Helgoland Roads over the whole year (Wiltshire & Dürselen 2004, Gebühr et al. 2009). Figure 1 shows the abundance data of *P. sulcata* from January 2007 to January 2008 with maximal cell numbers in winter (over 2000 cells ml^{-1}) and lower numbers in summer. This finding is in contrast to the literature where *P. sulcata* is mostly described as a winter algae (Hobson & McQuoid 1997). The multivariate statistical analysis on the long-term data set at Helgoland Roads exhibited that the changing environmental conditions lead to a shift in the ecological occurrence of *P. sulcata* from a winter diatom to a diatom occurring throughout the whole year (Gebühr et al. 2009) accompanied by niche shifts. Furthermore, the genetic diversity might either play a role in the general fitness and adaptation of the *P. sulcata* population or it could indicate species shifts. We assume that this observed shift in the ecological niche could be reflected in the genetic diversity of *P. sulcata*. To determine the genetic diversity a sampling campaign with the isolation of *P. sulcata* from the water column at different times of the year was started in 2007 at Helgoland Roads and *P. sulcata* cultures were analysed using 18S rDNA sequence and ISSR fingerprint method.

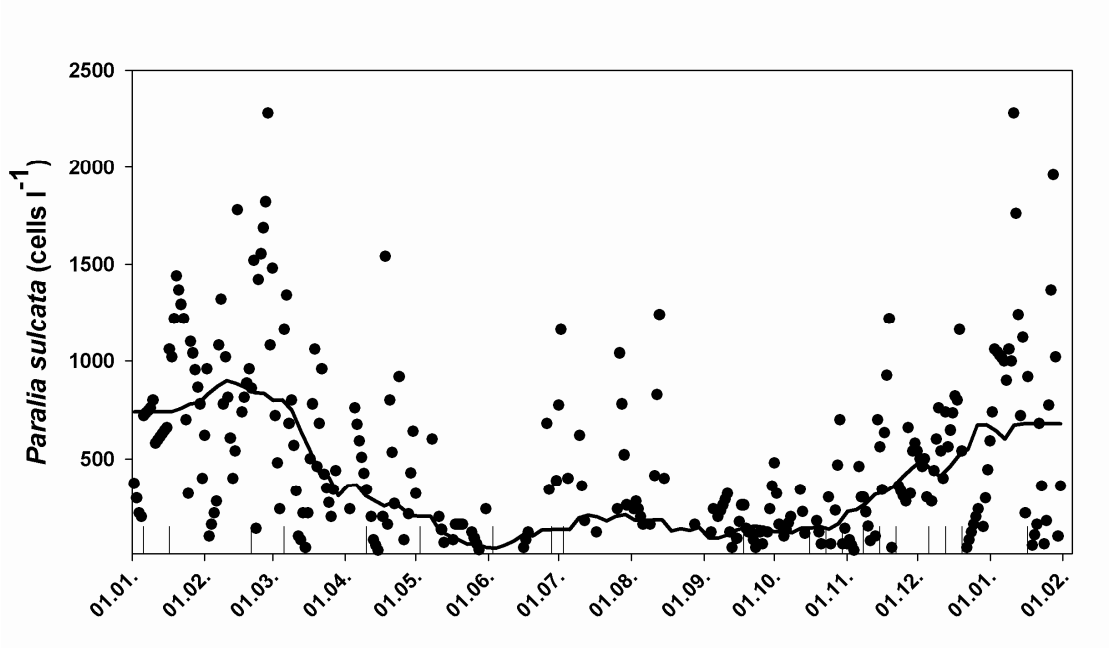

Figure 1: Abundance data of *Paralia sulcata* (cells l^{-1}) from January 2007 to January 2008 based on the long-term data set at Helgoland Roads, North Sea. The dots represent the abundance data, the running average are shown by the black line and the bars indicate the sampling dates of the water samples to isolate *P. sulcata*.

METHODS

Study site and sampling

Water samples were taken at Helgoland Roads (54°11.3'N; 7°54.0'E), German Bight. All *Paralia sulcata* strains were isolated from the surface water or from water sampled 1 m above the sea bottom between January 2007 and January 2008. Surface water samples were taken using 80 µm and 20 µm plankton nets. Water from 1 m above the sea bottom was taken with a 5 l Niskin bottle (HydroBios, Kiel, Germany) (named bottom water here). The environmental parameters temperature, Secchi, salinity, and concentrations of inorganic nutrients (nitrate, nitrite, ammonia, phosphate and silicate) were measured in parallel to the genetic samples. The abundance data of *P. sulcata* (Fig. 1) are from the daily sampling phytoplankton counting of the long-term data set at Helgoland Roads (Wiltshire & Dürselen 2004) at the same time.

Cultivation of *Paralia sulcata*

Single *Paralia sulcata* chains were isolated by micropipetting using a dissecting microscope and transferred into 6-well-plates containing f/2 medium (Guillard & Ryther 1962, Guillard 1975). After two weeks, growth was checked, *P. sulcata* chains were washed in f/2 medium and transferred into culture flasks with f/2 medium (73.5 ml) for further cultivation. All *P. sulcata* cultures were grown under controlled conditions at 12:12 hours light:dark photoperiods and 14 - 15 °C. *P. sulcata* cells were transferred into fresh medium every four weeks, when the cultures reached the stationary phase. Finally, all *P. sulcata* cultures were harvested on 2.0 µm filters (TTTP Isopore Membrane Filters, Millipore, Schwalbach, Germany) at the end of the exponential phase and stored at -20 °C until DNA extraction. At least 36 different *P. sulcata* strains were used for the molecular study (Table 1).

Table 1: List of the 36 *Paralia sulcata* clones isolated at Helgoland Roads from January 2007 till January 2008. 22 Strains labelled with an asterisk * were used for the statistical analysis with the performed composition pattern of all five ISSR primers using the software PRIMER. 26 *P. sulcata* strains labelled with # were used to perform the phylogenetic tree of the 18S rRNA based on the ARB similarity matrix (Fig. 2).

Sample No.	Isolation date	Origin	Water column
1*	05.01.2007	Helgoland Roads	surface
2*	05.01.2007	Helgoland Roads	surface
3* #	05.01.2007	Helgoland Roads	surface
4*	05.01.2007	Helgoland Roads	surface
5* #	05.01.2007	Helgoland Roads	surface
6* #	16.01.2007	Helgoland Roads	surface
7* #	16.01.2007	Helgoland Roads	surface
8* #	20.02.2007	Helgoland Roads	surface
9* #	06.03.2007	Helgoland Roads	surface
10 #	06.03.2007	Helgoland Roads	surface
11 #	10.04.2007	Helgoland Roads	surface
12 #	03.05.2007	Helgoland Roads	surface
13 #	03.06.2007	Helgoland Roads	surface
14 #	28.06.2007	Helgoland Roads	surface
15 #	28.06.2007	Helgoland Roads	surface
16* #	03.07.2007	Helgoland Roads	surface
17* #	03.07.2007	Helgoland Roads	surface
18* #	18.09.2007	Helgoland Roads	surface
19* #	23.10.2007	Helgoland Roads	surface
20*	23.10.2007	Helgoland Roads	surface
21* #	16.10.2007	Helgoland Roads	surface
22* #	30.10.2007	Helgoland Roads	surface
23* #	08.11.2007	Helgoland Roads	surface
24	15.11.2007	Helgoland Roads	surface
25	22.11.2007	Helgoland Roads	surface
26	22.11.2007	Helgoland Roads	surface
27 #	22.11.2007	Helgoland Roads	surface
28	22.11.2007	Helgoland Roads	surface
29	22.11.2007	Helgoland Roads	bottom
30 #	22.11.2007	Helgoland Roads	bottom
31 #	06.12.2007	Helgoland Roads	surface
32*	13.12.2007	Helgoland Roads	surface
33* #	13.12.2007	Helgoland Roads	surface
34* #	13.12.2007	Helgoland Roads	bottom
35* #	20.12.2007	Helgoland Roads	surface
36* #	17.01.2008	Helgoland Roads	surface

DNA extraction

DNA extraction was carried out following the method of Fawley & Fawley (2004). 500 µl Extraction buffer (70 mM Tris, 1 M NaCl, 30 mM Na_2EDTA [pH 8.6]), 2 spatula points with sterile silica beads (same volume of 1 mm and 0.1 mm silica beads) (BioSpec Products, Bartlesville, USA), 65 µl 10% DTAB (Sigma, Hamburg, Germany) and 500 µl chloroform (same volume as extraction buffer) (Merck, Darmstadt, Germany) were added to the filters and the cells were disrupted for 60 seconds using a mixing device. After cell disruption the homogenate was centrifuged for 5 minutes at 2000 x g. The supernatant was transferred to a new tube and the DNA was extracted again using phenol-chloroform-isoamylalcohol (25:24:1). Many samples had to be extracted twice due to the high protein content in the samples. After isopropanol precipitation, the DNA was washed in ethanol (75%), resuspended in sterile Millipore water (30 µl) and stored at -20°C until further analysis.

Previous to the PCR amplification the DNA extracts were analysed by agarose gel electrophoresis (0.7% agarose, 80 V, 50 min) in 0.5 TBE buffer (10 x stock solution: 900 mM Tris, 900 mM boric acid, 20 mM EDTA [pH 8]). After electrophoresis, gels were stained with ethidium bromide (0.5 mg l^{-1}) and images were captured with a ChemiDoc XRS System (BioRad, München, Germany). DNA concentration was measured photometrically using a nanoquant plate (Tecan, Grödig, Austria).

18S rRNA amplification

Two eukaryotic primers, EUK1F and EUK1528R (Thermo Fisher Scientific, Ulm, Germany), were used to amplify the 18S rRNA gene using 36 DNA-extracts from *Paralia sulcata* (Table 2) (Medlin et al. 1988). The PCR mixtures with a volume of 100 µl contained 15 µl Master Enhancer (Eppendorf, Hamburg, Germany), 10 µl 10 x Taq buffer (Eppendorf), 500 µM dNTPs (Promega, Mannheim, Germany), 0.6 µM of each primer, 2 U Taq DNA Polymerase (Eppendorf) and 60 ng DNA. The PCR reaction was carried out as described by Medlin et al. (1988) with the following modification: amplification started with a denaturing step at 94°C for 5 min followed by 35 cycles at 94°C for 2 min, 54°C for 1 min, 68°C for 2 min, and followed by 68°C for 10 min for the final extension (Eppendorf Mastercycler). 2 µl of each PCR product and a 1 kb DNA Ladder (GibCO BRL, Eggenstein, Germany) were visualized on 1.4%

agarose gels. Digital images were taken for documentation (ChemiDoc XRS System, BioRad).

After 18S rRNA gene amplification the products were purified using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) following the manual instruction. The purified 18S rRNA sequence fragments of all samples (fragment length approximately 1500 bp) were sequenced using the primers of the 18S rRNA gene and additionally a third eukaryotic primer (1055R) (Table 2).

Table 2: Primers and sequence of the primers used for the sequencing of the 18S rRNA gene of *Paralia sulcata*.

Primer	Sequence	18Sr RNA position	Reference
EUK1F	5'AACCTGGTTGATCCTGCCAGT3'	1	Medlin et al. (1988)
EUK1528R	5'TGATCCTTCTGCAGGTTCACCTAC3'	1800	Medlin et al. (1988)
1055R	5'ACGGCCATGCACCACCACCCAT3'	1200	Elwood et al. (1985)

In order to determine the closest relatives of *P. sulcata*, the 18S rRNA gene sequences were compared to sequences from the GenBank database using BLAST (Basic Local Alignment Search Tool, http://www.ncbi.nlm.nih.gov). The sequences were phylogenetically analysed using ARB software package (http://www.arb-home.de) (Ludwig et al. 2004). After the addition of the 18 S rRNA sequence data to the ARB 18S rRNA gene sequence database, the sequence alignment was carried out with the FastAligner integrated in the program. Additionally, the results of the alignment were compared with the sequences of the closest relatives of *P. sulcata* retrieved by BLAST. Sequences with approximately 1500 bp were used to calculate a phylogenetic tree. Partial sequences were added using the ARB parsimony-interactive tool. The phylogenetic relationship was deduced by the neighbour-joining method with the correction algorithm of Felsenstein (1993). Similarity matrices were calculated via neighbour-joining based on the phylogenetic distances of the *P. sulcata* strains using the correction 'similarity'.

ISSR amplification

Five ISSR primers (Thermo Fisher Scientific) without anchored ends were tested with DNA from 36 *Paralia sulcata* strains using specific annealing temperatures (Table 3). ISSR-PCR amplifications were done as described by Bornet & Branchard (2001) with the following modifications: PCR reaction mixtures with a volume of 25 µl contained 3.75 µl Master Enhancer (Eppendorf), 2.5 µl of 10 x Taq buffer (Eppendorf), 600 µM dNTPs (Promega), 0.8 µM of each primer, 1 U Taq DNA Polymerase (Eppendorf) and 15 ng DNA. The PCR amplification started with a denaturing step at 95°C for 6 min followed by 32 cycles at 95°C for 1 min (denaturation), specific annealing temperature for each primer for 1 min, 68 °C for 2 min (extension), and finished off with 68°C for 10 min for the final extension (Bornet et al. 2004). The annealing temperatures were adjusted for each of the five primers (Table 3) and ISSR-PCR was performed in triplicate (Eppendorf Mastercycler). Amplification products were separated on 2% agarose gels (170 min, 80 V). Ten or alternatively five µl of each PCR product was applied on the gel and a 100 bp DNA Ladder (GibcoBRL) was used as a standard to estimate the molecular size of the fragments. PCR products were visualised by ethidium bromide staining (0.5 mg l^{-1} for 5 min) and were documented (ChemiDoc XRS System, BioRad).

Table 3: List of the five ISSR primers, their annealing temperature (T_A), size range of amplified fragments and total number of fragments according to Bornet et al. (2001) and Bornet et al. (2004) (labeled with an asterisk *) and our results of the five ISSR primers used for the population diversity of 36 *Paralia sulcata* strains with size range of amplified fragments, total number of amplified fragments with the minimal and maximal range, the % of reproducibility of the strains for each ISSR primer within the replicates and PCR success in the *P. sulcata* strains.

ISSR primer	TA (°C)*	Size range of amplified fragments (bp)*	Total number of fragments*	Size range of amplified fragments (bp)	Total number of amplified fragments	Range of bands amplified	Reproducibility (%) within the replicates	PCR success in the *P. sulcata* strains
(ATG)5	45	100-2550	44	350 - 2000	45	3 - 27	82.9	36
$(CAA)_5$	52	180-2500	37	200 - 2550	50	5 - 27	88.4	24
$(CAG)_5$	60	100-2300	43	200 - 2300	61	2 - 20	83.0	30
$(CCA)_5$	57	200-2200	39	200 - 2700	60	3 - 31	84.8	36
(GACA)4	52	175-1850	37	100 - 2350	56	5 - 21	86.8	24

Data analysis

Comparative analysis of ISSR fingerprints was carried out with BioNumerics 5.0 (Applied Maths, Belgium). Clear bands were scored on the digitized gel images and a binary system was used to distinguish between absence ('0') and presence ('1') of a band. Normalisation of gels was carried out using the 100 bp Ladder as a reference in every profile using BioNumerics 5.0 (Applied Maths, Belgium). From the three replicates of each ISSR primer a consensus pattern was generated. Additionally, from the five consensus patterns a composite pattern (combined band-matching pattern) of all five ISSR primers was generated and only those 22 *Paralia sulcata* strains successfully showing all five primers in the ISSR-PCR were taken into account (22 strains, labelled with an asterisk in Table 1). The resulting band-matching table was imported into the software PRIMER 6.1.6 (PRIMER-E, Ltd., UK) and used to calculate Jaccard's similarities. Ordination of Jaccard's similarities was performed by non-metric MDS to visualise the genetic relationship of *P. sulcata* strains (Clarke & Warwick 2001). For clearness, the plots are presented two-dimensionally although three-dimensional plots usually displayed lower stress levels. Lower stress levels displays a better fit of the reproduced similarity matrix to the observed similarity matrix and therefore no random distribution of the data.

Hierarchical agglomerative clustering of Jaccard's similarities with group average was performed using the complete linkage method of the PRIMER 6.1.6. The similarity profile (SIMPROF) test described the similarities of the Jaccard's similarities in a group of unstructured samples and this generated profile was compared with the expected profile under the null hypothesis. The null hypothesis exhibited that no structure within the samples exists and the samples are not separated into groups (Clarke & Warwick 2001, Clarke et al. 2008). Thus, the SIMPROF test was performed to specific groups which can be identified with 1000 permutations to calculate the mean similarity profile of the Jaccard's similarities of the composition pattern of all ISSR patterns (biological data) with 999 permutations to generate the null distribution. The significance level was taken as 5% ($p < 0.05$) which was used to separate groups within these biological data and designated as a new factor, *"simgroups"*.

The PRIMER 6.1.6 subroutine ANOSIM (Analysis of Similarities) was used for estimating the degree of separation between groups of samples categorised by the factors *"month"* and *"simgroups"*. ANOSIM is a non-parametric technique designed to allow statistical comparisons for multivariate data sets in a manner similar to

univariate techniques (ANOVA) (Clarke & Warwick 2001). Complete separation is indicated by R = 1, whereas R = 0 suggests no separation among the groups with a significance level of p = 0.1% ($p < 0.001$).

To test whether the environmental parameters temperature [°C] (Temp), Secchi depth [m] (Sec), salinity (Sal), silicate concentration [µmol l^{-1}] (SiO_2), phosphate concentration [µmol l^{-1}] (PO_4), and total dissolved inorganic nitrogen concentration [µmol l^{-1}] (DIN) structure the *P. sulcata* populations, the PRIMER 6.1.6 subroutine BEST (Clarke & Warwick 2001, Clarke et al. 2008) was applied. For each sampling day, all data were normalised and Euclidean distances were calculated. The Jaccard's similarity matrix of the combined band-matching pattern of the ISSR and the Euclidean distance matrix of the environmental data were compared by using Spearman rank correlation and 999 permutations to identity the global BEST match rho ρ. The BVSTEP procedure selects the combination of environmental parameters which best explains the biotic data. The null hypothesis of the significance test implies that there is no relation between the similarity and the distance matrices indicating low rho values ($\rho = 0$). If there is a significant relation between the similarity and the distance matrices ρ will have values near one.

Additionally, an Analysis of Variance (ANOVA) with a Tukey's honestly significant difference (HSD) post-hoc test (STATISTICA 7.1, StatSoft Inc, USA) was performed to test for differences of the environmental parameters categorised by *"simgroups"* (SIMPROF).

RESULTS

18S rRNA gene analysis

In order to identify *Paralia sulcata* on the species level, sequence analyses of 18S rRNA of all strains was carried out. We successfully extracted sufficient DNA for sequencing from 26 selected *P. sulcata* strains isolated over one year (labelled with # in Table 1). All other strains were omitted from the analyses. Thus, in order to address the relationship among the strains a phylogenetic analysis based on the approximately 1500 bp long 18S rRNA gene was performed. According to Drebes (1974) and Hoppenrath et al. (2009) microscopic morphological characterisation identified our strains as *P. sulcata*. Based on the 18S rRNA gene, all 26 strains showed a similarity of over 99 % to a *P. sulcata* strain (EF 192995) using GenBank BLAST comparison

and were clearly identified as *P. sulcata* (Fig. 2). Moreover, based on the ARB similarity matrix data it was shown that all 26 *P. sulcata* strains from Helgoland are very closely related to each other. The phylogenetic ARB tree of the *P. sulcata* strains did not show clustered groups (Fig. 2) indicating a homogenous distribution of the population in the water column at Helgoland Roads throughout the year.

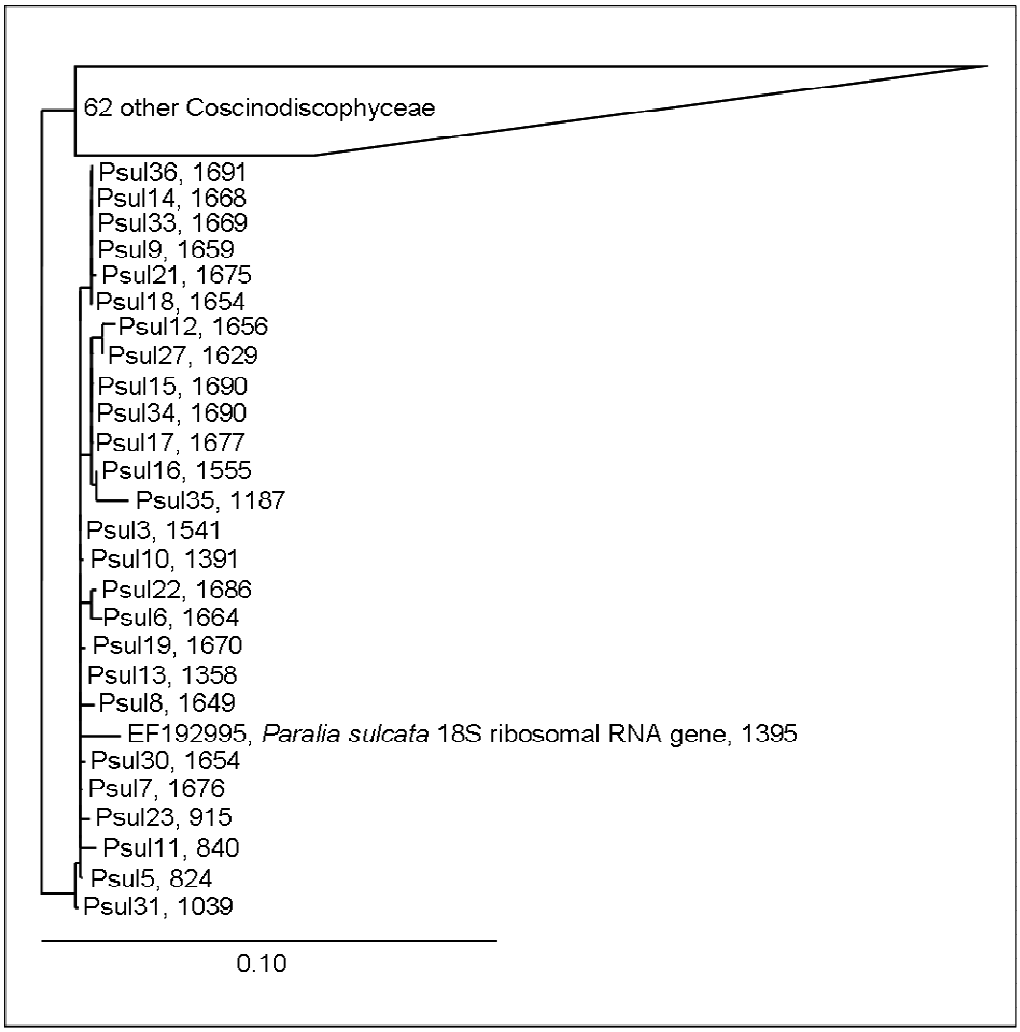

Figure 2: Phylogenetic tree of the 18S rRNA based on the ARB similarity matrix of 26 *Paralia sulcata* strains isolated at Helgoland Roads, North Sea.

ISSR amplification and genetic diversity of *Paralia sulcata*

ISSR analysis was used as a fingerprint method to differentiate a total of 36 *Paralia sulcata* strains. The results obtained for the five primers indicate different band pattern. Each ISSR primer showed clear band patterns between 100 and 2770 bp length for each of the 36 *P. sulcata* strains but the recovery success of each replicated ISSR primer was different (Table 3). A total of 272 bands of high intensity were selected as markers. All five ISSR markers were polymorphic for the analysed strains and displayed highly variable fragment patterns. Thus, 45 fragments were obtained overall with $(ATG)_5$ as a marker, 50 fragments with $(CAA)_5$, 61 fragments with $(CAG)_5$, 60 fragments with $(CCA)_5$ and 56 fragments with $(GACA)_4$ (Table 3). However, the results of the ISSR-PCR amplification were not reproducible within all 36 *P. sulcata* strains using the different primers due to fragment pattern on the agarose gels. Three of five primers exhibited no useable results for all 36 *P. sulcata* strains (($(CAA)_5$, $(CAG)_5$ and $(GACA)_4$).

PCR with two primers revealed the best results due to the visualisation on the agarose gels with all investigated *P. sulcata* strains: $(ATG)_5$ and $(CCA)_5$. The consensus pattern of the $(ATG)_5$ primer exhibited fragment pattern from 373 to 2017 bp varying from 3 to 27 fragments (Table 3). Whereas the consensus pattern of the $(CCA)_5$ primer showed a diverse pattern of fragment length (214 to 2767 bp) and ranged from 3 to 31 fragments (Table 3) in all 36 *P. sulcata* strains. The reproducibility for the three replicates of both ISSR primers displaying a mean of 82.9% for $(ATG)_5$ and 84.8% $(CCA)_5$ (Table 3) was generally high for all 36 *P. sulcata* strains. In contrast to the literature, not all primers were truly successful in the PCR for the replicated PCRs. Only two ISSR primers, $(ATG)_5$ and $(CCA)_5$, showed a PCR success within all 36 *P. sulcata* strains (100%). Thus, the PCR success within the *P. sulcata* strains of the other three ISSR primer was less with 83.3% for $(CAG)_5$ and 66.7% for $(CAA)_5$ and $(GACA)_4$ respectively (Table 3). Unfortunately, most of the spring and summer strains (March till June) and some samples from November of *P. sulcata* were not really PCR successful with these three ISSR primers. Consequently, further analyses and were performed with all five primers only 22 *P. sulcata* strains (labelled with an asterisk * in Table 1) were used where the success of the PCR for all five primers was 100%.

To analyse the ISSR fingerprints for the five ISSR primers the composition pattern of all five ISSR was generated and different statistical analyses were applied. At first, the Jaccard's similarities were calculated for the composition pattern of the five ISSR

primers with the 22 *P. sulcata* strains. It was apparent that the strains of the *P. sulcata* populations represented by the ISSR fingerprint method were separated in different degrees from each other. Thus, cluster analysis with the SIMPROF test created a new factor *"simgroups"* to test for a structure within the ISSR data and generated 4 *"simgroups"*.

The MDS analysis in combination with the SIMPROF test detected significant differences within the Jaccard's similarities of the *P. sulcata* strains between the factor *"simgroups"*. Moreover, the MDS plot based on the Jaccard's similarities of the composition pattern of the five ISSR primers of the *P. sulcata* strains revealed a well separated pattern between the strain isolated in September (strain no 18, *"simgroups"* group a); July (strains no 16 and 17) and October to December (strains no 19-23 and 32-35, *"simgroups"* group b); January (strains no 1-5, *"simgroups"* group c) and January (strains no 6, 7 and 36), February (strain no 8) and one strain from March (no 9, *"simgroups"* group d) (Fig. 3). As shown by the MDS, from the low stress level (0.18), the ordination was not randomly distributed but rather a clear separation of these data based on the Jaccard's similarities was displayed (Fig. 3). Thus, considering the MDS ordination and the cluster analysis (data not shown), the *P. sulcata* population showed clearly separated groups between the January isolates and the October to December strains. This was supported by the global R values using the ANOSIM analysis regarding the factors *"month"* and *"simgroups"* of the Jaccard's similarities (Table 4). The ANOSIM displayed significantly separated *P. sulcata* populations according to the ISSR patterns indicating high intraspecific diversity. Moreover, the Jaccard's similarities of the combined ISSR pattern and the factor *"simgroups"* displayed significant differences among the groups b + c ($R = 0.725$) and b + d ($R = 0.625$) suggesting a clear separation among these groups within the variability of the *P. sulcata* population (Fig. 3, Table 4).

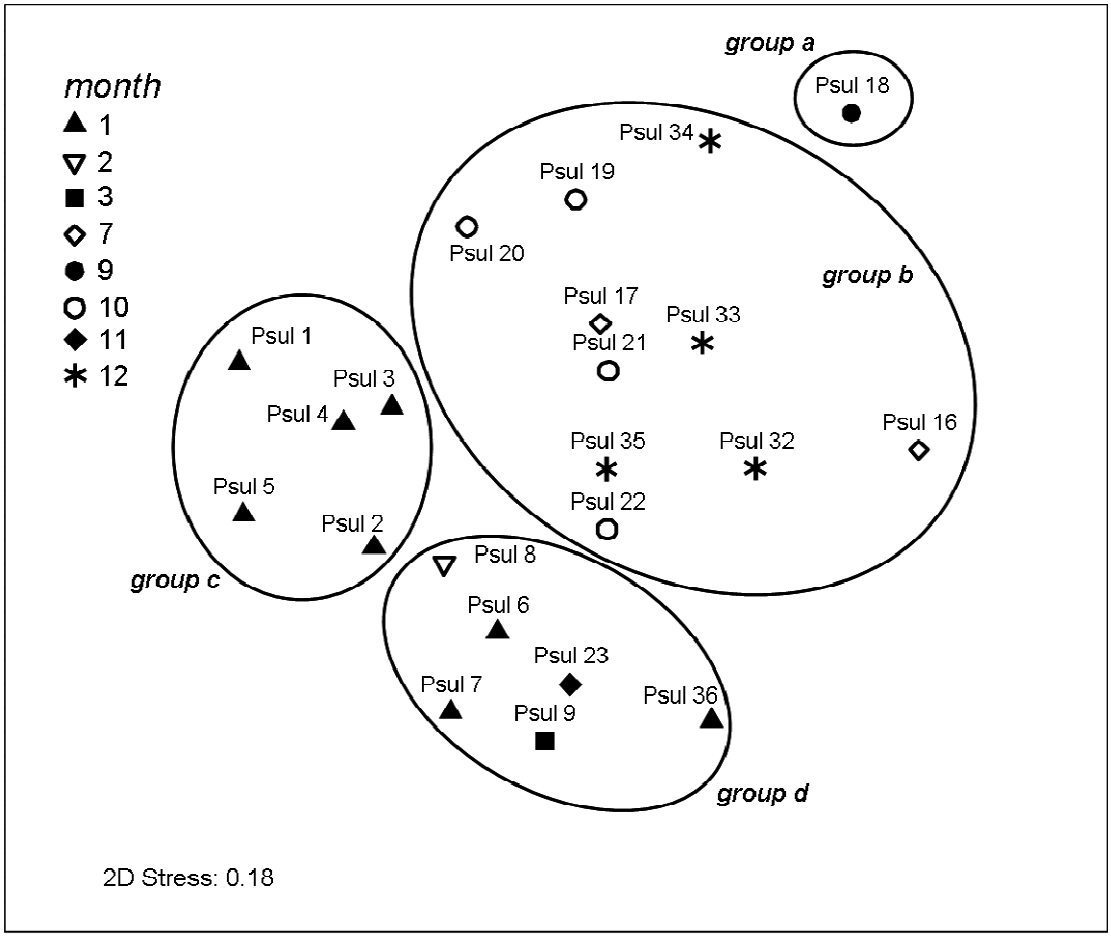

Figure 3: MDS 2-D plot based on the Jaccard's similarity of the combined band-matching pattern of five ISSR primers of 22 *Paralia sulcata* strains illustrating the genetic relationship of this diatom species over one year. The circles indicate significant separations of the *P. sulcata* strain based on the factor *"simgroups"* of the four groups generated by the similarity profile (SIMPROF) test ($p < 0.05$) suggesting no random distribution of these data.

When assigning the SIMPROF factors calculated on the basis of the ISSR patterns to the environmental parameters (Euclidean distance) again clearly separated groups were detected. High global R values of ANOSIM for the factors *"month"* ($R = 0.795$, $p = 0.1\%$; $p < 0.001$) and especially *"simgroups"* ($R = 0.98$, $p = 0.1\%$; $p < 0.001$) suggested a close biotic-abiotic linkage.

Table 4: ANOSIM (Analysis of similarity) statistics for the global R statistic based on the two factors *"month"* and *"simgroups"* and the pairwise SIMPROF test for the *"simgroups"* to compare the Jaccard's similarity (combined band-matching pattern of five ISSR primers within 22 *P. sulcata* strains) with 0 out of 999 permutations. Only significant values for the groups are shown, significance level p = 0.1 % (p < 0.001).

Factor	R statistic	p – value
"month"	0.551	0.1 %
"simgroups"	0.702	0.1 %
Pairwise SIMPROF test for "simgroups"		
b ↔ c	0.725	0.1 %
b ↔ d	0.625	0.1 %

To further analyse this possible linkage BEST analysis was performed. The BEST analysis selects environmental variables "best explaining" community patterns, by maximising a rank correlation between the respective resemblance matrices. The BEST rho value ($\rho = 0.45$) indicated a significant correlation (p = 0.1%; p < 0.001) of environmental parameters with the ISSR patterns of the *P. sulcata* strains. Specifically, the phosphate concentration, the Secchi depth and the temperature exhibited significant influence on the *P. sulcata* populations (p < 0.001). Furthermore, by analysis of variance (ANOVA) with Tukey's HSD post-hoc test, the environmental parameters were compared using SIMPROF classification (*"simgroups"*) of the biotic data. Using this classification as categories in ANOVA, the Tukey's HSD post hoc test revealed significant differences in phosphate concentration (p = 0.0011) and Secchi depth (p = 0.0087) between the groups b and c (shown as mean ± SE in Fig. 4). For all other environmental parameters no significant differences (DIN, SiO_2, Sal and Temp) among the groups were observed.

Summarising the results, the genetic diversity of *P. sulcata* strains were highly and mostly influenced by phosphate concentrations, Secchi depth and temperature. Additionally, the analysis with the ISSR primers indicated a high genetic variability and explained the clear separation of the *P. sulcata* strains isolated in January and October to December at Helgoland Roads, North Sea.

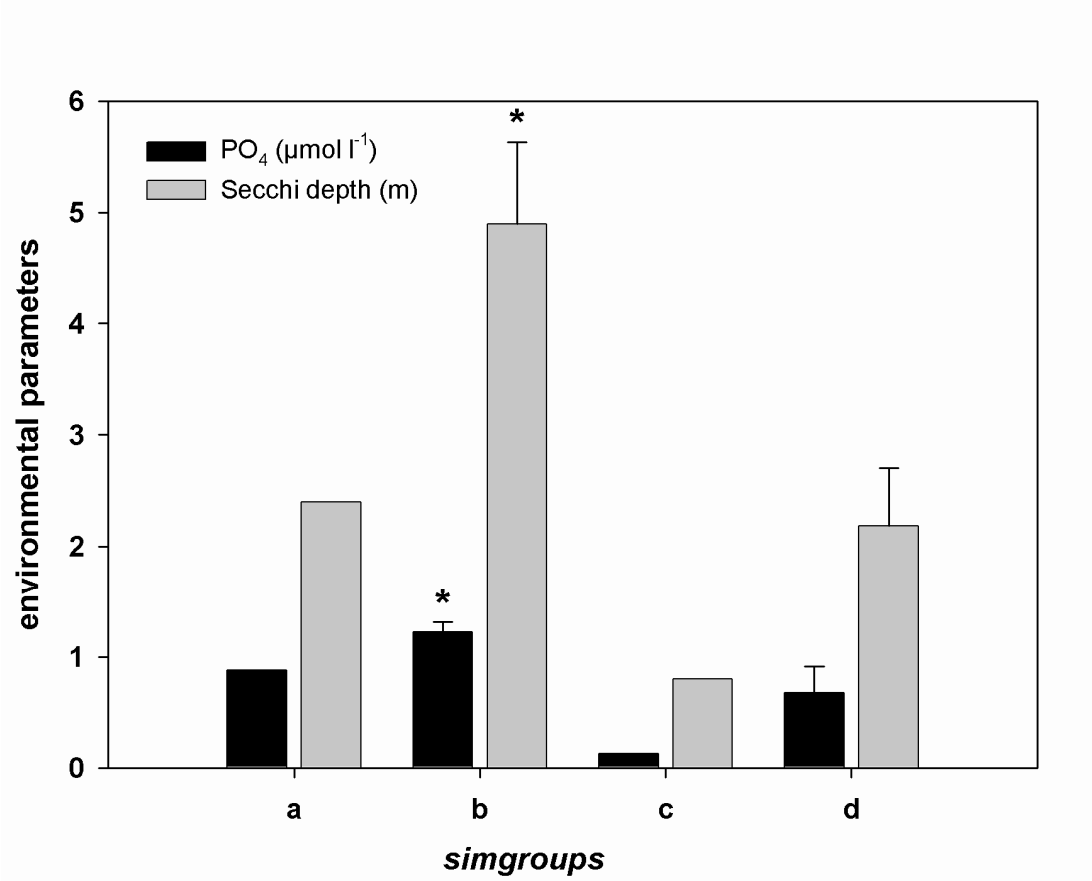

Figure 4: Mean and standard error (SE) of the significant phosphate concentration and Secchi depth extracted from the ANOVA (Analysis of variance) involving comparisons within the factor *"simgroups"* determined by similarity profile (SIMPROF) test (ANOVA, Tukey's HSD post-hoc test for different sample size, $p < 0.05$) of the Jaccard's similarity (combined band-matching pattern of five ISSR primers within 22 *P. sulcata* strains). Significant differences between the groups of each environmental parameter alone is labelled with an asterisk * significance level $p < 0.05$. Group a and group c shows no SE because the *P. sulcata* strains were isolated on only one day.

DISCUSSION

18S rRNA phylogeny and determination of *Paralia sulcata*

Traditionally diatoms have been identified and classified based on morphological characteristics (Round et al. 1990). However with the molecular techniques now also available it is important to establish the major clades of diatom phylogeny based on both morphological and molecular features (Evans et al. 2007, Alverson 2008). The 18S rDNA sequence is a highly conserved region and is used for basic reconstruction of diatom phylogeny. It provides insights into the evolution of diatoms and 18S rDNA sequences are available for most representatives of the diatoms (Medlin et al. 1996, Beszteri et al. 2001, Fox & Sorhannus 2003). Beszteri et al. (2001) characterised the phylogenetic structure within a clade of six diatoms belonging to the *Naviculaceae* in order to assess the monophyly using the 18S rDNA. Other studies focussed on intragenomic nucleotide variations among different *Skeletonema* species or the phylogenetic relationship among phytoplankton species which were distinguished by their 18S rDNA sequences (e.g.Sorhannus 2004, Alverson & Kolnick 2005).

In our study we used the 18S rRNA gene to determine the intraspecific variation of 26 selected *Paralia sulcata* strains over one year and to verify the phylogenetic relationship among the strains. Our analysis demonstrated that all strains displayed a high similarity (more than 99 %). After comparison with the reference sequence of the *P. sulcata* strain (EF 192995) (Rampen et al. 2007), all isolated strains were clearly identified as *P. sulcata*. Thus, all strains belonged to the same population of *P. sulcata* and showed a lower intraspecific variation regarding the 18S rRNA gene. This low differentiation indicated a homogenous distribution of the *P. sulcata* population in the water column at Helgoland Roads throughout the year.

ISSR amplification and genetic diversity of *Paralia sulcata*

In order to investigate and to infer the intraspecific phylogenetic relationship of phytoplankton species, molecular markers are an important tool. Thus, ISSR-PCR has been frequently used for differentiation of phytoplankton species within the last decade (Zietkiewicz et al. 1994, Bornet & Branchard 2001). In this study the ISSR fingerprinting method was used to examine the genetic diversity among 36 *P. sulcata* strains for the first time. The fragment patterns of all ISSR primers were highly variable and no primer has a similar fragment pattern within the *P. sulcata* strains.

Three ISSR primers were not successful for all replicates. The most applicable primers seem to be $(ATG)_5$ and $(CCA)_5$ due to the wide range in fragment length and amplified fragment numbers. Indeed the primer $(ATG)_5$ is described as the best primer for intraspecific interactions among phytoplankton species (Bornet et al. 2004). Interestingly, all primers showed a higher number of total amplified fragments as described in the literature (Bornet et al. 2004) (Table 3). This result indicates a high genetic diversity within the genome of *P. sulcata* probably due to more abundant simple sequence repeats (SSRs) within the genome and due to a highly variable fragment pattern within the ISSR primers.

Similar results were shown by Zhang et al. (2006) analysing *Carchesium polypinum* (Ciliophora) from diverse Chinese lakes. The authors found a high genetic diversity within this population but the genetic differentiation among the populations was low (Zhang et al. 2006). 34 ISSR primers were used and PCR with eight of these primers exhibited highly polymorphic band pattern such as the $(GACA)_4$ and the $(CAA)_5$ primer which were also used in our study. In contrast to our study, Zhang et al. (2006) showed a lower range in amplified fragment lengths (from 200 to 1400 bp) which could indicate less abundant SSRs in the genome of *C. polypinum* compared to a higher complexity within the *P. sulcata* genome as shown in this study. Thus, the genome size, the complexity of the genome and the distribution of the SSRs as binding sites for the ISSR primers are important factors to determine the genetic diversity on *P. sulcata*.

In a study on genetic diversity on *Arabidopsis thaliana* it has been reported that ISSR markers are not suitable in comparison with CAPS (cleaved amplified polymorphic sequence) due to the uneven distribution of ISSR markers within the genome (Barth et al. 2002). Zietkiewicz et al. (1994) pointed out that the genome of *A. thaliana* is smaller than most other culture plants and therefore reflected a low number of observed amplified ISSR fragments due to a lower genome complexity.

ISSR-PCR shows high reproducibility and efficiency for study the intragenetic variability in phytoplankton species (Nagaoka & Ogihara 1997, Galvan et al. 2003, Bornet et al. 2004). In our study the reproducibility of all five ISSR primers was between 83 to 88 %. This was less than the observed results from a previous study by Charters et al. (1996) which exhibited a high reproducibility (100 %) of the ISSR-PCR with the primers in *Brassica napus* and *B. rapa* cultivars. However, similar to our results, the study of McGregor et al. (2000) displayed a low reproducibility of ISSRs

with 87 % in 39 different potato cultivars. The authors postulated that competition for priming sites within the genome, which was also the possible reason for low reproducibility of RAPDs (Hallden et al. 1996) may also be the cause of the low reproducibility of ISSRs (McGregor et al. 2000). This problem with the lower reproducibility could limit the application of the ISSR primers as fingerprinting method for *P. sulcata*.

Due to the lower reproducibility of the replicates and to the missing data of some ISSR primers all statistical analyses were performed with the composition pattern of all five ISSR for 22 *P. sulcata* strains. The MDS analysis in combination with the factor *"simgroups"* detected a clear separation of the *P. sulcata* population (Fig 3). Moreover the ANOSIM displayed significantly separated *P. sulcata* populations according to the ISSR patterns indicating high intraspecific diversity. The clear separation of the January to March strains (*"simgroups"* group d, c) from all other strains in the MDS plot for *P. sulcata* strains (*"simgroups"* group b) based on the Jaccard's similarities (Figs 3) is notable. These results indicate that there is a high genetic variability within the *P. sulcata* population over one year. Unfortunately, the ISSR-PCR with three primers was not successfully in all *P. sulcata* strains and, especially the spring to summer strains were missing (April to June). Due to these missing data in the analysis we could not make ultimate conclusions for a shift in the *P. sulcata* population from winter (December to February) to a summer population (June to August).

However, we might explain our observed separation in the *P. sulcata* population with the ISSR primer fragment patterns as well as with the environmental parameters. Helgoland Roads is a temperate marine system with a typical seasonal pattern. In general, spring to summer are characterised by warmer water temperatures and higher water transparency (Secchi depth) and autumn to winter by higher concentrations of nutrients due to recycling and resuspension processes in the water column (Wafar et al. 1983, Gebühr et al. 2009). As expected, this seasonal pattern of the environmental parameters was observed with the calculation of the Euclidean distance of the environmental parameters. The isolated *P. sulcata* strains were well correlated with the environmental parameters displayed by the BEST analysis and as well as with the changes in the environmental parameter due to the different seasons. The significant separation of the *P. sulcata* strain based on the factor *"simgroups"* indicates this well correlation with the environmental parameters and a more or less seasonal distribution of the isolates during the year. Thus, we can support our hypothesis of the existence of

genetically variable *P. sulcata* populations at Helgoland Roads. This result pointed out that the North Sea provided a good adaptation of the *P. sulcata* population on its changing marine habitat.

Ecological conclusion for *Paralia sulcata*

Our results indicated a high genetic diversity within the *Paralia sulcata* population and a clear separation between January and strains isolated in October to December detected via the ISSR fingerprinting method. Furthermore, we have shown (Gebühr et al. 2009) that *P. sulcata* is now a non seasonal diatom species being occurring all the year round at Helgoland Roads. In winter the abundance of *P. sulcata* increases probably due to lower competition with other algae and its successful adaptation to lower temperatures and light conditions. In spring, as bloom-forming species become abundant and nutrients rapidly become limiting (Sommer et al. 2007), *P. sulcata* might survive in the sediment due to its tychopelagic life cycle. McQuoid & Hobson (1998) described that *P. sulcata* can easily be sloughed off the sediment bottom during storm activities leading to a re-dispersal of the population over the year.

The hydrography of Helgoland Roads is highly variable in the winter and late summer period with a lot of mixing (Wiltshire et al. 2008, Stockmann et al. 2010) which would positively influence the *P. sulcata* population in the water column. Our results showed that the population could be separated in January to March and October to December strains visible by the MDS based on the Jaccard's similarities and was distinguishable with the ISSR fingerprinting method. However, the mixing water column in summer times could lead to a homogenous distribution of the *P. sulcata* population in autumn and winter times of the year as based on the Jaccard's similarities some strains from July were observed in the same group with the October and December strains (*"simgroups"* group b, Fig. 3).

Furthermore, in a previous study it was shown that the concentration of phosphate, temperature and Secchi depth influenced the growth of *P. sulcata* in a significant manner (Gebühr et al. 2009). These findings were confirmed by the results of the BEST analysis in this study due in that these environmental parameters explain the structure within the Jaccard's similarities (*P. sulcata* population) and the Euclidean distances in terms of abiotic factors. Additionally, we know that the ecological niche of this diatom is broad at Helgoland Roads (Gebühr et al. 2009). A high genetic

diversity therefore, reflects the good adaptation of *P. sulcata* to its frequently unstable environment indicating a more general ecological niche of this diatom species within the marine habitat. Adaptation to the changing environment of *P. sulcata* may occur due to the high genetic variability. *P. sulcata* probably appears over the whole year in the water column and in the sediment at Helgoland Roads as a result of its tychopelagic life-style making it very adaptable to different environment conditions. This assumption is supported by a variety of studies which showed that *P. sulcata* is capable of growing under a wide range of environmental conditions throughout the year and may have an advantage at low light conditions with cooler water temperatures (Roelofs 1984, Hobson & McQuoid 1997, Zong 1997, McQuoid & Hobson 1998). Furthermore *P. sulcata* is often found in fossil records and therefore serves as paleoindicator (McQuoid & Hobson 1998, McQuoid & Nordberg 2003a). This diatom species is very old and thus, due to the frequently changing environment the genetic adaptation could be used to understand any shifts in the ecological niche. However, the tychopelagic lifestyle and the fast adaptation to a wide range and to fast changing of environmental conditions make ecological interpretations difficult (McQuoid & Hobson 1998) especially to reconstruct the past climates.

Summarising the results of the analysis with the ISSR primers it can be shown that *P. sulcata* has a high genetic diversity during one year isolated at Helgoland Roads and a well separation according to the changing environmental parameters and due to the seasonal periods in the marine system was found. Furthermore, the *P. sulcata* population is strongly influenced by phosphate concentrations, water transparency (Secchi depth) and temperature. These environmental parameters were also the main explaining factors for the shift in the ecological niche of this diatom species at Helgoland Roads over the last 10 years (Gebühr et al. 2009). These results pointed out that the North Sea provided a good adaptation of the *P. sulcata* population on its changing marine habitat.

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CHAPTER IV

How important is *Paralia sulcata* within its marine food web and as possible food source for copepod grazers?

How important is *Paralia sulcata* within its marine food web and as possible food source for copepod grazers?

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ABSTRACT

Mesocosms experiments were performed during the spring bloom 2009 at Helgoland Roads, North Sea to investigate the effects of different food qualities on grazing and selectivity of a copepod predator. Nutrient depletion during phytoplankton spring blooms affects the food quality of planktonic prey organisms. A natural spring bloom was induced and the response of the copepod *Temora longicornis* to the planktonic community under three different treatments (natural, enriched with nutrients or with *Paralia sulcata*) was monitored. The grazing of *T. longicornis* on phytoplankton in the nutrient-enriched treatment was significantly higher than for both other treatments. In contrast, the grazing on the microzooplankton was considerably higher in the *Paralia* treatment compared to the natural and nutrient treatment. The results showed that *T. longicornis* grazed on centric diatoms (grazing rate: 2.72 ± 0.83 d^{-1}) and ciliates (2.40 ± 0.35 d^{-1}) in the nutrient enriched treatment, on pennate diatoms (1.37 ± 0.18 d^{-1}) and ciliates (1.42 ± 0.38 d^{-1}) in the non-enriched natural treatment and on dinoflagellates (1.62 ± 0.89 d^{-1}) and ciliates (1.23 ± 0.46 d^{-1}) in the *Paralia* treatment. We suggest that the higher grazing rates of *T. longicornis* on microzooplankton was due to the decreasing food quality of the non-enriched phytoplankton treatments (natural and *Paralia*) during the spring bloom succession. The microzooplankton is able to stabilise the limitation of the phytoplankton due to the enrichment and synthesis of essential fatty acids and a better stoichiometric ratios.

Keywords: mesocosms, microzooplankton, North Sea, *Paralia sulcata*, phytoplankton, selective grazing, spring bloom, *Temora longicornis*

INTRODUCTION

Although our knowledge concerning marine food web has increased in the last decades, it is still not straightforward to assess the effects of changing environmental conditions on organisms and especially their interactions. Thus, it is important to understand the relationships between predator and prey species in the food web, especially when the latter comprises both autotrophs and heterotrophs.

Seasonal succession in the plankton of temperate coastal waters is initiated with the phytoplankton bloom in spring. It is triggered by strong vertical mixing which favours the dominance of chain-forming diatoms, whereas later the water column stratification favours the strategy of dinoflagellates and other flagellates that can swim to zones rich in light or nutrients (Casas et al. 1999). Furthermore, the phytoplankton spring bloom is triggered by light availability (season and latitude) and higher amounts of nutrients in temperate coastal waters. The limiting nutrient sets the upper limit to the amount of biomass that can be produced (Sommer et al. 1986, Sommer 1996). The rapid growth of phytoplankton, in the North Sea typically diatoms, starts with very low densities, as very few phytoplankters survive the winter, and is supported by the high nutrient contents of the seawater in spring. The nutrients are rapidly taken up by the phytoplankters in the beginning of the spring bloom and thus with increasing phytoplankton biomass nutrient availability decreases. This changes the stoichiometry and thus potentially the food quality of phytoplankton. The increase in phytoplankton biomass is followed by an increase in the numbers of predators. First to appear in the plankton succession are the unicellular microzooplankters with fast growth rates, followed by larger mesozooplankton such as copepods (Sommer et al. 1986, Sommer 1996, Sommer et al. 2007, Wiltshire et al. 2008).

Microzooplankton is an important link between phytoplankton and copepods, providing an important food source for copepods particularly when phytoplankton quality is low (Malzahn et al. 2010). It is known that microzooplankton can consume up to 60% -75% of the primary production (Calbet & Landry 2004) and it is therefore considered a key component in the control of phytoplankton blooms (e.g.Irigoien et al. 2005). Moreover, microzooplankton is generally considered a high quality food, containing high levels of nutrients and lipids essential to copepod grazers (Stoecker & Egloff 1987, Klein Breteler et al. 1999).

Copepods are the most important mesozooplankton grazers during the spring bloom. The majority of copepods are capable of selective feeding and are known to actively

choose specific phytoplankton groups or species (Kiørboe et al. 1996, Jansen 2008). In addition to grazing on phytoplankton, copepods are considered effective microzooplankton consumers. The calanoid copepod *Temora longicornis* occurs in the North Sea throughout the year and plays an important role in the food web (Maar et al. 2004, Gentsch et al. 2009). *T. longicornis*, an omnivorous grazer feeding on phytoplankton, ciliates and other microzooplankton, is known to be capable of food selectivity (Tackx et al. 1990). Since this copepod species is not able to store lipids as an energy source it relies on the continuous availability of food (Dam & Lopes 2003, Gentsch et al. 2009). It is therefore possible that the copepod can switch its feeding mode from phytoplankton to microzooplankton diets depending on the quality of the food. To investigate this in the context of the spring bloom was one of the main focus points of this study.

By investigating *in situ* plankton community from Helgoland Roads, North Sea, we examined the effects of inorganic nutrients and the addition of phytoplankton biomass on the trophic interaction between phytoplankton, microzooplankton and copepods. Several studies have focused on the marine system at Helgoland Roads, concerning particularly bacteria and interactions with diatoms (Sapp et al. 2007b, Sapp et al. 2008), bacteria-copepod interactions (Brandt et al. 2010), phytoplankton and microzooplankton (Wiltshire & Dürselen 2004, Wiltshire & Manly 2004, Löder 2010a) and zooplankton (Greve et al. 2004).

We conducted a mesocosm experiment simulating a natural spring bloom succession. To estimate the effect of additionally phytoplankton biomass we enriched one treatment with *Paralia sulcata*, a marine centric diatom species (McQuoid & Nordberg 2003a). Multivariate statistical analysis on the long-term data set of Helgoland Roads illustrate that the changing environmental conditions have led to a shift in the ecological niche of *P. sulcata* from a winter diatom to a diatom occurring throughout the whole year (Gebühr et al. 2009), making it to a good food source for micro- and mesozooplankton. *P. sulcata* occurs as pre-spring bloom species and during the spring bloom in higher abundances in the water column alongside the fast growing spring bloom species. But nothing is known of the ecological role of this diatom within the marine food web at Helgoland Roads. The question of whether *P. sulcata* is eaten by copepods is to be tested. We suggested that due to the occurrence of *P. sulcata* in the water column throughout the year this diatom could be a continuous food source for micro- and mesozooplankton. Therefore, we suggested that when *P. sulcata* is eaten,

this diatom will also be a good pre-spring bloom species. We hypothesised that with increasing abundance of *P. sulcata* in the water column during the spring bloom the grazing on this diatom will also have increased. Additionally, we tested whether copepods (*Temora longicornis*) selectively feed on heterotrophic organisms (especially dinoflagellates and ciliates) when the food quality of the autotrophic fraction (centric and pennate diatoms) declines. We hypothesised that with severe nutrient-limitation of the phytoplankton during the course of spring bloom copepods would shift their feeding mode from a predominately herbivore diet to a diet comprising mainly microzooplankton. Phytoplankton usually reflect the nutrient ratios of their surrounding environment and the micro- and mesozooplankton reflect much more constant nutrient ratios with respect to their body C:N:P ratios which is referred to as homeostasis and leads to a frequent imbalance between food supply and the demand of consumers such as copepods (Elser et al. 2000). In contrast, when nutrients were added to the plankton community the copepods were expected to feed more on the phytoplankton due to an increase in food quality of the autotrophic fraction.

MATERIALS AND METHODS

Description of the mesocosms

To observe the natural spring bloom development under near *in situ* conditions the mesocosms experiment was run from 16 March to 16 April 2009, encompassing the spring bloom. Three mesocosms were set up in a temperature-controlled room at the Biologische Anstalt Helgoland, Alfred Wegener Institute.

The water for the filling of the mesocosms was scooped from the surface water at Helgoland Roads, North Sea (54°11.3'N; 7°54.0'E) from board of the RV Uthörn. The water was pre-screened over a 200 µm plankton net to exclude bigger mesozooplankton groups (for more details see Löder 2010a). Each mesocosm was filled with 750 l water which was stirred with propellers (107.5 rpm, 15 min mixing, followed by 15 min pause) to ensure a homogenous distribution of the plankton community and to avoid sedimentation.

The temperature in the culture room was kept at 6°C with a starting temperature of 4.2°C and an end temperature of 6.8°C. Light was provided by computer-controlled light units (Profilux II, GHL Groß Hard- and Software Logistics, Kaiserslautern, Germany) which received instructions for brightening and dimming from an external

control computer (Programme 'Prometheus', GHL, modified version 'Copacabana', for details see Sommer et al. 2007). The light cycle and intensity were adjusted daily to account for changes in the photoperiod during the experimental run according to the geographical position of Helgoland following the model by Brock (1981). The light regime was adapted to simulate the average light intensity at 1.5 m depth at Helgoland Roads (for more details to the description of the mesocosms set-up seeLöder 2010a).
The chlorophyll *a* concentration (µg l^{-1}) was measured daily in all three mesocosms to observe the bloom development *in situ*. A water sample (50 ml) was taken from each of the mesocosms and the chlorophyll *a* concentration was determined in the laboratory using a cuvette multialgal fluorometer (BBE Moldaenke, Kiel, Germany).

Grazing experiment

Copepods of the genus *Temora longicornis* were collected for the grazing experiment from 500 µm plankton net samples taken at Helgoland Roads, North Sea, shortly before the start of the experiment. *Paralia sulcata* was sampled from the surface waters with 80 µm plankton net in February 2007 at Helgoland Roads. Cultures of *P. sulcata* were grown in f/2 medium (Guillard & Ryther 1962, Guillard 1975) under controlled conditions with 12:12 hours light:dark photoperiods and 14 - 15°C. *P. sulcata* cells were transferred into fresh medium every four weeks, when the cultures reached the stationary phase and were used for the grazing experiment during their exponential phase.

Treatments

For the grazing experiment water from all three mesocosms was used. Three different treatments were set up: (1) natural: without nutrient addition, treated as natural spring bloom development, (2) nutrients: with nutrient-addition and (3) *Paralia*: with an additional amount of *Paralia sulcata*. The nutrient treatment consisted of an addition of the mineral, metal and vitamin stock solutions used for f/2 medium according to Guillard & Ryther (1962). The concentrations added corresponded to 25% of the concentrations in full f/2 medium and to 10 times the nutrient concentrations found at Helgoland Roads in winter months. This ensured that the potentially limiting nutrients were added in excess of requirements. The density of *P. sulcata* was determined by

counting of the culture stock and the volume necessary for a final concentration of 4000 cells l^{-1} was added to each mesocosm subsample according to the treatments. This concentration corresponds to the annual mean abundance in winter months with slightly decreasing abundances in spring. Prior to the feeding experiments the copepods were incubated under experimental conditions for 24 hours. For this purpose water was taken from each mesocosms and subsamples were either enriched or remained untreated according to the treatment procedure. The copepods were acclimated at 6°C and 12:12 hours light:dark cycle. Afterwards, the copepods were removed by gentle pouring over a sieve and transferred to the incubation bottles of the grazing experiment.

For the grazing experiment three 5 l samples were taken from each mesocosm. Of these subsamples one per mesocosm was enriched with nutrients to change the stoichiometry of the phytoplankton, one was enriched with a final concentration of 4000 *P. sulcata* cells l^{-1} to increase the phytoplankton biomass and one subsample per mesocosm remained unchanged to represent the natural composition of the phytoplankton.

Once all of the mesocosm subsamples had been prepared three replicate 500 ml glass bottles were filled with copepods for the grazing experiment and three replicate bottles for the microzooplankton grazing control. The microzooplankton bottles contained no copepods and were used to calculate the gross growth rates of the phytoplankton and microzooplankton, and subsequently the grazing rates. Ten of the pre-conditioned copepods (female) were added to the bottles for the grazing experiment. After filling the bottles were sealed with parafilm to ensure no air bubbles were included. The bottles for the grazing experiment and the control bottles without copepods (each N = 3) were mounted on a plankton wheel and rotated for 24 hours at 1 rpm at 6°C and 12:12 hours light:dark cycle. The temperature corresponded to the water temperature in the mesocosms.

Prior the grazing experiment plankton samples were taken in 100 ml brown glass bottles and fixed with 1 ml of Lugol's iodine solution. These samples served as the start "zero" samples against which the changes in phytoplankton and microzooplankton in the control and grazing set-ups was compared. Additionally, samples were filtered as "zero" samples for the analyses of C, N and P of the seston. After 24 hours of incubation the bottles were removed from the plankton wheel and copepods were carefully sieved out of the 500 ml bottles over a 250 µm net to avoid

sampling the copepods with the seston. Again, samples for plankton and nutrients were taken as described above.

Data analysis

Counting of the plankton community

The plankton community was counted according to the method of Lund (1958) in 25 ml (for phytoplankton) and 50 ml (for microzooplankton) in Utermöhl settling chambers (HydroBIOS, Kiel, Germany) using an inverted microscope (Axiovert 135, Zeiss, Germany). Phytoplankton (diatoms and flagellates) were identified up to genus level or separated into size classes by microscopically measuring species size. Different size fractions were used to count *Chaetoceros* species, centric and pennate diatoms and flagellates. Furthermore, the microzooplankton was differentiated to the species or genus level or when undetermined pooled to size-dependent morphotype.

Determination of grazing rates and selectivity index

The cell abundance data were extrapolated to cells l^{-1}. In addition, the species-specific biovolume was calculated according to Hillebrand et al. (1999). The cell numbers were converted into pg carbon $cells^{-1}$ and transformed to biomass with the help of the biovolume according to specific conversions factors: ciliates pg C μm^{-3} = 0.19 (Putt & Stoecker 1989), diatoms pg C $cell^{-1}$ = 0.288 x $V^{0.811}$, dinoflagellates pg C $cell^{-1}$ = 0.760 x $V^{0.819}$ and all other protists pg C $cell^{-1}$ = 0.216 x $V^{0.939}$ (last three: Menden-Deuer & Lessard (2000), where V refers to biovolume in μm^3). Rotifer carbon was estimated according to McCauley (1984) and Park & Marshall (2000).

Growth and grazing parameters were calculated according to Frost (1972). The carbon biomass was used to calculate the gross growth rate μ (day^{-1}) of the phytoplankton and microzooplankton:

$$\mu = \ln(control) - \ln(zero) \qquad (1)$$

where control is the carbon biomass after 24 hours in the treatments without copepods and zero is the carbon biomass at the beginning of the experiment. The net growth rate r (day^{-1}) was calculated according to Landry & Hassett (1982) as follows:

$$r = \ln(copepod) - \ln(zero) \qquad (2)$$

with the carbon biomass from the treatments with copepods after 24 hours (copepod) and the carbon biomass at the beginning of the experiment (zero). The grazing rates per day on the phytoplankton and microzooplankton species (g_p) were calculated from the gross growth rate (1) and net growth rate (2):

$$g_p = \mu - r \quad (3)$$

As the copepods and the microzooplankton both feed on the phytoplankton, the grazing rates of the copepods on the phytoplankton had to be corrected for the microzooplankton grazing. Nejstgaard et al. (2001) described a method for correcting the copepod grazing on the phytoplankton with values from simultaneously performed microzooplankton grazing experiments. Thus, the copepod grazing rates were corrected for each prey type:

$$g_{corr,p} = g_p + k_p \quad (4)$$

where $g_{corr,p}$ is the corrected copepod grazing for each prey type p, g_p is the calculated uncorrected copepod grazing rates per day (3) and k_p is the correction for the loss of microzooplankton grazing on each prey type p in the copepod bottles (Nejstgaard et al. 2001) which is calculated with the microzooplankton grazing coefficient for each prey type. The microzooplankton grazing data and the mean carbon concentration of microzooplankton was obtained from simultaneously performed dilution experiments (Löder 2010a).

Thereafter, the filtration rate (F) of the copepods was calculated (Frost 1972):

$$F = V * g_{corr,p} / N \quad (5)$$

where V is the volume in the bottle, $g_{corr,p}$ is corrected grazing rate of the copepod (4) and N is the total number of copepods in the bottle. Negative filtration rates were set to zero. With the filtration rate (F) and the mean prey density (p_{mean}) the ingestion rate (I) for the copepod can be calculated (Frost 1972):

$$I = p_{mean} * F \quad (6)$$

The determination of the mean prey density (p_{mean}) was calculated according to Frost (1972):

$$p_{mean} = [C_1 * (e^{(\mu - g_{corr,p})*(t_2-t_1)}-1)] / [(t_2-t_1)*(\mu-g_{corr,p})] \quad (7)$$

where C_1 is the concentration of prey at the beginning, μ is the gross growth rate (1), $g_{corr,p}$ is the grazing rate of the consumers (microzooplankton and copepods) (4) and t_1, t_2 are the time points at the beginning and after 24 hours.
After this, the Chesson selectivity index α (Chesson 1983) was calculated. We used the formula for α with the constant food density which takes account of the mean prey density for the ingestion rates.

$$\alpha = (r_i/n_i) / (\Sigma r_i/n_i) \quad (8)$$

In this formula r_i is the frequency of prey *i* in the diet and n_i is the frequency of prey in the environment, divided by the sum of all relationships between the frequency of prey in the diet and in the environment. Thus, the selectivity index α is a relative measurement of the preference of the copepod for a special prey type relative to another prey type which is present in the diet in relation to its abundance in the environment. The selectivity index was calculated for each prey type (phytoplankton and microzooplankton groups) and for each treatment.

Analytical procedures

The carbon and nitrogen content of the samples was measured with an Elementar vario MICRO cube CHN analyser (Elementar Analysensysteme, www.elementar.de).
Phosphorus was analysed as orthophosphate, after the method described by Grasshoff et al. (1999), following oxidative hydrolysis. The samples were treated with an oxidation agent ($K_2S_2O_8$, H_3BO_3, NaOH in distilled water) under high pressure and at high temperature (120 °C) in an autoclave to convert the phosphorus compounds to the ortho-phosphate form. Molybdate-antimony-solution (containing ammonium molybdate $(NH_4)_6Mo_7O_{24}$ x $4H_2O$, antimony potassium tartrate $K(SbO)C_4H_4O_6$ x $0{,}5H_2O$) and ascorbic acid were added to the solute before the P-content was measured photometrically.

Statistical analysis

To test for significant effects of the factors treatments, microzooplankton and phytoplankton and the interaction within these parameters a two-factorial analysis of variance (ANOVA) was used for the total carbon content, the net growth rates, the grazing rates and the selectivity index as dependent variables (STATISTICA, Statsoft 7.1). The Fisher's least significant difference (LSD) post-hoc test was used for equal sample sizes.

RESULTS

Mesocosms succession

The phytoplankton bloom started right after the filling of the mesocosms and culminated within the next five days to the maximal concentration of 10.2 µg Chl *a* l^{-1} (Fig. 1a). Parallel to the fast increase in phytoplankton biomass, a rapid depletion of the nutrients within the mesocosms was observed from day 5 onwards, with silicate and phosphate concentrations below detection limit. The grazing experiment was carried out during the late-bloom period of the phytoplankton bloom from the 31.03.2009 to the 01.04.2009 (Fig. 1a).

The stoichiometry of the seston reflected the nutrient depletion over the course of the spring bloom (Fig. 1b). The C:N ratio of the seston increased from 5.5 ± 0.2 (mean ± SE) on the first day to a maximum value of 11.5 ± 0.3 on the 3rd of April. Due to the duration of the spring bloom the C:N ratio changed within the mesocosms and thus, on the day of the experiment, the C:N ratio was 11.4 ± 0.4 in the natural treatment, 11.3 ± 0.8 in the *Paralia* treatment and 9.8 ± 0.7 in the nutrient-enriched treatment. Even though the nutrient addition lowered the C:N ratio, the difference to the C:N in the natural and *Paralia* treatment was not significant. Furthermore, the C:P of the seston showed a rapid depletion of the P in the mesocosms. Thus, the highest C:P value of 1862 ± 164 was measured on the 31st of March, one day before the grazing experiment. The nutrient addition resulted in a significantly lower seston C:P ratio of 277 ± 16 compared to the C:P of 1711 ± 146 in the natural treatment and the C:P of 927 ± 64 in the *Paralia* treatments (ANOVA, LSD post-hoc test, $p < 0.001$) (Fig. 1c). As a result, the C:N ratio remained at a high level, indicating continuous N-limitation in the seston while the C:P ratio decreased again to a level of around 500 during the

post-bloom period. *Paralia sulcata* itself has a C:N ratio of 7.5 ± 1.1 and a C:P of 113.7 ± 22.1 which showed values near the Redfield ratio.

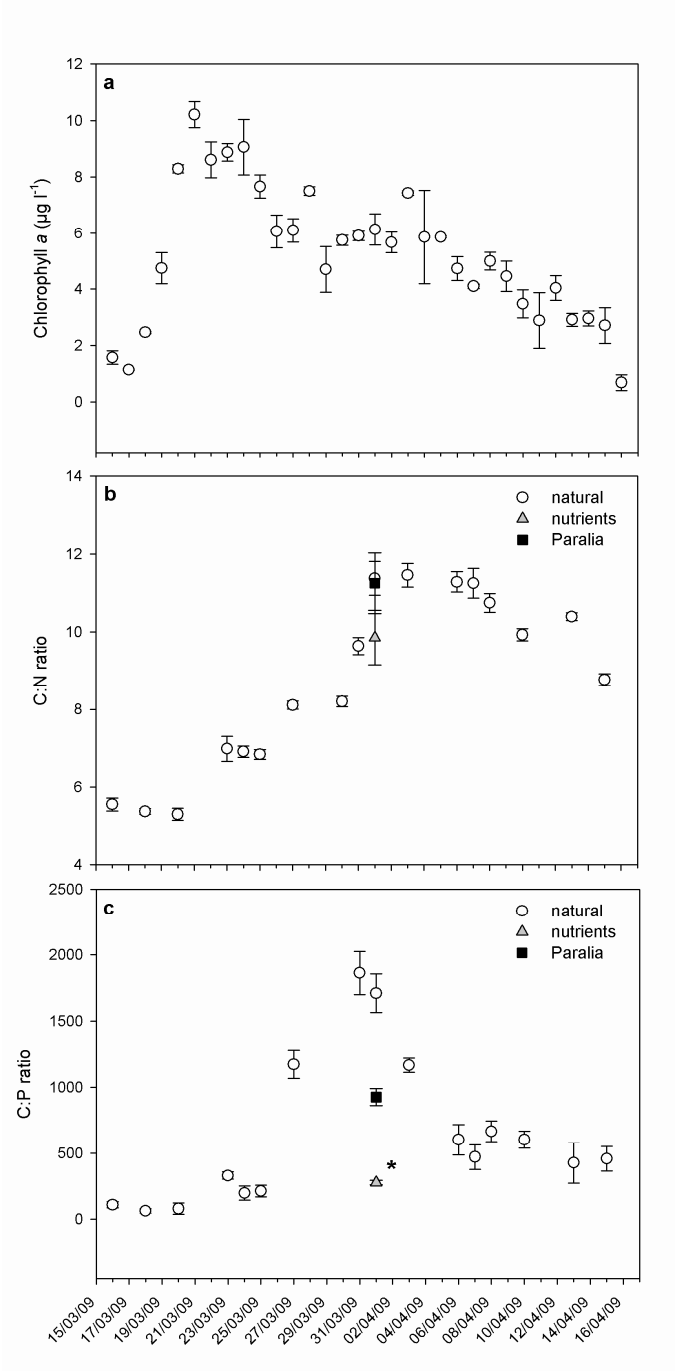

Figure 1: a) Total chlorophyll *a* (µg l^{-1}) concentration over the investigated time period (16.03.-16.04.2009) in all three mesocosms. B) C:N ratio of the seston in the mesocosms as natural (open circles), nutrient treatment (grey triangle) and *Paralia* treatment (black square) from the grazing experiment. C) C:P ratio of the seston from the mesocosms as natural, nutrient treatment and *Paralia* treatment from the grazing experiment. All values are means with standard error (N = 3). The asterisk * indicates a significant difference of the nutrient treatment with the two other treatments (ANOVA, LSD post-hoc test, $p < 0.001$).

Plankton community

The phytoplankton biomass accounted for around 80% of the diet of *T. longicornis* in the three treatments while microzooplankton made up 20% of the copepods' diet. Furthermore, the biomass of phytoplankton and microzooplankton did not differ significantly between all three mesocosms, making them good replicates. All results are presented as mean values over the three mesocosms (N = 3). The plankton community consisted of diatoms (centrales and pennales, Table 1) whereas the centric diatoms *Rhizolenia hebetata* (around 22%), *Thalassiosira nordenskioeldii* (around 37%) and *Thalassiosira rotula* (around 11%) dominated the community in terms of biomass. The microzooplankton community was composed of dinoflagellates (mainly *Ceratium* spp., *Protoperidinium* spp., *Peridinium* spp., *Diplopsalis* spp., *Scrippsiella* spp. and *Gyrodinium* spp.), ciliates (mainly *Myrionecta* spp., *Strombidium* spp., *Acineta* spp., *Euplotes* spp., *Laboea strobila*, *Leegaardiella sol*, *Lohmanniella oviformis*, *Rimostrombidium* sp., *Tintinnopsis* spp. and *Tontonia gracillima*), thecate amoeba and rotifers.

For further analyses the phytoplankton and microzooplankton were pooled into groups. The total carbon (pg C cells^{-1}) was calculated for each species and genus and the sum of the carbon was calculated for each group of the plankton community. Significant decreases in phytoplankton (ANOVA, LSD post-hoc test, $p < 0.05$) (Fig. 2a) and microzooplankton ($p < 0.01$) (Fig. 2b) biomass from the start compared with the copepod after 24 hours were observed in all three treatments.

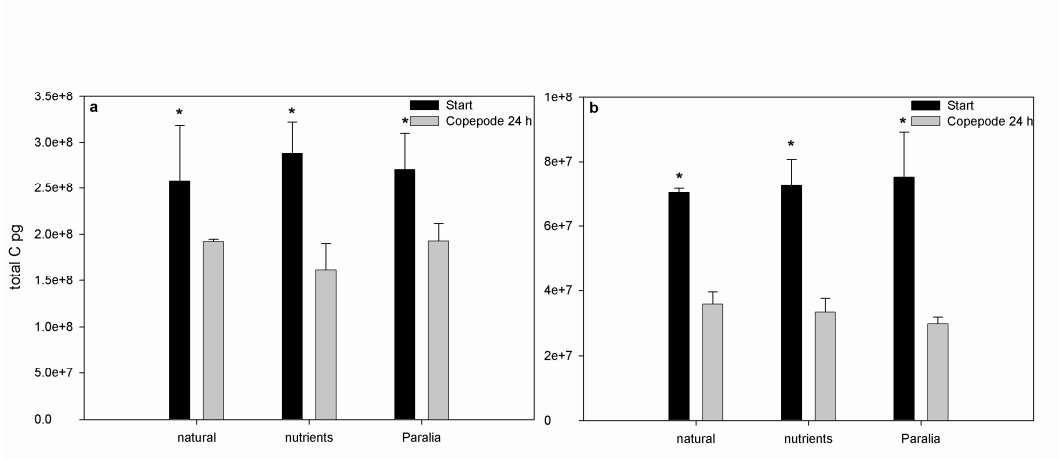

Figure 2: Total carbon (pg cells^{-1}) in the phytoplankton (a) and microzooplankton (b) community in the natural, nutrient and *Paralia* treatment at the beginning and after 24 hours with *Temora longicornis*, mean with standard error (N = 3). Significant differences (labelled with an asterisk *) were shown in the total carbon in the phytoplankton and microzooplankton community (two-way ANOVA, LSD post-hoc test) and in comparison of the phytoplankton ($p < 0.001$) and microzooplankton ($p < 0.05$) between start and after 24 h. Note the different scaling of the y-axis.

Furthermore, net growth rates per day were calculated as described above (Table 1). Negative net growth rates indicated a decrease in carbon biomass in the phytoplankton groups due to grazing by *T. longicornis* and microzooplankton. Positive net growth rates indicated that the growth of this plankton group was higher than the loss by grazing and occurred only in the natural treatments for *R. imbricata*, *T. rotula*, *C. didymus* and flagellates (Table 1). Within the phytoplankton community a significant difference in the net growth rates (day^{-1}) between the three treatments were observed (two-way ANOVA, LSD post-hoc test, $p = 0.026$). The higher net growth rates of phytoplankton in the natural treatment resulted in lower grazing and were lowest within the nutrients treatment (Table 1). Furthermore, no significant differences in the net growth rates within the microzooplankton community among the three treatments were found with an exception for athecate dinoflagellates, ciliates and rotifers (ANOVA, LSD post-hoc test, $p < 0.05$). However, thecate dinoflagellates, especially *Protoperidinium* sp., *Myrionecta* sp. and rotifers displayed positive net growth rates (Table 1). Thus, these results showed a higher grazing on the microzooplankton community due to better food quality in general for *T. longicornis*.

Table 1: Net growth rates (day^{-1}) and grazing rates (day^{-1}) in the plankton community in the natural, nutrient and *Paralia* treatments, mean with standard deviation (N = 3). Significant differences in the net growth rates and grazing rates between natural, nutrient and *Paralia* treatments in the plankton groups are found (two-way-ANOVA, LSD post-hoc test, $p < 0.001$). Same letter displayed no significant differences, whereas different letters indicated significant differences in the plankton groups among the treatments.

	net growth rate (day^{-1})			grazing rates (day^{-1})		
phytoplankton	**natural**	**nutrients**	**Paralia**	**natural**	**nutrients**	**Paralia**
Rhizosolenia hebetata	-0.346 ± 0.222^{a}	-0.956 ± 0.338^{b}	-0.625 ± 0.466^{ab}	0.183 ± 0.302^{a}	0.574 ± 0.159^{b}	0.455 ± 0.156^{ab}
Rhizosolenia imbricata (20;300 µm)	0.125 ± 0.216^{a}	-0.417 ± 0.201^{b}	-0.228 ± 0.423^{ab}	0.041 ± 0.071^{a}	0.382 ± 0.101^{b}	0.115 ± 0.200^{ab}
Thalassiosira nordenskioeldii	-0.417 ± 0.190	-0.521 ± 0.302	-0.347 ± 0.395	0.265 ± 0.145^{a}	0.629 ± 0.222^{b}	0.383 ± 0.182^{ab}
Thalassiosira rotula	0.306 ± 0.201	-0.772 ± 0.410	-0.273 ± 0.209	0.100 ± 0.122^{a}	0.482 ± 0.340^{b}	0.413 ± 0.101^{ab}
Chaetoceros debilis	-0.046 ± 0.281	-0.122 ± 0.306	-0.251 ± 0.252	0.211 ± 0.110	0.368 ± 0.367	0.160 ± 0.140
Chaetoceros decipiens (20;20 µm)	-0.138 ± 0.500	-0.354 ± 0.234	-0.185 ± 0.146	0.156 ± 0.155	0.328 ± 0.326	0.124 ± 0.111
Chaetoceros danicus	-0.190 ± 0.462	-0.556 ± 0.163	-0.125 ± 0.254	0.066 ± 0.055	0.241 ± 0.346	0.019 ± 0.033
Pseudo-nitzschia sp.	-1.197 ± 0.387^{a}	-1.226 ± 0.195^{a}	-0.182 ± 0.131^{b}	1.227 ± 0.214^{a}	1.129 ± 0.315^{a}	0.307 ± 0.315^{b}
Chaetoceros didymus	0.145 ± 0.285^{a}	-0.1582 ± 0.160^{ab}	-0.401 ± 0.617^{b}	0.106 ± 0.184	0.080 ± 0.071	0.029 ± 0.050
Diatomaceae pennales (15;60 µm)	-0.697 ± 0.248^{a}	-0.045 ± 0.205^{b}	-0.568 ± 0.201^{a}	0.301 ± 0.249	0.389 ± 0.288	0.386 ± 0.157
Nitzschia longissima	-0.366 ± 0.634^{a}	-0.862 ± 0.605^{a}	0.00^{b}	0.158 ± 0.105^{a}	0.784 ± 0.289^{b}	0.097 ± 0.0^{a}
Paralia sulcata	0.00^{a}	0.00^{a}	-1.299 ± 0.815^{b}	0.091 ± 0.0^{a}	0.091 ± 0.0^{a}	0.770 ± 0.735^{b}
Flagellate indeterminata (10;20 µm)	0.008 ± 0.089	-0.042 ± 0.061	-0.145 ± 0.285	0.211 ± 0.130	0.195 ± 0.124	0.417 ± 0.229
Diatomaceae pennales (20 µm)	-0.410 ± 0.095	-0.298 ± 0.158	-0.345 ± 0.043	0.201 ± 0.081	0.199 ± 0.111	0.287 ± 0.071
Flagellate indeterminata (6;10 µm)	0.166 ± 0.159	-0.139 ± 0.173	0.108 ± 0.042	0.027 ± 0.047	0.163 ± 0.047	0.167 ± 0.103
Chaetoceros minimus	-0.006 ± 0.354	-0.036 ± 0.127	-0.073 ± 0.324	0.024 ± 0.042	0.049 ± 0.046	0.055 ± 0.096

microzooplankton	net growth rate (day^{-1})			grazing rates (day^{-1})		
	natural	nutrients	Paralia	natural	nutrients	Paralia
Ceratium sp.	-0.960 ± 0.196	-0.508 ± 0.479	-0.573 ± 0.743	0.00	0.144 ± 0.203	0.468 ± 0.661
Protoperidinium sp.	0.711 ± 0.585	0.526 ± 0.436	0.010 ± 0.772	0.00[a]	0.00[a]	0.932 ± 1.203[b]
other Dinoflagellates thecate	0.135 ± 0.395	0.246 ± 0.517	0.579 ± 0.719	0.00	0.065 ± 0.112	0.378 ± 0.365
Dinoflagellates athecate	-0.416 ± 0.232[ab]	-0.310 ± 0.348[a]	-0.747 ± 0.145[b]	0.222 ± 0.129	0.461 ± 0.334	0.798 ± 0.165
Myrionecta rubra	0.333 ± 0.448	-0.134 ± 0.651	0.288 ± 0.472	0.00	0.165 ± 0.173	0.070 ± 0.121
Strombidium sp.	-2.480 ± 0.657	-3.045 ± 0.538	-2.758 ± 0.170	1.250 ± 0.767	1.566 ± 0.673	0.885 ± 0.927
Ciliates	-0.269 ± 0.190[a]	-1.320 ± 0.065[b]	-0.476 ± 0.436[ab]	0.168 ± 0.171	0.671 ± 0.216	0.270 ± 0.263
thecate amoeba	-0.970 ± 0.195	-0.573 ± 0.608	-0.644 ± 0.336	0.393 ± 0.143	0.350 ± 0.073	0.392 ± 0.340
Rotifers	0.000 ± 1.099[a]	0.327 ± 0.634[a]	-1.596 ± 0.203[b]	0.170 ± 0.295[a]	0.00[a]	0.924 ± 1.059[b]

Additionally, grazing rates were calculated. All negative grazing rates were set to zero for the subsequent calculations. Positive grazing rates indicate grazing on specific plankton groups. When considering single groups of phytoplankton (especially larger phytoplankton species such as *Rhizolenia* spp., *T. rotula*, and smaller species e.g. *Pseudo-nitzschia* sp., *N. longissima* and *P. sulcata*) a significant difference in the grazing rates (day^{-1}) between the three treatments (two-way ANOVA, LSD post-hoc test, $p = 0.00047$) was observed. Higher grazing rates were detected on the *Rhizolenia* spp. and *Thalassiosira* spp. in the nutrient and *Paralia* treatments, indicating a higher grazing pressure on these phytoplankton groups (Table 1). Furthermore, *P. sulcata* was grazed in significantly higher amounts in the *Paralia* treatment. This was also indicated by the significantly lower net growth rates (Table 1). Thus, significantly higher proportions of phytoplankton were consumed by *T. longicornis* in the nutrient enriched treatment (ANOVA, LSD post-hoc test, $p < 0.05$) when compared to non-enriched natural and *Paralia* treatments (Fig. 3a). In contrast, significantly higher grazing rates on the microzooplankton were observed in the *Paralia* treatment compared with the two other treatments ($p < 0.05$) (Fig 3a).

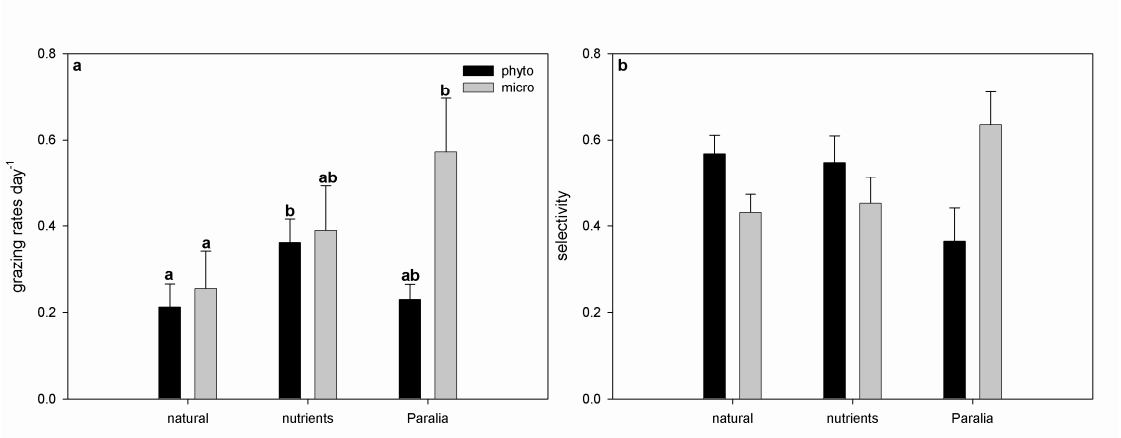

Figure 3: a) *Temora longicornis* grazing rates (day^{-1}) on the total plankton community in the natural, nutrient and *Paralia* treatment, mean with standard error (SE). Significant difference between the treatments in the phytoplankton community (ANOVA, LSD post-hoc test, $p < 0.05$), and microzooplankton community ($p < 0.05$) were indicated by different letters, same letters displayed no significant difference. b) Selectivity index of the feeding behaviour of *T. longicornis* on the total plankton community in the natural, nutrient and *Paralia* treatments (mean ± SE). No significant differences were shown within the treatments and the comparison between phytoplankton and microzooplankton.

A look at Chesson's selectivity index for the copepods' feeding behaviour on phytoplankton and microzooplankton indicated no significant differences between all three treatments. In general, *T. longicornis* displayed a slight preference for phytoplankton over microzooplankton within the natural and nutrient treatments and a slight but non significant preference for microzooplankton in the *Paralia* treatment (Fig 3b).

In general, the grazing rates on specific plankton groups (Fig. 4a) within the natural treatment was significantly lower compared to both other treatments (two-way ANOVA, $p < 0.05$) and significant differences within the grazing rates on the plankton groups (two-way ANOVA, $p < 0.001$) and interactions between treatments and groups existed (two-way ANOVA, $p < 0.01$). Taking a closer look at the grazing rates of *T. longicornis* on specific plankton groups we found an interesting pattern: ciliates, centric diatoms and also to a slight degree thecate dinoflagellates were grazed in higher amounts compared to the other plankton groups. Especially centric diatoms and ciliates in the nutrient treatment were grazed in significantly higher amounts by *T. longicornis* than other groups compared to the natural and *Paralia* treatments and thecate dinoflagellates were grazed in significantly higher quantities in the *Paralia* treatment compared to both the other treatments (two-way ANOVA, LSD post-hoc test, $p < 0.05$) (Fig. 4a, Table 2). In contrast to that finding the grazing rates on flagellates, dinoflagellates, pennate diatoms, amoeba and rotifers showed that these plankton groups were not preferentially grazed food items (Fig 4a).

The selectivity index for the feeding preference of *T. longicornis* on different plankton groups displayed slightly different patterns compared to the grazing rates (Fig 4b**,** Table 3). Generally, no significant differences were detected among the three treatments, but significant differences between the plankton groups (two-way ANOVA; $p < 0.001$). Taking a closer look revealed that centric diatoms and ciliates were significantly preferred in the diet of *T. longicornis* within the nutrient treatment*,* as shown by the grazing rates. Furthermore, within the natural treatment higher preferences for the pennate diatoms and ciliates were observed for *T. longicornis* and higher selectivity on thecate dinoflagellates were visible in the *Paralia* treatment (two-way ANOVA, LSD post-hoc test, $p < 0.05$) (Fig. 4b).

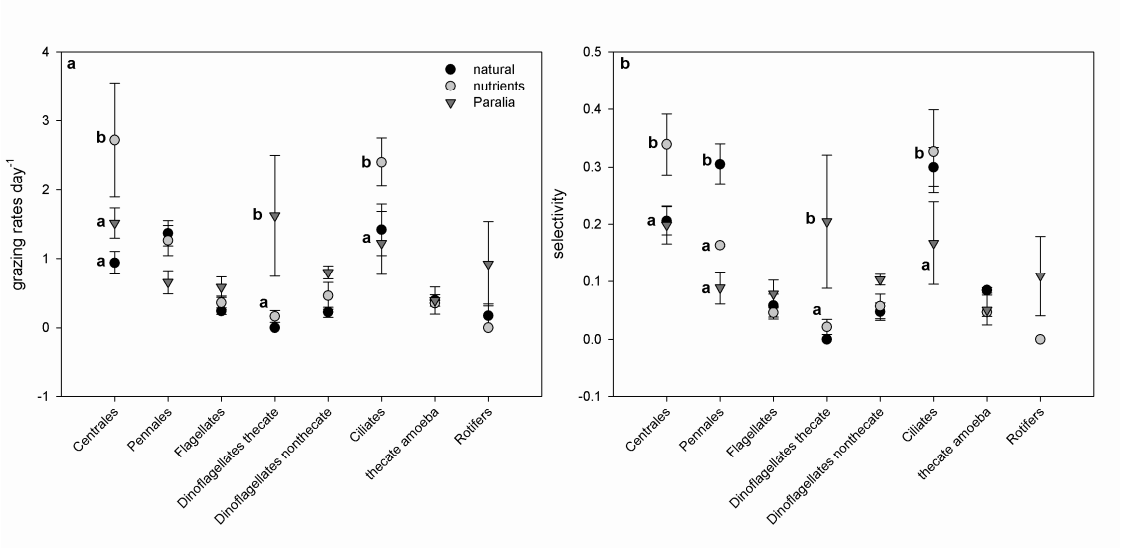

Figure 4: a) *Temora longicornis* grazing rates (day^{-1}) on the plankton groups in the natural, nutrient and *Paralia* treatments. Significant differences between the treatments (two-way ANOVA, LSD post-hoc test $p < 0.05$) between the plankton groups ($p < 0.0001$) and interaction of the treatments and groups ($p < 0.01$) were found. Different letters indicated significant differences between the treatments in the plankton groups (Table 2). b) Selectivity index for *T. longicornis* on the plankton groups in the natural, nutrient and *Paralia* treatment. No significant difference between the treatments, but significant differences between the groups (two-way ANOVA, LSD post-hoc test $p < 0.001$) and interaction of treatments and groups ($p < 0.001$) were detected. Different letters indicate significant differences between the treatments in the plankton groups (Table 3).

In summary the results showed different feeding behaviour of *T. longicornis* due to the three treatments. Thus, the copepod fed preferentially on centric diatoms and ciliates in the nutrient enriched treatment, on pennate diatoms and ciliates in the non-enriched natural treatment and on thecate dinoflagellates and ciliates in the *Paralia* treatment.

Table 2: Results of the two-way ANOVA (LSD post-hoc test) of *Temora longicornis* grazing rates (day^{-1}) on the plankton groups in natural, nutrient and *Paralia* treatments. Significant interactions between treatments and the plankton: n.s. no significance, * $p < 0.05$, ** $p < 0.01$ (Fig. 4a).

	groups	nutrients								Paralia								natural							
		centrales	pennales	flagellates	thecate dino	athecate dino	ciliates	amoeba	rotifers	centrales	pennales	flagellates	thecate dino	athecate dino	ciliates	amoeba	rotifers	centrales	pennales	flagellates	thecate dino	athecate dino	ciliates	amoeba	rotifers
nutrients	centrales																								
	pennales	**																							
	flagellates	**	n.s.																						
	thecate dino	**	*	n.s.																					
	athecate dino	**	n.s.	n.s.	n.s.																				
	ciliates	n.s.	*	**	**	**																			
	amoeba	**	n.s.	n.s.	n.s.	n.s.	**																		
	rotifers	**	**	n.s.	n.s.	n.s.	**	n.s.																	
Paralia	centrales	*	n.s.	*	**	*	n.s.	*	**																
	pennales	**	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.															
	flagellates	**	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.														
	thecate dino	*	n.s.	**	**	*	n.s.	**	**	n.s.	*	*													
	athecate dino	**	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.												
	ciliates	**	n.s.	n.s.	*	n.s.	*	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.											
	amoeba	**	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	*	n.s.	n.s.	*	n.s.	n.s.										
	rotifers	**	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.									
natural	centrales	**	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.								
	pennales	**	n.s.	*	*	n.s.	*	*	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.							
	flagellates	**	*	n.s.	n.s.	n.s.	**	n.s.	n.s.	**	n.s.	n.s.	**	n.s.	*	n.s.	n.s.	n.s.	*						
	thecate dino	**	**	n.s.	n.s.	n.s.	**	n.s.	n.s.	**	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.					
	athecate dino	**	*	n.s.	n.s.	n.s.	**	n.s.	n.s.	**	n.s.	n.s.	**	n.s.	*	n.s.	n.s.	n.s.	*	n.s.	n.s.				
	ciliates	**	n.s.	*	*	*	*	*	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	*	**	*			
	amoeba	**	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	*	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	*		
	rotifers	**	*	n.s.	n.s.	n.s.	**	n.s.	n.s.	**	n.s.	n.s.	**	n.s.	*	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	*	n.s.	

Table 3: Results of the two-way ANOVA (LSD post-hoc test) for *Temora longicornis* selectivity index on the plankton groups in natural, nutrient and *Paralia* treatments. Significant interactions among treatments and plankton: n.s. no significance, * $p < 0.05$, ** $p < 0.01$ (Fig. 4b).

	groups	nutrients								Paralia								natural							
		centrales	pennales	flagellates	thecate dino	athecate dino	ciliates	amoeba	rotifers	centrales	pennales	flagellates	thecate dino	athecate dino	ciliates	amoeba	rotifers	centrales	pennales	flagellates	thecate dino	athecate dino	ciliates	amoeba	rotifers
nutrients	centrales																								
	pennales	**																							
	flagellates	**	*																						
	thecate dino	**	*	n.s.																					
	athecate dino	**	n.s.	n.s.	n.s.																				
	ciliates	n.s.	**	**	**	**																			
	amoeba	**	*	n.s.	n.s.	n.s.	**																		
	rotifers	**	**	n.s.	n.s.	n.s.	**	n.s.																	
Paralia	centrales	*	n.s.	*	**	*	*	*	**																
	pennales	**	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.															
	flagellates	**	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	*	n.s.														
	thecate dino	*	n.s.	**	**	*	*	**	**	n.s.	*	*													
	athecate dino	**	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.												
	ciliates	**	n.s.	*	*	n.s.	**	*	**	n.s.	n.s.	n.s.	n.s.	n.s.											
	amoeba	**	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	*	n.s.	n.s.	**	n.s.	n.s.										
	rotifers	**	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.									
natural	centrales	*	n.s.	**	**	*	*	**	**	n.s.	*	*	n.s.	n.s.	n.s.	**	n.s.								
	pennales	n.s.	*	**	**	**	n.s.	**	**	n.s.	**	**	n.s.	**	*	**	**	n.s.							
	flagellates	**	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	*	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	**	**						
	thecate dino	**	**	n.s.	n.s.	n.s.	**	n.s.	n.s.	**	n.s.	n.s.	**	n.s.	**	n.s.	n.s.	**	**	n.s.					
	athecate dino	**	*	n.s.	n.s.	n.s.	**	n.s.	n.s.	*	n.s.	n.s.	**	n.s.	*	n.s.	n.s.	n.s.	**	n.s.	n.s.				
	ciliates	n.s.	*	**	**	**	n.s.	**	**	n.s.	**	**	n.s.	**	*	**	**	n.s.	n.s.	**	**	**			
	amoeba	**	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	**	**	n.s.	n.s.	n.s.	**		
	rotifers	**	**	n.s.	n.s.	n.s.	**	n.s.	n.s.	**	n.s.	n.s.	**	n.s.	**	n.s.	n.s.	**	**	n.s.	n.s.	n.s.	**	n.s.	

DISCUSSION

The main factor controlling the succession in phytoplankton and microzooplankton communities is the selective feeding of the mesozooplankton (Vanderploeg & Scavia 1979). To examine the influence of copepods' feeding behaviour on natural spring bloom succession during a mesocosms experiment different treatments were used; natural, and enriched with nutrients or *Paralia sulcata*, respectively. Hence, in this study we investigated not only the effect of nutrient limitation of phytoplankton on the grazing behaviour of copepods, but also the effect of additional diatom biomass. On the one hand we hypothesised that the addition of nutrients would positively change the nutrient content and thereby the food quality of the phytoplankton, making the phytoplankton more attractive to the copepod predator. Therefore, we expected to see increased feeding on phytoplankton compared to the microzooplankton after nutrient addition. Our results indicated significantly higher grazing rates of *Temora longicornis* on the phytoplankton after to the nutrient enrichment compared with both the other treatments. Although higher phytoplankton biomass was available in the *Paralia* treatment, the microzooplankton was strongly grazed by the copepod. This might represent a trend to a higher selectivity of *T. longicornis* on this plankton group. Especially the grazing rates showed that *P. sulcata* was grazed in a significantly higher amount compared to the two other treatments (Table 1) and also compared to the other phytoplankton species within this treatment. Therefore, when *P. sulcata* was higher in biomass, the grazing pressure of the copepod on this diatom increased making *P. sulcata* a potential food source. Generally, higher grazing rates were observed within the nutrient and *Paralia* enriched treatments compared to the natural one. Thus, dependent on the food sources *T. longicornis* exhibited a clear shift within the food spectrum showing a switch in its feeding behaviour. Our results were confirmed by studies of Sommer et al. (2005) showing a preference of *T. longicornis* for dinoflagellates and ciliates, but a preference for only ciliates (Vincent & Hartmann 2001, Jakobsen et al. 2005) was also exhibited.

Based on the biochemical composition of the phytoplankton the diatoms seemed to be lower in food quality and have lower nutritional value than other plankton groups (Perissinotto 1992, Sommer 1996). Our hypothesis that the phytoplankton would be significantly more eaten by *T. longicornis* due to the enrichment of nutrients in one treatment was shown in the grazing rates and the selectivity on the phytoplankton groups. This was especially visible for the centric diatoms, indicating the active

selection of this plankton group (Fig. 4a, b). The centric diatoms in our mesocosms consisted mainly of *R. hebetata*, *T. rotula*, some *Chaetoceros* species and *P. sulcata* (in the *Paralia* treatment). Thus, the positive selection towards these large, chain-forming diatoms was in line with the results of a mesocosms study by Sommer et al. (2004), who reported a high grazing by copepods on *Rhizolenia* and *Thalassiosira* species. Furthermore, grazing experiments with mesozooplankton, mainly *Acartia tonsa* and natural plankton communities demonstrated the dominance of *P. sulcata* as a food source for the mesozooplankton community (Diodato & Hoffmeyer 2008). These authors showed a biomass of around 363 pg C cell^{-1} for *P. sulcata*, which is in line with our size of *P. sulcata* (323 ± 51 pg C cell^{-1}) found at Helgoland Roads. Kasim & Mukai (2009) showed that *P. sulcata* was found in high amounts in the gut content of benthic and suspension feeders as *Crassostrea gigas* and *Ruditapes philippinarum* during all seasons indicating a high preference for *P. sulcata* even when its abundance was low in the water column.

Consequently, it appears that *T. longicornis* was able to distinguish between nutrient enriched phytoplankton species and non-enriched phytoplankton species as shown in this study. It has been reported that some copepod species can distinguish between high and low quality food. In this context, the copepod *Acartia tonsa* selected for faster growing, higher quality diatoms of the species *Thalassiosira weissflogii* (Cowles et al. 1988, Arendt et al. 2005). The fast-growing diatom type contained more total protein, chlorophyll and dissolved amino acids and had a lower C:N ratio than the slow growing type, making it a high quality food source from the copepods' perspective (Cowles et al. 1988).

Due to its intermediary trophic position between phytoplankton and mesozooplankton, the microzooplankton plays an important role in the marine food web (e. g. Klein Breteler et al. 1999, Veloza et al. 2006, Gentsch et al. 2009). Microzooplankton is generally regarded as food of higher quality for mesozooplankton due to higher contents of essential nutrients, polyunsaturated fatty acids and amino acids as well as low C:N ratios (Stoecker & Capuzzo 1990, Dam & Lopes 2003, Saba et al. 2009). Furthermore, microzooplankton is capable of enriching or synthesising essential macromolecules such as fatty acids (Stoecker & Capuzzo 1990, Kleppel 1993) through trophic upgrading (Klein Breteler et al. 1999). For copepods it is important to obtain these unsaturated fatty acids and sterols from their diet, as their ability for *de novo* synthesis is limited (Goad 1981, Stoecker & Capuzzo 1990, Arendt et al. 2005).

Therefore, the diet of many copepods often comprises large amounts of microzooplankton (ciliates, dinoflagellates and rotifers) relative to phytoplankton, and some copepod species are known to preferentially select for microzooplankton at certain times of the bloom (Stoecker & Egloff 1987, Verity & Paffenhöfer 1996, Dam & Lopes 2003). This was confirmed by our results showing that *T. longicornis* fed preferentially more on ciliates and dinoflagellates (especially *Protoperidinium* spp.) during the natural spring bloom, particularly when enriched with more phytoplankton biomass as shown in the *Paralia* treatment. The study of Gentsch et al. (2009) also showed a selective feeding of *T. longicornis* on dinoflagellates. In contrast, we did not find a tendency for higher grazing of *T. longicornis* on dinoflagellates in the nutrient enriched treatment. Corresponding to our results within the natural treatment the study of Löder (2010a) also exhibited an increase in the grazing of *T. longicornis* on microzooplankton due to nutrient-limitations in natural spring blooms and the lower food quality of the phytoplankton. In fact, grazing rates and the selectivity index of *T. longicornis* were significantly lower for dinoflagellates in the natural and nutrient treatments, while showing a preference for diatoms and ciliates. The ciliates and dinoflagellates were however grazed significantly more in the *Paralia* treatment compared to the other two.

The stoichiometry of the seston as a whole changed significantly after the enrichment with nutrients and *P. sulcata*. Especially the low C:P ratio within the *Paralia* treatment can be explained by the higher carbon input due to the increase in phytoplankton biomass in this treatment. Furthermore, the stoichiometry in the nutrient enriched treatment was significantly lower indicating a much more balanced food in terms of the carbon:nutrient ratio and therefore of higher quality to the consumers in the nutrient and *Paralia* treatments. However, while mesozooplankton may be able to buffer stoichiometric imbalances of their food to a certain extent, the homeostatic ability of microzooplankton is limited. Changes in the nutrient composition of their phytoplankton prey are therefore rapidly reflected in the stoichiometry of the microzooplankton (Andersen et al. 1986), making them also food of variable quality for higher trophic levels. The quality of the plankton as a whole increased as a result of the nutrient addition, making it a more interesting food source for the copepods. This would explain why in our experiment the microzooplankton in the nutrient treatment was also heavily grazed. Similar results to the ones found in our experiment have also

been reported in studies by Stoecker & Egloff (1987), Merrell & Stoecker (1998) and Malzahn et al. (2010).

However, this result of the grazing experiment poses some questions, e.g. why did *T. longicornis* significantly feed on more microzooplankton, when additional phytoplankton biomass was added? Diatoms in general have an average C:N ratio of 7.3 ± 1.2 (Sarthou et al. 2005), which is close to the 6.6 C:N ratio reported by Redfield et al. (1963), whereas N:P ratio (10 ± 4) showed that more than 90% of the values are lower than the Redfield ratio of 16 (Sarthou et al. 2005). Moreover, the molar C:N (7.5 ± 1.1), C:P (113.7 ± 22.2) and N:P (7.0 ± 1.6) ratios of *P. sulcata* showed only a slight limitation of nitrogen and phosphate within the *P. sulcata* cells used during the grazing experiment which was to a slight degree different from that reported by the mean ratios by Sarthou et al. (2005) for diatoms. Unfortunately, nothing is known about the stoichiometry of *P. sulcata* itself from other grazing experiments indicating a lack of knowledge about the food quality and therefore the food preference for micro- and mesozooplankton in general.

Conclusions for the feeding behaviour of *Temora longicornis* and *Paralia sulcata*

The results of the grazing experiment showed that *T. longicornis* is a selective omnivorous copepod feeding on different prey types. Nutrient-rich phytoplankton is preferentially eaten by *T. longicornis*. The higher grazing and selectivity on the microzooplankton in the nutrient-enriched treatment indicates that the quality of the microzooplankton increased along with that of the phytoplankton. Our results also showed that the highest grazing rates of *T. longicornis* occurred on the diatoms and ciliates, and this pattern was reflected in the selectivity index for *T. longicornis*. The feeding behaviour changed within the *Paralia* treatment mainly due to higher grazing on dinoflagellates and ciliates, indicating a preference for microzooplankton due to the high food quality.

Furthermore, it was shown that *Paralia sulcata* was significantly grazed within the *Paralia* treatment making it a possible food source for copepods. Two other studies have shown that *P. sulcata* represents a good food source for mesozooplankton in the pelagic (Diodato & Hoffmeyer 2008) and for bivalves in the benthos (Kasim & Mukai 2009). The occurrence of *P. sulcata* in the water column especially in spring and

summer times means that this diatom is an important pre-spring bloom species available as continuous food source for copepods and should not be neglected within the food web in the marine system around Helgoland. Rousseau et al. (2002) reported from the southern North Sea that *P. sulcata* and other small colony forming species characterise the early spring diatom community. This typical pre-spring bloom with the winter species goes along with low light and temperature conditions and higher concentrations of nutrients and coincides with the results found in our grazing experiments. This shows that the ecological importance of *P. sulcata* in the marine food web in the North Sea is not fully understood.

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GENERAL DISCUSSION

In this study the primary objective was the investigation of the ecological role of the marine centric diatom species *Paralia sulcata* at Helgoland Roads, North Sea also in order to provide a detailed description of its ecological niche. To do this we investigated three levels of organization: 1) the individual level, to determine the autecology of *P. sulcata*. These investigations included the growth experiments shown in Chapter II, 2) the population level including the multivariate analysis of the long-term data set (Chapter I, II) as well as the genetic diversity of *P. sulcata* (Chapter III) and 3) the community level where we specified *P. sulcata*'s ecological importance in the marine food web at Helgoland Roads (Chapter IV). The most important insights into the ecological role of *P. sulcata* can be summarised as follows:

1) The results from the laboratory experiment were not at all in agreement with the results found at the field. The autecological study on *Paralia sulcata* revealed an optimal growth at higher temperatures (ranging from 10 to 16°C) especially when silicate concentrations were not limiting. No growth occurred at 4°C. This is in contrast to the temperature range in field observations where highest abundances of *P. sulcata* were found in autumn and winter demonstrating that they can tolerate lower temperatures very well. However, the adaptation to low light and the positive correlation of *P. sulcata* with higher nutrient concentrations (especially silicate) is in agreement in, the laboratory and field observations.

2) The ecological behaviour of *Paralia sulcata* changed significantly over the last decades influenced mainly by temperature, light availability as well concentrations of silicate and phosphate. Thus, the changing temperature, light, nutrients and in general the hydrography regime at Helgoland Roads seemed to be responsible for the recent increase in abundance of *P. sulcata* especially in summer.

3) A shift in the ecological niche of *Paralia sulcata* was detected from a more specialised niche in the 1980s to a more generalised niche since the middle of the 1990. The seasonal observations at Helgoland Roads indicated a wide range of different environmental parameters that could be tolerated and therefore also possible changes in the marine environment whereas the smaller niche breadth in autumn and winter only indicated a narrow tolerance of the given environmental parameters.

4) One possible explanation for the occurrence of *Paralia sulcata* in the summer period could be the introduction of new clones adapted to warmer water temperatures. This was shown by the high genetic diversity within the *P. sulcata* population and the separation between winter and late summer-autumn isolates.

5) The observed trend towards a less seasonal appearance of *Paralia sulcata* leads to the conclusion that this diatom is now a 'baseline' diatom occurring in the water column throughout the year. Grazing experiments with an omnivorous copepod showed significant grazing on *P. sulcata* and therefore particularly the higher abundances in winter and early spring could mean that this diatom now provides a continuous food source for higher trophic levels.

To characterise the ecological niche of *Paralia sulcata* and to summarise the important environmental parameters influencing the occurrence of this diatom species in the next sections all results will be discussed in a general context with the available literature.

What are the long-term trends and effects of changing environmental conditions on the occurrence and autecology of *Paralia sulcata*?

Since 1996/1997 the occurrence of *Paralia sulcata* at Helgoland Roads has changed from a winter diatom to a less seasonal diatom, which occurs in the water column throughout the year. This means that the ecological niche of *P. sulcata* has changed from a more specialised species in the 1980s to a more generalised one in the mid 1990s (which was discussed in detail in Chapter I). The possible reasons for this changed pattern will be discussed in the following sections.

The main hypothesis that the changing weather conditions and also temperature, light and nutrient conditions at Helgoland Roads lead to an increase of the abundance of *Paralia sulcata* in the last decades was tested in this thesis using statistical methods.

The hydrography of Helgoland Roads is highly variable in the winter and late summer period with a lot of mixing (Wiltshire et al. 2008, Wiltshire et al. 2010) which seemed to positively influence the *P. sulcata* population in the water column. A significant increase in the mean and the maximal wind speed over the last decades at Helgoland Roads (own analysis, Table 1, and see also Wiltshire et al. 2008, Wiltshire et al. 2010) led to higher storm activities and therefore, to higher vertical mixing and turbulences

within the water column especially in summer. This mixing in turn led to a resuspension of *P. sulcata* from the sediment into the water column and is reflected in increasing abundances especially in summer times during the last 40 years. Due to this mixing, the nutrient concentrations were also highly available within the water column which supported the growth and the development. *P. sulcata* might survive on the sediment as well as in the pelagial in temperate coastal waters due to its tychopelagic life cycle (Roelofs 1984, Hobson & McQuoid 1997, Zong 1997, McQuoid & Nordberg 2003a). In agreement with the results found at Helgoland Roads, McQuoid & Hobson (1998) also described that *P. sulcata* can easily be sloughed off the sediment bottom during storm activities leading to a re-dispersal of the population over the year.

The higher abundances of *P. sulcata* especially during the summer coupled with the warming trend of the North Sea of 1.7°C over the last 40 to 50 years (Wiltshire et al. 2010) leads to the conclusion that the optimal temperatures for growth in this species are not necessarily the colder winter temperature but the warmer ones. The better growth at warmer temperatures was shown in the laboratory experiments and suggests that the optimal growth temperatures ranged between 10°C to less than 20°C. Low winter temperatures (in a mean of 4°C) can be tolerated but no growth of *P. sulcata* occurs. Long-term trends showed that warmer winter temperatures favour the growth of *P. sulcata* but higher summer temperature might limit the growth (Table 1). Despite the findings described above, the influence of temperature is complex and not all the results of this thesis are in agreement with the available data in the literature. The study of Hobson & McQuoid (1997) found that *P. sulcata* was present throughout the year with higher abundances in the winter months i.e. the abundance increased with cooler winter temperatures, short day lengths and higher salinity in the surface water, whereas Sancetta (1989) found only a low abundance of *P. sulcata* within the water column in winter times. An interesting contrast was shown by Choudhury & Pal (2010) were *P. sulcata* occurred with low cell abundances only in warmer summer months (April to July) with temperatures between 28°C and 32°C in the water column at the coast at the Bay of Bengal (Eastern India). The results from these studies and the results from this thesis showed that *P. sulcata* has a wide range in temperature tolerance and can cope with cooler water temperatures in the same way as with much warmer ones, but the optimal temperatures seemed to be in a range between 10°C to 20°C. Temperature seems to be one of the main factors influencing phytoplankton community.

Table 1: Significant correlations (Pearson correlation coefficient) of the abundance of *Paralia sulcata* with different environmental parameters measured at Helgoland Roads during the 2 years sampling campaign ("bottom" and "surface" water samples) and the long-term data from 1962 to 2008 ("long") for the four seasons. Significance level: $p < 0.05$, "+" indicated a positive correlation, "-" indicated a negative correlation, "0" means no correlation.

environmental parameter	Spring			Summer			Autumn			Winter		
	bottom	surface	long	bottom	surface	long	bottom	surface	long	bottom	Surface	long
temperature (°C)	0	0	-	0	-	0	0	0	-	+	0	+
salinity	-	0	+	0	0	+	0	0	+	0	0	0
Secchi depth (m)	-	0	-	-	0	-	-	0	-	-	0	-
silicate (µmol l^{-1})	+	0	+	0	0	-	0	0	+	0	0	-
phosphate (µmol l^{-1})	+	0	+	0	0	-	0	0	+	0	0	+
nitrogen (µmol l^{-1})	+	0	0	0	+	-	0	0	+	0	0	0
mean wind speed (Bft)	0	0	+	0	0	+	0	0	+	0	0	+
maximal wind speed (m sec^{-1})	0	0	+	0	0	+	0	0	+	0	0	+
sunshine (h)	0	0	-	0	0	0	0	0	-	0	0	-

Although important, temperature is of course not the only driver of *P. sulcata* distribution and abundance. For instance, laboratory experiments showed that higher concentrations of nutrients have a positive influence on the growth of *P. sulcata*. It was shown that *P. sulcata* was strongly dependent on the silicate concentrations reflected by the best growth when silicate concentration was not limited independently from the temperature (10°C or 16°C). This fact could be explained by the high silicate demand due to the strongly silicified valves of this diatom species (Crawford 1979a) and the requirement of silicate for its growth (Lewin 1962, Egge & Aksnes 1992, Bidle & Azam 1999). Furthermore, the field observations also confirmed that especially in the "growth" seasons (spring and autumn) for *P. sulcata* the nutrient concentrations were positively correlated with the abundance of this diatom species (Table 1). In contrast to this result is the significant decrease in the concentration of phosphate and dissolved inorganic nitrogen during the spring from 1962 to 2008. But the laboratory experiments showed that limiting phosphate concentrations at 10°C did not inhibit growth leading to the conclusion that *P. sulcata* is able to cope with limiting phosphate concentrations as long as silicate is available. Furthermore, Abrantes (1988a) and Bao et al. (1997) also showed a negative correlation of nutrient concentrations with the abundance of *P. sulcata*. However, not only the temperature but also other environmental parameters affected the spring bloom dynamics at Helgoland Roads.

Taking into account the long-term data set at Helgoland Roads the phenology of the spring bloom dynamic was investigated due to changes in nutrient concentration, weather conditions and zooplankton indicating a significant shift of the start of the spring bloom (Wiltshire et al. 2008).

How light availability affected the growth of *P. sulcata* was investigated also in an experimental set-up as well as on the field with the bottom sample. That humic substances can inhibit the growth of phytoplankton (dinoflagellates and pelagic diatoms) at higher concentrations due to the increased amount of yellow substances absorbing the light in coastal waters was shown by a study of Prakash & Rashid (1968) and Prakash et al. (1973). This is in contrast to the results found out in this thesis were high concentrations of humic acids positively influenced the growth of *P. sulcata*. The higher cell abundances of *P. sulcata* in the treatments with humic acid addition obtained from our growth experiments indicated an optimal growth at low light conditions which could be underpinned by the long-term data set analysis exhibiting a negative correlation to high light intensities (expressed as Secchi depth and sunshine duration) over 40 years (Table 1) and by e.g. Hobson & McQuoid (1997). Taking into account that *P. sulcata* as benthic species is highly adapted to live on the sediment; higher humic acid concentrations can be tolerated.

These studies from the literatures showed the occurrence of *P. sulcata* at different sampling sites and only for a short investigation period. Unfortunately, nothing is concluded about the shifts in the ecological role of this diatom species. In comparison to that, it was shown the regime shift in the North Sea strongly influenced the waters around Helgoland Roads over the last 50 years (Reid et al. 2001, Weijerman et al. 2005, Stockmann et al. 2010). Especially variations in the hydrography led to strong changes (e.g. warmer water masses coming more from the in the northwest, higher storm activities) in the North Sea where the phytoplankton species have to cope with the different environmental conditions. Therefore, the ecological investigations on *P. sulcata* can give a short insight into a shift in the ecological behaviour.

Despite the differences described above, some common patterns between our results and the literature occur and can be illustrated as follows:

1) Higher abundances were detected in winter times with temperatures more than 5°C and low light conditions in British Columbia fjords (Roelofs 1984, Sancetta 1989, Hobson & McQuoid 1997) and at Helgoland Roads (Table 1, Chapter I, II).

2) A positive relation with vertical water mixing and patchy nutrient concentrations were detected at the continental shelf of the west coast of Portugal (Abrantes 1988a), at the Bay of Vigo (northwest Spain) (Margalef 1969) and at Helgoland Roads (Table 1, Chapter I, II).
3) Furthermore, increasing relative abundance of *P. sulcata* in sediments and surface waters with increasing salinity were shown in British Columbia fjords (Roelofs 1984, Hobson & McQuoid 1997), Hudson Estuary (Weiss et al. 1978) and at Helgoland Roads, North Sea (Table 1, Chapter I, II).

Do genetically different populations of *Paralia sulcata* occurs at Helgoland Roads?

The shift in the ecological niche of *Paralia sulcata* over the last 40 years can also be explained by a second important hypothesis that especially the changing hydrographical conditions in the North Sea, e.g. regime shifts and thus, changing water masses at Helgoland Roads (Stockmann et al. 2010, Wiltshire et al. 2010) are responsible for the new occurrence of *Paralia sulcata* in summer time due to the introduction of a new population adapted to warmer water temperatures.
On one hand it is known, that new species can be introduced in the North Sea. The identification and monitoring of the phytoplankton species is currently mostly based on microscopic investigations of taxonomic characteristics. Moreover, morphological features can often be very similar and thus, differentiation next to impossible. Despite this, morphological characteristics of species are an important tool in phytoplankton taxonomy. Some examples for the introduction/ invasion of species into the North Sea are known, e.g. *Coscinodiscus wailesii* appeared for the first time at the end of the 1970s in the North Sea (Wiltshire & Dürselen 2004) and *Mediopyxis helysia* in the Wadden Sea (Kühn et al. 2006).
On the other hand introductions cannot not only occur at the species level but also within a species it is possible that new differently adapted clones can be introduced, causing a change in intra- rather than interspecific diversity. To investigate this, genetic markers can provide a fast and specific means for identifying differences in the intraspecific diversity and dynamics of phytoplankton population both on a spatial and a temporal scale (Medlin 1990). The hypothesis that different genetic populations of *P. sulcata* exist and therefore a high intraspecific diversity at Helgoland Roads maybe

due to the introduction of a warm-water adapted population of *P. sulcata* was evaluated with a molecular approach. A high genetic diversity was indeed detected and the ISSR-fingerprint analysis showed a separation between the January isolates from strains isolated from September to November (autumn) which could indicate the presence of two 'seasonal clones' adapted to different environmental conditions (Chapter III). However, a further potential reason for the changing *P. sulcata* distribution, namely the increasing storm activity within the summer period should not be neglected. Therefore, it could be possible, that naturally a high genetic diversity within the *P. sulcata* population exist and the increasing storm activity only lead to a well mixing of this present population. This could be a reason why *P. sulcata* occurs in the water column also in summer times, especially within the last 15 years. This higher mixing resuspends *P. sulcata* in the water column throughout the whole year, which might also be an explanation for a naturally existing high genetic diversity of this population within one year (Chapter II, III).

Another example for distinct populations of one species in relatively small location was shown by the study of Rynearson & Armbrust (2004). They discovered a high genetic diversity of *Ditylum brightwellii* populations using microsatellites and identified three distinct *D. brightwellii* populations related to different sampling locations. Furthermore, investigations on the species *Thalassiosira rotula* including the evaluation of the genetic diversity, physiological and morphological variability in response to changing environmental conditions were performed showing differences in the growth response of the *T. rotula* clones when cultured at different salinities (pers. comm. A. Kraberg).

These results mentioned above compared with the results of this thesis showed that genetically diverse populations of one species can exist in small areas and also on more global scale where diatom species are composed of many genetically and physiologically distinct populations. At the species level the worldwide distribution of *P. sulcata* could be related on one hand on the mixing processes, advection and other physical features in the marine environment. But it appears that within this globally distributed species distinct populations could develop, which might be an adaptation of *P. sulcata* to special local environmental conditions facilitating survival in geographic regions governed by very dissimilar environmental characteristics (e.g. occurrences in the North Sea compared with the British Columbia waters).

How important is *Paralia sulcata* within its marine food web and as possible food source for copepod grazers?

As *Paralia sulcata* is now occurring almost throughout the year at Helgoland Roads, one focus of this study was the question whether *P. sulcata* could be used as possible food source for copepods within a phytoplankton community during a spring bloom mesocosm experiment (Chapter IV). The results showed that *P. sulcata* is grazed significantly by copepod predators which is also in line with results found by Diodato & Hoffmeyer (2008). Unrevealing such biotic interactions is vital for an assessment of potential changes in the local plankton community as a result of climate change.

Rousseau et al. (2002) reported that *P. sulcata* occurred in the early spring bloom community present in mid March and within the autumn diatom community in September and October in Belgian coastal waters (Southern North Sea) which supports our observations of the occurrence of *P. sulcata* at Helgoland Roads. The notion that *P. sulcata* is could be a baseline (stable) food source throughout the year, providing a continuous food source of micro- and mesozooplankton predators especially in the pre-spring bloom situation and should not be neglected within the food web in the marine system around Helgoland. The occurrence of *P. sulcata* at the highest nutrient conditions, moderate water temperature (mean range from 7.5 to 14.9°C) and light regimes (mean range from 12 to 26 µE m^{-2} s^{-1}) recorded at the same periods (early spring and autumn) in Belgian coastal waters (Rousseau et al. 2002) are very well in agreement with our observations at Helgoland Roads.

Summary of the ecological role of *Paralia sulcata* at Helgoland Roads, North Sea

Based on the results of the experimental and long-term analysis (two years monitoring campaign and multivariate statistical analysis) I will now sum up the most important environmental parameters determining the occurrence of *Paralia sulcata* at Helgoland Roads. Based on the results discussed in the previous sections, I suggest a distinct change in the annual phase of *P. sulcata* at Helgoland Roads, which is influenced by different environmental parameters and also shows the adaptation of this diatom due to the changing habitat conditions (Fig. 1).

As shown by the growth experiments the best temperature and nutrient conditions were detected in spring and autumn with moderate temperature and higher amounts of

nutrients in the water column favouring the growth and development of *P. sulcata*. I suggest that two phases of *P. sulcata* can be observed at Helgoland Roads related to the benthic-pelagic life style of this diatom. It seems to be the case that spring a change from the pelagic to benthic phase and in autumn a change from the benthic to the pelagic phase takes place. Especially the increasing storm activity in autumn leads to a well mixed water column again and therefore to a resuspension of *P. sulcata* from the sediment which can explain the higher abundances of *P. sulcata* in the water column in autumn and winter.

As described in the literature and also shown by the results of our long-term analysis *P. sulcata* is adapted to live at lower temperatures, especially when nutrient concentrations were high and the light intensities were lower with short day lengths. In winter periods the occurrence of *P. sulcata* was supported by higher storm activities and mixing of the water column in the surface water. Typical for temperate coastal waters like the North Sea, there are generally lower abundance of phytoplankton in the water column (Wiltshire & Manly 2004). Hence, the biotic interactions e.g., competition with other diatoms is less or reduced. Thus, *P. sulcata* is able to survive in the water column very well (Table 1, Fig. 1, Chapter I, II).

In spring the increasing light conditions near the surface water have negative effects on the occurrence of *P. sulcata* (Table 1, Fig. 1) and thus, leading to a shift from the pelagic to the benthic phase. Factors that seemed to exert a positive effect on *P. sulcata* abundance were the high nutrient concentrations (especially silicate is needed for the growth) as well as to a slight degree the increasing temperature (see Chapter II, growth experiments). The high abundance of *P. sulcata* provided a continuous food source from the winter to the spring transition as pre-bloom species (see Chapter IV). Further, the biotic interactions in the water column strengthen due to competition for nutrients and a high grazing of the micro- and mesozooplankton on the phytoplankton community as a whole. The ecological tolerance of *P. sulcata* in this period is wider compared to the tolerance in the other seasons indicating that in this time *P. sulcata* can cope with a wide varying range of environmental conditions (Chapter II).

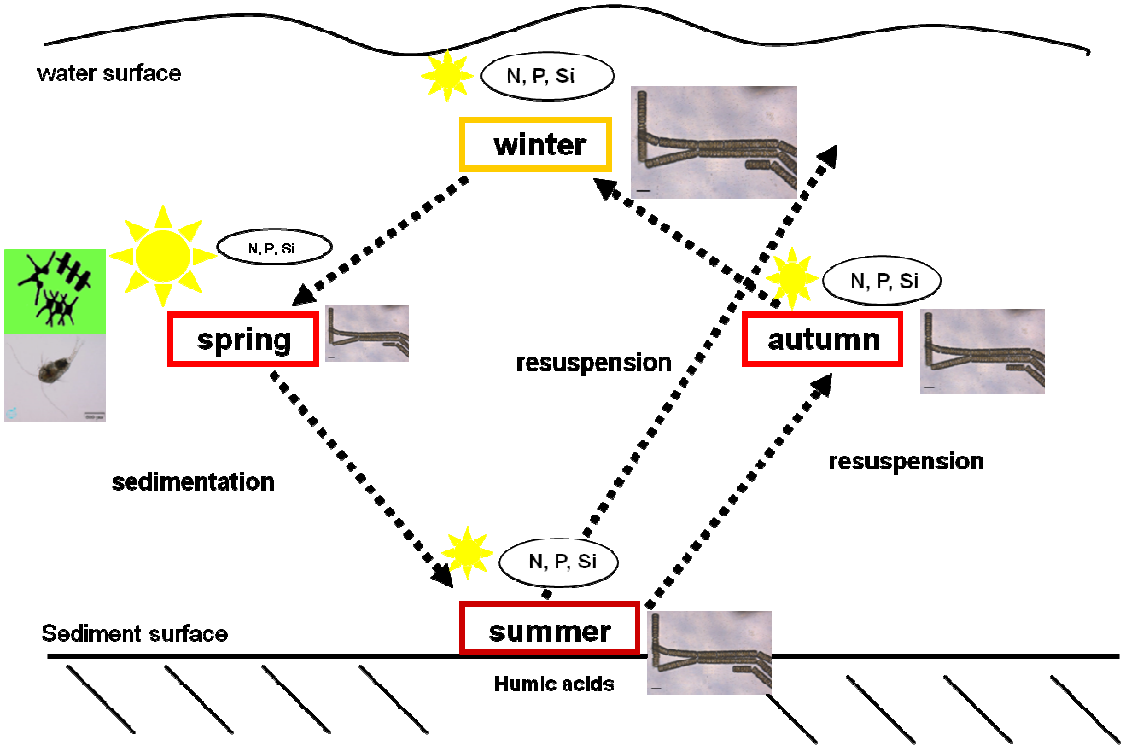

Figure 1: Schematic description of the results from the experimental and long-term data analysis of the ecological role of *Paralia sulcata* in the water column and sediment (as tychoplankter) and the most important environmental parameters influencing the occurrence at Helgoland Roads separated by the different seasons. The colour of the squares indicated the temperature whereas the orange colour means codler or decreasing temperatures (autumn-winter) and the red colour mean higher or increasing temperatures (bright and dark red) (from winter-spring-summer-autumn). The black dashed line from the sediment to the water surface indicated the resuspension of *P. sulcata*. The light conditions are shown as sun: low light conditions expressed as small sun symbol, higher light conditions are shown as larger sun symbols, note that the light conditions are spring is higher compared with the autumn. The nutrients (N = nitrogen, P = phosphate, Si = silicate) in bold indicate high concentrations, nutrients in plain font mean low concentrations. Biotic interactions were shown as copepod (grazing) and phytoplankton (competition). The abundance of *P. sulcata* is reflected in large or small pictures of this diatom in the different seasons.

In summer higher cell numbers were detected in the bottom water samples compared with the surface water samples. The summer period could therefore be characterised by the benthic phase of *P. sulcata* which is supported by the low light intensities on the bottom of the sediment surface. Temperature displayed no significant influence but also higher temperatures did not inhibit the growth of *P. sulcata*. Interestingly, all nutrients exhibited a significant negative influence on the abundance of *P. sulcata* at

this time. One possibility of this correlation could be the occurrence of humic acids in the sediment, providing benthic diatoms species with additional nutrients due to the formation of complexes with nutrient ions which could affect the transport of the ions to the diatoms (Lund 1990). We suggested that the nutrients can be taken up very fast by benthic diatoms (such as *P. sulcata*) so they were not detectable within the water column as dissolved inorganic nutrients. Furthermore, on the sediment re-mineralisation processes by bacteria are an important process (Bidle & Azam 1999) and quickly provides nutrients for the benthic diatoms. The nitrogen and phosphate re-mineralisation in the microbial loop is supported by the micro- and mesozooplankton (biologically mediated) and very rapidly (Officer & Ryther 1980). The recycling of silicate is strongly dependent on bacterial activity because the latter promote the silicate recycling near the sea surface bottom especially at higher temperatures (Bidle & Azam 1999).

The autumn is characterised by increasing storm activity and nutrients due to re-mineralisation processes as well as decreasing temperature and light conditions. Therefore, good conditions for the growth of *P. sulcata* were provided and a change from the benthic to the pelagic life style occurred. Due to turbulence the abundance of *P. sulcata* increased within the water column which is in line with the increasing nutrient amounts supporting the growth and development of this species. Furthermore, the day length and light availability decreased provided favourable conditions within the water column for *P. sulcata*.

What are the ecological implications of these findings? - An Outlook

Up to now the biotic interactions within the annual cycle of *Paralia sulcata* at Helgoland Roads and therefore its ecology were not well understood. A more detailed look at the food quality and preference for different predators should give new insights into the ecological role of *P. sulcata* within the food web at Helgoland Roads. Taking into account that e.g. the warming trend of the North Sea led to a shift of the start of the spring bloom (delay of spring bloom species) and therefore, the potential mismatch of food resources for predators (Wiltshire & Manly 2004, Wiltshire et al. 2010) *P. sulcata* could be one species that opposes this possible trend and becomes a more reliable food source. Another trend for a changing occurrence at Helgoland Roads was observed in a typical summer species (warm water temperatures adapted), *Guinardia*

delicatula, which widen the range of occurrence from only a summer species in the beginning of the 1960s and 1970s to a species occurred from March till November in the water column at Helgoland Roads in the end of the 1990s (Wiltshire et al. 2010).
Because *P. sulcata* now occurs throughout the year in the water column leading to the assumption that not only food resources will missing during special times of the year but rather a new food source can be provided. With regard to climate change, which is likely to cause a further warming of the water and taking into account the results of our experiments it could be assumed that *P. sulcata* is slowly approaching optimal growth conditions and therefore this diatom species might become more and more important within the marine food web at Helgoland Roads. To evaluate this more investigations are needed, especially in combination with other phytoplankton species and the changing environmental conditions at Helgoland Roads.
This thesis pointed out that changing optima in growth/ occurrence of *P. sulcata* occurred over a long investigation period of around 40 years and that this is strongly related to changing environmental conditions (temperature, light regime, and nutrient concentrations). The worldwide distribution and the adaptation to a wide range of environmental parameters make it interesting to try to differentiate populations of *P. sulcata* genetically. It can be hypothesised that genetically different populations existed due to different location (geographical) and altered adaptations on the environmental parameters (biological). Another molecular method (e.g. ITS regions) might be used as well as ISSR or microsatellites to determine the different strains to get more details about the geographical distribution of *P. sulcata* along the coastal areas within the oceans. Integrated methods such as multivariate analysis of long-term data sets combined with laboratory studies and especially genetic investigations provide an important approach to investigate the ecology of species.
One implication of the broad range of environmental conditions this species can tolerate is that it might actually be quite difficult to use it as specific paleoindicator to reconstruct past climates. Thus, a careful use of *P. sulcata* with the interpretation of the ecological niche is necessary.

SUMMARY

This thesis investigates the ecological role of *Paralia sulcata*, a ubiquitous centric marine diatom species, in its marine habitat at Helgoland Roads, North Sea. In order to determine the ecological role of a species it is important to understand its ecological niche. In order to characterise the ecology of a species it is important to understand the habitat and environmental conditions in which this species lives. To achieve this long-term data sets are absolutely crucial. Taking this into account, one focus of this thesis was to investigate the autecological behaviour of *P. sulcata* in more detail with laboratory experiments and compare the results with a field sampling campaign as well as the long-term data set provided at Helgoland Roads. The main questions were: What are the long-term trends and effects of changing environmental conditions on the occurrence and autecology of *P. sulcata*? Do genetically different populations of *P. sulcata* occur at Helgoland Roads? How important is *P. sulcata* within the marine food web and as a possible food source for copepod grazers?

A multivariate statistical analysis of the long-term data was used to determine the important environmental parameters influencing the occurrence and therefore the ecological niche of *P. sulcata*. Moreover, a detailed two year sampling campaign compared the abundances of *P. sulcata* in different water depths. Laboratory experiments to determine the autecological role and therefore the optimal living conditions of *P. sulcata* were carried out. The autecological study on *P. sulcata* revealed an optimal growth at higher temperatures (ranging from 10 to 16°C), especially when silicate concentrations were not limiting. No growth occurred at 4°C. This is in contrast to the temperature range in field observations where highest abundances of *P. sulcata* were found in autumn and winter demonstrating that this diatom species can tolerate lower temperatures very well. However, the adaptation to low light and the positive correlation of *P. sulcata* abundances with higher nutrient concentrations (especially silicate) is in agreement in the laboratory and field observations. Furthermore, the ecological range of *P. sulcata* changed significantly over the last decades from a more specialised species in the 1980s to a more generalised species, adapted to a wide range of environmental parameters. Since the middle of the 1990 *P. sulcata* is influenced mainly by temperature, light availability as well concentrations of silicate and phosphate.

These results of the laboratory and especially the field sampling data revealed different behaviours of *P. sulcata* during the seasons. As a consequence, a new hypothesis was

developed to answer whether genetically different populations of *P. sulcata* occurred at Helgoland Roads. Using an ISSR (inter simple sequence repeat) fingerprint method, a high genetic diversity of different *P. sulcata* strains and a separation between January and September to December strains was detected at Helgoland Roads. On one hand a possible explanation for the occurrence of *P. sulcata* especially in the summer period seemed to be the introduction of new clones adapted to warmer water temperatures. On the other hand, the increasing storm activity which occurred at Helgoland Roads lead to a thorough mixing of the water column and therefore, to a mixing of the existing *P. sulcata* populations, which consists of naturally highly diverse clones.

Additionally, the community structure especially during the spring bloom development was investigated and the role of *P. sulcata* as food source in the marine food web in the North Sea was estimated during a mesocosm spring bloom experiment. The observed trend towards a less seasonal appearance of *Paralia sulcata* leads to the conclusion that this diatom is now a 'baseline' diatom occurring in the water column throughout the year.

Integrated methods such as multivariate analysis of long-term data sets combined with laboratory studies and especially genetic investigations provide an important approach to investigate the ecology of species.

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DECLARATION

Herewith, I declare that this thesis is my own work and effort and has been written independently. All other sources of information and literatures have been used, these have been cited and are shown in the references. Moreover, I declare that this work has not been submitted to any University for the conferral of a Degree.

Christina Gebühr