The coastal ecosystem of Kongsfjorden, Svalbard. Synopsis of biological research performed at the Koldewey Station in the years 1991 - 2003

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Introduction

Ny-Ålesund (78°55' N, 11°56' E), the world's northernmost permanent human settlement, is situated at Kongsfjorden on north-western Spitsbergen, the largest island of the Svalbard archipelago. Surrounded by mountains and glaciers, Kongsfjorden itself consists of several zones from calving glacier fronts to rocky shores and soft bottom, providing a rich variety of different habitats and, thus, excellent opportunities for marine research. Ample research possibilities are also provided in the terrestrial environments in the Kongsfjorden area: The vegetation varies from bleak 'Arctic desert' to lush tundra and grassland communities composed of many species occurring also in the European Alps.

For half a century, the commercial basis of Ny-Ålesund was coal mining. Since over 30 years, however, Ny-Ålesund has been used as a research base for natural sciences. In 1970, the research station of the Norsk Polar Institutt began operation. Since then, the location became an important place for research of many nations and disciplines. The research activities increased, especially in the 90s, when new permanent and seasonally active stations came into operation. A few years ago, Ny-Ålesund was part of the Large Scale Facility Programme of the European Community. An advantage of Ny-Ålesund is that it is easily accessible and has a well-developed infrastructure. Combined with the unique natural surroundings, Ny-Ålesund is an optimal base for intense scientific activities in the Arctic environment.

Almost exactly 13 years ago, in August 1991, the Koldewey-Station in Ny-Ålesund was opened as the permanent research station of the Alfred Wegener Institute for Polar and Marine Research in Ny-Ålesund, representing the national German research platform in the Arctic. The station was named after Carl Koldewey, the leader of the first German Expedition to the Arctic in the year 1868. A major focus of research at Koldewey Station is monitoring of the atmosphere. Since 1992, the Koldewey Station is part of the global "Network for Detection of Stratospheric Change" (NDSC). It supplies key long-term observation data essential for improving our understanding of chemical and physical processes in the atmosphere. In 1995, a new laboratory for this purpose entered service, featuring a special roof design that permits the installation of optical equipment. In 2003, the German and French stations in the Ny-Ålesund area joined and formed a Joint French – German Polar Research Platform in order to conduct joint research projects in the areas of atmospheric chemistry, climate change and marine and terrestrial biology. The Ny-Ålesund Marine Laboratory, jointly planned by eight research institutions from different nations interested in marine biological studies, will be opened in 2005. It will be the northernmost marine laboratory in the world, meet high international standards and serve as the basis for marine biological projects in Kongsfjorden. So far, biological research has been performed in the "Nansen Laboratory", a small laboratory owned by the Norsk Polar Institutt and consisting of three relatively small rooms in the Old Power Station.

As explained above, although the main research focus of the Koldewey Station was initially concerned with atmospheric studies, biologists used this research platform since the opening of the station in 1991. A few years later, studies on the effects of global changes mainly on marine biota were performed in collaboration with the atmospheric working groups. So it seems appropriate to summarise the biological work, which has been performed within a large number of projects at Koldewey Station since the opening of this German Arctic research platform in conjunction with the related research fields and in collaboration with scientists from Argentina, Australia, Austria, Norway, Russia, Spain, Sweden and The Netherlands.

Chapter 1 addresses the environment of Kongsfjorden and links biological research to physical studies. Stratospheric ozone depletion is one major issue as it determines the UV radiation to which organisms on land and in the water are exposed. Two papers deal with this topic. The third paper focuses on the seasonal development of sea ice and its optical surface properties. Although Polar Regions are believed to belong to remote places, radionuclide contaminants can be measured here as shown in the last paper of this chapter.

Chapter 2 focuses on ecological studies of the structure and function of the ecosystem. The first paper deals with the temperature demands and the phylogeny of snow algae, the second with the structure and zonation of marine benthic macroalgae, representing the major primary producers in Kongsfjorden. This paper relates closely to the next one on the interactions between macroalgae and herbivores. Palatability and chemical ecology of invertebrates of Kongsfjorden is the focus of paper five. A sponge community composed of several species new for Svalbard is described in the next paper. Two papers on soft bottom community structure and diversity complete this chapter.

Chapter 3 on ecophysiological studies on key organisms in the ecosystem contains three papers. The first focuses on a comparison between the physiological differences between terrestrial plants with an Arctic – Alpine distribution. The second examines the enzymes involved in nutrient assimilation of macroalgae from Kongsfjorden and the last describes the ecophysiological characteristics of two diatom species epiphytic on marine benthic macroalgae.

Chapter 4 summarises the most striking results on the effects of enhanced UV radiation on Kongsfjorden biota, a major research topic for the last 10 years. The first paper focuses on the dependence of photosynthetic and bacterial activity in the water column on the irradiation conditions. The following seven papers deal with the impact of UV radiation on macroalgae from the cellular up to the organismic level. The first two of these focus on the damaging impact of UV radiation on photosynthesis and the DNA and give insight into the acclimation processes and repair mechanisms. How red algae are protected by UV-absorbing mycosporine-like amino acids against UV radiation is described in the next paper, followed by two papers on how marine macroalgae cope with reactive oxygen species formed under various stress conditions including exposure to UV. The balance between the various damaging effects of UV

radiation and the repair and protection mechanisms is shown in the integrative parameters growth and reproduction as explained in papers 6 an 7 of this series. The last paper of this chapter shows that herbivorous amphipods from Kongsfjorden exhibit a better UV tolerance than carnivorous species because herbivorous amphipods were able to accumulate UV protecting substances from macroalgae.

Chapter 5 contains two papers on the feedback mechanism from the biosphere to the atmosphere. Whereas the effects of the atmosphere (e.g. enhanced UV radiation) on the biosphere have often been studied in the past, relatively little is known about the interaction of the biosphere and atmosphere in the opposite direction. The two papers of this chapter examine the biogenic production of organohalogenic compounds in macro- and microalgae, compounds, which can potentially participate in the destruction of the stratospheric ozone layer.

My hope is that this book is not only an overview about the biological research performed so far at the Koldewey Station. It should also present a baseline for future research in collaboration with scientists from other institutions working together in the new Marine Laboratory in Ny-Ålesund. I would like to thank all contributors for submitting their papers to this synopsis and all involved referees for their constructive criticism.

Bremerhaven, 17. July 2004

The Editor

1. THE ENVIRONMENT OF KONGSFJORDEN

Stratospheric Ozone Losses over the Arctic

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Introduction

Since the detection of the Antarctic ozone hole in 1985 (Farman et al., 1985) the polar regions were in the focus of ozone research. After two decades of active research the mechanisms that lead to the formation of the Antarctic ozone hole are largely understood today. Rapid ozone loss in the polar lower stratosphere is due to chlorine and bromine catalysed reaction cycles (WMO, 2003). Nearly all of the chlorine and a substantial fraction of the bromine in the stratosphere originates from the breakdown of man-made chlorofluorocarbons (CFCs) and halons. Beside the anthropogenic origin of these substances there are, however, also natural sources of volatile organohalogens (Laturnus, this issue). Effective ozone destruction occurs in the presence of reactive chlorine radicals and sunlight. In extra-polar regions nearly all of the stratospheric chlorine is tied up in passive molecules that do not react with ozone. But during polar winter the stratosphere cools and large low pressure systems form over the polar regions, the polar vortices, which are encompassed by strong jet streams that isolate the air mass inside from mid-latitude air. When temperatures inside the vortex drop below ~195 K mixtures of H_2SO_4, HNO_3, and water condense to droplets or crystals which form so called Polar Stratospheric Clouds (PSCs). Chemical reactions on the surface of PSC particles release chlorine radicals from the passive reservoir species and convert a large fraction of the total available chlorine into reactive radical species. Rapid ozone loss starts in spring when intensive sunlight returns to these air masses. The most effective process to deactivate chlorine and to stop ozone loss requires the presence of gas phase HNO_3 and sunlight. But during winter PSC particles can grow to large sizes and may fall out of the relevant layer of air if conditions are sufficiently cold over an extended period. With the sedimentation of HNO_3 containing PSC particles most of the available HNO_3 can be removed from the layer irreversibly, a process that is called denitrification. Under highly denitrified conditions effective deactivation of chlorine radicals is not possible and ozone loss continues until ozone is completely destroyed. Only then another deactivation mechanism becomes effective and the passive chlorine reservoir is restored. During the transition to the stratospheric summer circulation the polar vortex breaks down and mixing in of ozone rich air from mid-latitudes fills the ozone hole.

By the late eighties it was not clear whether ozone depletion can take place not only over the Antarctic but also above the Arctic and whether chemical loss of ozone plays a role there. Wave activity in the Arctic stratosphere is much stronger compared to the Antarctic, and the Arctic polar vortex is less stable, warmer and breaks up earlier than its Antarctic counterpart. Temperature and vortex strength show a pronounced year to year variability (e.g. Pawson et al.,

1995). In some Arctic winters the vortex breaks up in mid-winter, causing dramatic increases in polar temperatures (Scherhag, 1952; Naujokat 1992).

To study the Arctic ozone layer the Alfred Wegener Institute started regular balloon borne ozone soundings at the Koldewey station in 1988 (Schrems, 1992). The ozone sensors are launched with standard radio sondes and measure the ozone partial pressure and meteorological parameters up to 30-38 km altitude, where the sounding terminates by the burst of the helium filled balloon. The data are transmitted to the ground station during ascend. Ozone volume mixing ratios can be calculated from the measurements with a vertical resolution of about 150 m. The overall amount of ozone present in a vertical column of air between the ground and the space can be calculated when a correction for the small amount of ozone in the atmosphere above the burst level of the balloon is made. This quantity is termed the total ozone column and is measured in Dobson Units (DU; 1 DU = 2.69 x 10^6 $cm^{-2)}$. 100 DU correspond to a 1 mm thick layer of pure ozone under standard surface pressure and temperature.

Chemical loss of ozone in the Arctic stratosphere

The Arctic ozone layer is very variable and measurements from any individual station reflect the combination of short term fluctuations by advection of variable amounts of ozone, long term net transport effects and potential in situ chemical loss of ozone. The three profiles shown in Figure 1 illustrate the dramatic effect that short term advection can have on the local ozone profile. Fluctuations like the one shown in Figure 1 are common and on a time scale of a few days or a couple of weeks they completely hide any signal from chemical loss. On longer time scales net transport effects become important: net downward transport advects air with high ozone mixing ratios from above where it is replaced by ozone rich air from lower latitudes. At low levels air relatively poor in ozone leaves the polar regions. This polar subsidence is a net source of ozone at each individual level and in the total column and can mask the effect of chemical loss. The main difficulty in assessing chemical ozone loss in the Arctic is to separate the ozone changes induced by chemical reactions from these transport effects.

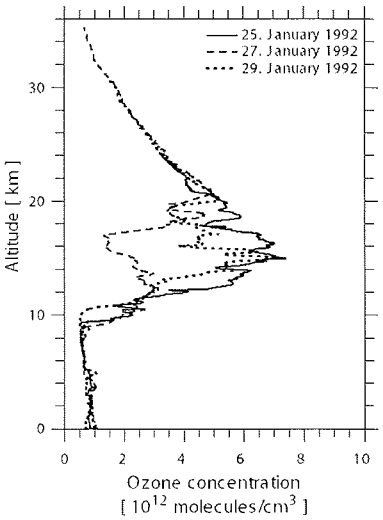

Figure 1 Three ozone profiles measured within four days in January 1992 at the Koldewey Station (78.9°N, 11.9°E). The rapid fluctuation seen here is due to dynamical effects.

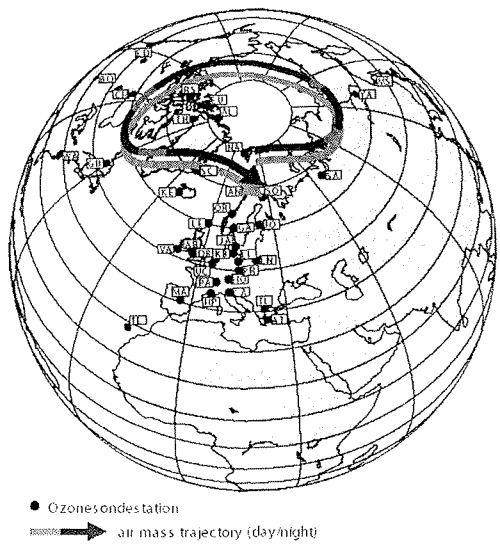

Figure 2 Illustration of the match technique and map of the participating ozonesonde stations. The arrow shows the path of an air mass that was first probed by a sonde from Ny Alesund (NA) and five days later again by a sonde from Sodankylä (SO).

To precisely measure the rate of chemical ozone loss in the Arctic stratosphere we have developed the so called Match approach. The technique is based on the statistical analysis of a large number of 'matches'. A match is defined as a pair of ozonesonde measurements, where both sondes probed the same air parcel at different times, as it passed over the respective sounding site. The basic idea of the Match approach is illustrated in Figure 2. To identify the matches, calculated air parcel trajectories that take into account modelled diabatic subsidence rates were used to track the motion of the air parcels between the measurements. The approach can be applied in two ways: (a) for post campaign analysis of a very large number of uncoordinated ozonesonde soundings by selecting soundings which are linked by chance based on calculated trajectories [Rex, 1993; von der Gathen et al., 1995; Rex et al., 1998], or (b) much more effectively as a joint effort by a large number of stations that perform coordinated ozonesonde launches during a 'Match campaign' [e.g. Rex et al., 1997; 1999; 2002, Schulz et al., 2001]. The analysis includes various quality controls on ozone sonde data as well as trajectory data. The main advantage of this Lagrangian approach is that chemical and dynamical effects can be separated to a high degree. The ability of the Match technique to account for dynamical changes in ozone without introducing a systematic bias is demonstrated by a statistical analysis that shows that inferred change of ozone is close to zero during periods of darkness along the trajectories [Rex et al., 2003]. Match results, based on the coordinated launches of 600 to 1400 ozonesondes per winter, are now available for 10 winters between 1991/1992 and 2002/2003. A map showing the locations of the participating stations is also shown in Figure 2.

With the Match approach we were able to prove that chemical ozone loss indeed occurs in the Arctic (Rex, 1993; von der Gathen et al., 1995). The method allows the quantification of the vertical distribution and seasonal variation of ozone loss rates with a vertical resolution of a couple of kilometers and a time resolution of about two weeks. We found that periods of rapid

chemical loss of ozone were always triggered by cold periods with extensive PSC formation. During warm winters, when the PSC formation temperature was hardly reached no significant loss occurred (e.g. winter 1998/1999). The largest ozone loss on record occurred in winter/spring 2000, the coldest winter since 1991/1992. Figure 3a shows an altitude time section of the accumulated ozone loss between early January and late March 2000. Dotted lines show how air masses subside through the figure. A profile of the overall ozone loss in the subsiding air masses at the end of the winter is shown in Figure 3b. Figure 3c shows the vertical integration of the accumulated ozone loss, i.e. the loss in the total ozone column.

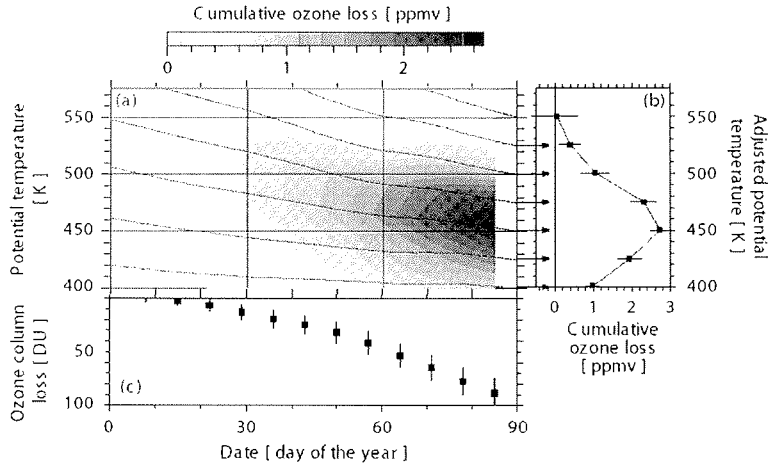

Figure 3 (a) Evolution of the accumulated ozone loss in subsiding air masses; (b) Profile of the accumulated ozone loss end of March 2000; (c) Accumulated chemical ozone loss in the partial column between Θ=400 and 575 K. Potential temperature (Θ) is a convenient vertical scale in the stratosphere. Θ=400 and 575 K correspond approximately to 15.5 and 23.5 km altitude.

Figure 4 (left panel) shows the vortex averaged ozone profile at the end of the Arctic winter 1999/2000 (solid curve). From our measurements of accumulated chemical ozone loss we can reconstruct the ozone profile that would have been present at the end of the winter without chemical loss (dotted line). The shaded area illustrates the substantial impact chemical loss had on the Arctic ozone profile in spring 2000. At about 19 km altitude, where usually the maximum of the ozone profile is reached, about 70% of the local ozone was lost. But even in the extremely cold Arctic winter 1999/2000 the situation in the Arctic was quite different from the typical ozone loss in the Antarctic, which is illustrated in the right panel of Figure 4.

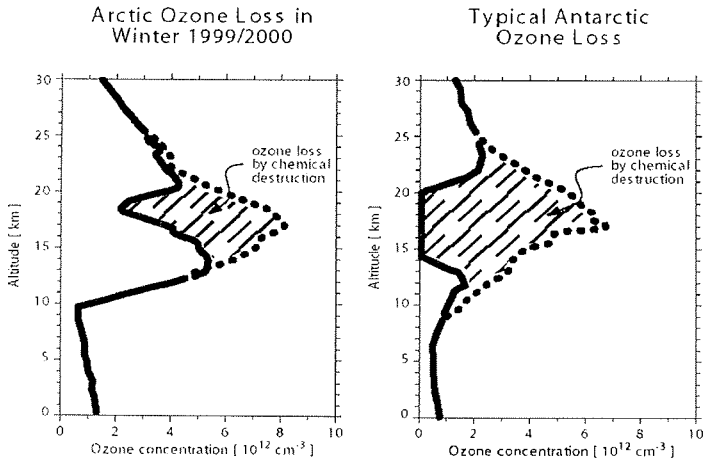

Figure 4 Comparison of ozone loss in the Arctic winter of 1999/2000 (left) with typical Antarctic losses (right). The solid lines give the average ozone profile at the end of the winter. The ozone profile that would have been present at the same time in the absence of chemical loss is indicated by the dotted line. The shaded area illustrates the impact of chemical ozone loss on the late winter ozone profile.

Impact on total ozone

The quantity that is most directly linked with UV levels at the ground is total ozone. Figure 5 shows the seasonal and interannual variation of the total ozone column at the Koldewey station compared to data from the Neumayer station in the Antarctic.

During summer and fall both data sets are quite similar, showing total ozone columns around 290 DU with little interannual variation. In winter and spring the time series are dramatically different. In the Antarctic the evolution of the total ozone column is dominated by the formation of the ozone hole – a drop of the total ozone column to values around 100-150 DU with only one exception in 2002 when a

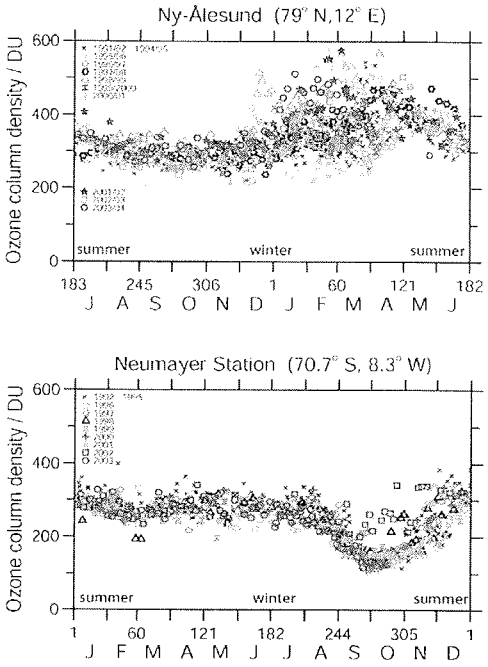

Figure 5 Seasonal and interannual variation of the total ozone column above the Arctic (Koldewey Station, Ny Alesund) and the Antarctic (Neumayer station).

midwinter warming occurred in the Antarctic for the first time since observations began in 1957. In the Arctic the winter/spring total ozone column is characterized by tremendous interannual variability with March average total ozone columns ranging from about 300 to 500 DU for the different years. On average, Arctic winter/spring is characterized by an increase in total ozone that is the result of dynamical supply of ozone to high latitudes with the slow poleward and downward motion of air with the stratospheric residual circulation. We can now separate the individual contributions of chemical loss and dynamical supply to the overall total ozone evolution through the winter. Figure 6 shows the dynamical and chemical contributions to the change of the total ozone column between October and late March and their respective year to year variability. Both quantities are very variable. Chemical loss destroyed between zero and about 100 DU ozone over the course of the different winters. So far chemical loss was always at least balanced by dynamical supply of ozone to the Arctic, that ranged between about 100 and 200 DU. Hence the total abundance of the ozone above the Arctic does not decrease over the course of the winter despite substantial chemical loss. In contrast to the Antarctic the effect of chemical loss in the Arctic is rather to cut off the climatological seasonal peak of total ozone in spring rather than to produce an ozone hole.

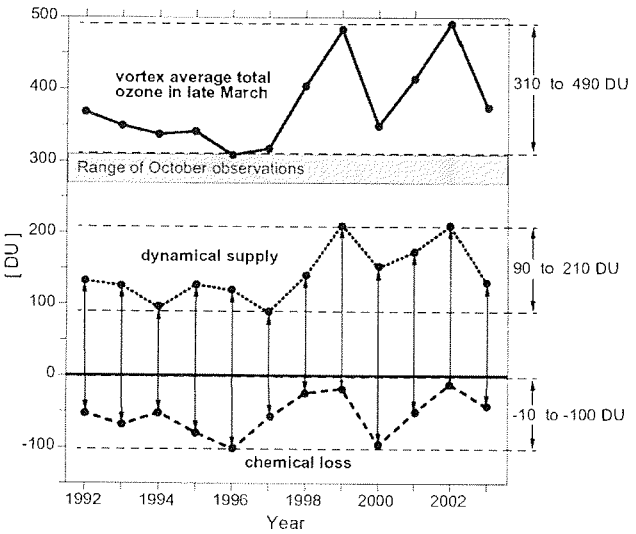

Figure 6 Dynamical (dotted line) and chemical (dashed line) contributions to the interannual variability of the late winter Arctic ozone column (solid line).

Long term evolution and thoughts about the future

Work by Crutzen, Molina, and Rowland in the 1970ies led to early concerns that the emissions of CFCs can harm the ozone layer. The discovery of the Antarctic ozone hole in 1985 (Farman et al., 1985) rapidly led to international agreements for the protection of the ozone layer. In the Montreal protocol from 1987 and various later amendments (e.g. Kopenhagen 1992, Montreal 1997) the

production of CFCs, Halons and a number of other chlorine and bromine containing substances (HCFCs, methylbromide) were strictly controlled and the worldwide production of the main anthropogenic suppliers of chlorine and bromine to the stratosphere basically phased out over the recent years. As a result of these protective measures stratospheric chlorine loading has peaked around the year 2000 and will now slowly decrease. But during the next decades the slow breakdown of CFC molecules that have already been released into the atmosphere over the past decades will continue to be a source of stratospheric chlorine. Since the atmospheric lifetime of CFCs is extremely long, it will take about half a century until chlorine levels will eventually fall below critical values. If the international agreements for the protection of the ozone layer hold and are strictly enforced we can expect that polar ozone loss will come to an end sometime around the middle of the current century. But on the time scale of a couple of decades the variability of Arctic ozone loss is entirely driven by the variability of stratospheric temperatures in Arctic winter. Figure 7 shows the interannual variability of total column loss and the average volume of air below the PSC formation threshold (V_{PSC}) over the past decade. A close quantitative relation exists between these two quantities. The slow variation of the stratospheric chlorine loading is not very relevant on these time scales. Hence, the future of the Arctic ozone layer over the next few decades will mostly depend on the evolution of stratospheric temperatures and V_{PSC}.

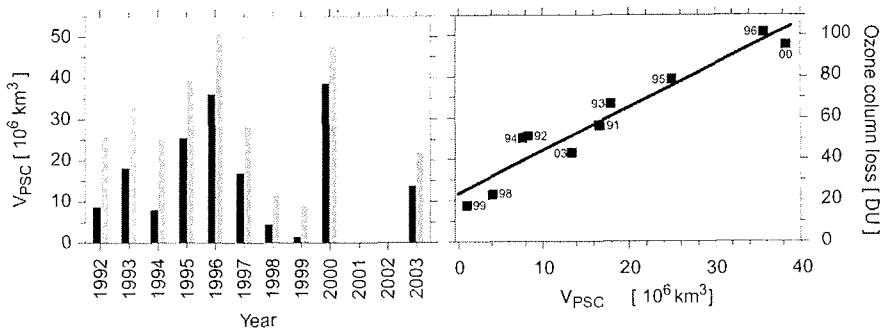

Figure 7 left panel: Variation of V_{PSC} (black bars) and ozone column loss (gray bars; scale is on the left side) over the past decade. Right panel: scatter plot of ozone column loss versus V_{PSC}.

Figure 8 shows the long term evolution of V_{PSC} over the past four decades as calculated from meteorological data. While the frequency of warm winters with little PSC occurrence has not changed, cold winters became significantly colder since the 1960ies. The maximum values of V_{PSC} reached during cold winters increased by a factor of three during this period. Large ozone losses during some winters in the 1990ies were the result

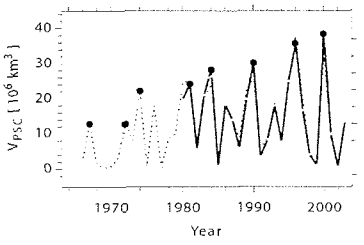

Figure 8 Long term evolution of V_{PSC} from ECMWF data (solid line) and FU Berlin data (dashed line). The maximum values during any five year intervals are marked and a linear fit through these points is shown (gray).

of the cooling trend. Had the climate conditions not changed since the 1960ies, Arctic ozone loss would not be of much concern to us today.

Cooling of the Arctic stratosphere is qualitatively consistent with the direct radiative effect of increasing greenhous gas levels in the atmosphere. However, Arctic stratospheric temperatures are the result of radiative and dynamical effects and it is currently not possible to unambiguously attribute the observed cooling trend to rising greenhouse gas concentrations. Should the cooling continue into the future, Arctic ozone loss may become worse over the next couple of decades before the healing effect of the Montreal Protocol will eventually unfold.

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The radiation, temperature and salinity regime in Kongsfjorden

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Introduction

The basic geographical and geophysical characteristics of the Arctic seas are low temperatures, pronounced seasonal variations of the light regime, salinity, temperature coupled with long periods of ice and snow cover. Due to influx of warm, nutrient rich water from the south (the so-called Spitsbergen current), the area of Spitsbergen is relatively mild and humid. This is one reason that the seas of the European Arctic belong to the most productive seas in the world (Orheim et al. 1995). The study site, the Kongsfjord, is a unique marine coastal system located at the north-western coast of Spitsbergen (78°55'N 11°56'E, Norway). An underwater flora composed of at least 50 macroalgal species (Wiencke et al., this issue) exists, similar to the vegetation of the more southerly located Isfjorden described by Svendsen (1959). For a better insight into the marine underwater light environment, a multidisciplinary research program has been performed in Kongsfjorden (Hanelt et al. 2001). In this context, the seasonal variation of the daily solar irradiance, light transmittance into the water body, with emphasis on the UV-B radiation range, are described to estimate possible implications on the primary productivity of the benthic macroalgae in further studies. Salinity and temperature measurements within the water body were also included in order to characterize the strong influence of melt water on the light regime in the fjord.

Solar radiation is a prerequisite for life on earth. Plants use a certain wave-band (about 400–700 nm) of the impinging radiation on earth to supply photo-synthesis with energy which is called photosynthetically active radiation (PAR). This range is dependent on the absorption characteristic of the pigments in-volved in photosynthesis. PAR constitutes about 45 % of the energy in the direct solar beam at the Earth's surface when the solar elevation is more than 30° (Kirk 1994). The wavelengths longer than the PAR range consist mainly of the infra-red-band and the shorter wavelengths of the ultraviolet-band (UV). UV is only minimally used for photosynthetic energy supply of plants, but has an important controlling function or, especially in the case of UV-A, is necessary for e.g. DNA repair processes. The UV-radiation is divided in three wavelength ranges: UV-C (200–280 nm), UV-B (280–315 nm) and UV-A (315–400 nm) according to the CIE definition (Commission Internationale de l' Eclairage, 1935). However, for practical reasons most biological and environmental researchers define UV-B as a range between 280 – 320 nm as radiation filtering material is generally available with a cut off of wavelengths around 320 nm (Franklin et al. 2003). Moreover, no quanta in the range of 280 – 290 nm are detectable in the solar spectrum reaching the earth's surface (Jordan 1996, and see chapter below).

Thus, the shortest wavelengths in the above defined UV-B range does not affect the ecosystems on earth. Depletion of stratospheric ozone over the Arctic region (Müller et al. 1997, Rex et al. 1997) may cause an increase in harmful UV-B radiation at this high latitude and could affect algal distribution patterns. Stratospheric ozone depletion results in very specific increases of UV-B radiation between 290 and 315 nm (Holm-Hansen et al. 1993). However, the impact of UV-B < 300 nm is much stronger than the small increase of the irradiance in this range would suggest (Frederick et al. 1989). Moreover, a shift of some nanometers of the impinging radiation to shorter wavelengths was observed during strong ozone depletion in Antarctica (Holm-Hansen et al. 1993). Many macromolecules have a different absorption within the UV-B range so that for biological systems the wavelength dependency of the response needs to be based on a spectral biological weighting function (BWF) (Wängberg et al. 1996). The BWF is comparable to an action spectrum which describes the biological sensitivity of organisms to UV-B, and was determined for photoinhibition by Jones and Kok (1966) for a general plant response (Caldwell 1971) or for DNA damage (Setlow 1974). Although those investigations were partly done when UV-research started, these spectra are still used for calculations of BWF's. Calculation of the biological effective radiation shows that although the increase in irradiance and the wavelength shift in the UV-B band is small, the effect on plants is tremendous and much stronger as the visible change in the solar spectrum due to ozone depletion shows.

Area of investigation and radiation measurements

The Kongsfjord is part of the north-western coast of Svalbard (78°55'N, 11°56'E) with a length of about 26 km. It extends from north-west to south-east into the inland. The width ranges from about 3 km to about 8 km with a maximal depth of about 400 m. The coast is mostly steep and rocky with shallower soft bottom parts caused by strong deposition of sediments from four glaciers. Rivers and glaciers discharge high amounts of sediment and freshwater loaded with fine sediments resulting in high water turbidity and salinity stratification of the water body during the summer months. The tidal range in the fjord is about 2 m and its current is weak (Ito and Kudoh 1997). The polar day in the Kongsfjorden region begins on the 21st of April and ends on the 22nd of August, the polar night lasts from the 26th of October up to the 14th of February.

The inner fjord is generally free of ice cover, at least during summer, due to the mild climate influenced by the Westspitsbergen current. In the middle and outer part of the fjord, approximately from the 100 m depth contour outwards, a stable ice cover does not develop in winter during most years. Thin pack ice is shifted out of the fjord by wind, and the fjord surface maintains open water characteristics throughout the winter (Ito and Kudoh, 1997). Air temperature is higher than usual for those at the high latitudes, with an annual mean temperature ranging from -15°C in winter to about 5°C in summer (Svendsen et al. 2002). The annual mean water temperature is generally slightly above 0°C (Ito and Kudoh, 1997).

With the instruments of the Baseline Surface Radiation Network (BSRN) of the AWI, global radiation (305-2800 nm) is measured continously with a CM11-pyranometer (Kipp & Zonen, Delft, Netherlands), UV radiation (300-370 nm) using

a TUVR-photodiode detector (Eplab, Newport, VT, USA) and sunshine duration (SSD) using a Solar 111 sunshine detector (Haenni, Switzerland). The measurements are carried out about 15 m above sea level, in co-operation with the Norsk Polar Institute in Ny Ålesund, Spitsbergen (for further description see Koenig-Langlo and Marx 1997). The highest yearly fluence of visible and UV radiation within a 3-year measuring period occurred in 1998 (Tab.1). Maximal irradiance always occurs during June and July caused by the high sun angle with a maximum of the daily average of 170 for visible, 16.8 for UV (300-370 nm) and 0.27 W m^{-2} for only UV-B radiation in air, which means a maximal daily fluence for UV-B of 23.3 kJ m^{-2}. This seems to be a low value compared to stations at lower latitudes. Using a GUV instrument (Ground-based Ultraviolet Radiometer, Biospherical Instruments, San Diego, U.S.A.) Dahlback (2002) measured about two times/five times higher CIE-weighted UV dose rates (mW m^{-2}) in Oslo and in Izaña (Tenerife) compared to Ny Ålesund under similar atmospheric and surface conditions. However, the GUV measured generally only at five small band UV-wavelength channels so that the irradiance needs to be extrapolated about the whole wavelength range. UV-B was only measured with two channels at 305 and 312 nm, which restricts the significance.

	Daily integrated dose calculated from March 1 until October 31			Yearly fluence (MJ m^{-2})		Yearly
Year	Visible (MJ m^{-2}) (370-695 nm)	UV (MJ m^{-2}) (300-700 nm)	SSD (hours)	Visible (370-695nm	UV (300-700 nm)	SSD (hours)
1996	3.98	0.48	3.0	972.3	116.6	722.1
1997	4.14	0.51	5.4	1009.8	123.8	1327.0
1998	4.69	0.54	3.8	1145.7	131.0	931.1

Tab.1 Averaged radiation data measured at Ny Ålesund , BSRN-Station (Baseline Surface Radiation Network; SSD: Sunshine duration).

Such biologically weighted UV dose rates are often used to study biological effects of UVradiation. Single monochromator or filter radiometers are not sufficient, because the cutting edge of the UV spectrum at the short wavelength decreases while the biological weighting functions action spectra (BWF) increase. As a result, the decrease of intensity below 320 nm by about 6 orders of magnitude is opposed to an increase of the biological sensitivity also by some orders of magnitude. Therefore, UVB-radiation (280-320 nm) in air is continuously measured at the Koldewey-Station using 32 channel quanta counting spectroradiometer developed at AWI (Hanken and Tüg 2002). The instrument counts the number of quanta impinging on a cosine diffuser and is installed on the roof of the NDSC building (Network for the Detection of Stratospheric Change; Koldewey Station). UV-B-radiation in the water column of Nansen Bay was determined in parallel with a similar but 2 ¶ diffuser equipped device enclosed in a water-tight housing. The instrument has been fixed to a pulley anchored to the ground of the fjord and floated in the water column at about 3 m depth. Depth profiles of UV-B penetration can be recorded by pulling the instrument into different depths and counting the impinging quanta at 2 min intervals.

Depth is determined by an internal pressure sensor. For measuring PAR, LI-COR dataloggers (LI-COR, LI-1000) equipped with different air and underwater sensors were used (e.g. LI 192 SA). In addition, underwater light spectra were determined during SCUBA-diving with a battery-powered Kruse underwater-spectroradiometer (Kruse, Bremerhaven, FRG) and data were stored within the instrument by datalogging simultaneously with the depth data detected by an internal pressure sensor. Such a different set of instruments is necessary to fully cover the wavelength range between 280 and 700 nm.

UV penetration into the water body depends strongly on the water characteristics. Therefore, the diffuse vertical attenuation coefficients of downward irradiance (K_d) should been determined using following formula (after Kirk 1994):

$$K_d = \ln (Ed_{(z_2)} / Ed_{(z_1)}) * (z_1-z_2)^{-1}$$

with $Ed_{(z_1)}$ and $Ed_{(z_2)}$ as the respective irradiance at depth z_1 and z_2. Logarithmic dependencies of light attenuation on water depths can be proven by non-linear regression over a depth profile of several meters. Optical stratification of the water body is visible if the data deviate from its logarithmic regularity. As the vertical attenuation coefficients is a logarithmic derivative, a low K_d-value, for example 0.1 m^{-1} means, that there is about 10 % light attenuation per meter and characterizes clear water. A value of 1 m^{-1} means very turbid water and a strong light attenuation of about 63 % per meter. The euphotic depth is defined to be at 1% PAR of the subsurface value and represents the lower threshold where significant phytoplankton photosynthesis can take place (Kirk 1994). Maximal UV-B transmittance in oceanic waters was found to be at about 60-70 m, whereas the threshold for biological effective irradiances occurs generally in clear oceanic water at about 10-20 m, in coastal areas, like the Kongsfjord, at 5-6 m, and in very turbid waters at 0.2–1 m water depth (Hanelt et al. 2001, and see below).

The absolute UV radiation measured in air depends also on the solar altitude or sun angle, and thus, on the daily course of the sun position of the respective latitude. E.g. closer to the Equator, the irradiance at noon is always higher than in the Arctic, because the pathlength of the solar rays through the atmosphere decreases. A hypothetical ozone depletion of 20 % would result in a maximal UV-B irradiance of 1.5 W m^{-2} in April on Spitsbergen (78° North, highest sun altitude (upper culmination point) about 26.4°) (Svendsen et al. 2002). However, on the island of Helgoland at 54° North (upper culmination point 50.4°), about 2.9 W m^{-2} are measured under normal stratospheric ozone concentrations as the sun stays much higher in its zenith. This means that already twice the UVB radiation is impinging on the earth's surface in this more temperate region even without any destruction of the ozone layer, which needs to be considered for discussing the UV problem in polar regions. However, polar algae may be more sensitive to UV-radiation and this will be discussed in some chapters of this issue.

As mentioned above, irradiance of wavelengths below 295 nm is very low and can be hardly measured already at the water surface (Fig.1). These wavelengths will certainly not affect organisms under water as irradiance is further

decreased by absorption and scattering within the water column. This may be a reason why DNA damage, which is especially caused by wavelengths below 300 nm, was scarcely found in macroalgae growing in the sublittoral in Kongsfjorden (van de Poll et al. 2002).

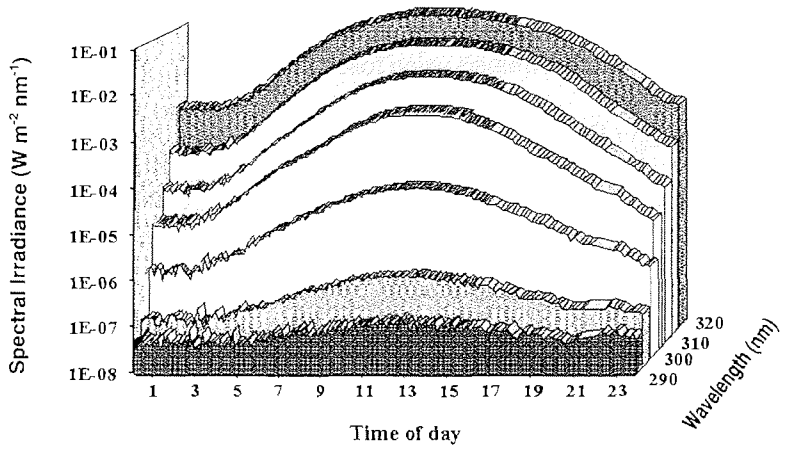

Fig.1 Daily course of different UV-B wavelengths (in 5 nm steps; ordinate with logarithmic scale). Measured on the 3.Sept. 1995 on the roof of the NDSC building (Network for Detection of Stratospheric Change). The irradiance measured at 290 nm is already close to the noise signal of the spectroradiometer, as it is very low.

The underwater light environment and salinity

Excessive solar radiation can affect plant communities negatively (Hanelt 1998). To investigate such effects on the marine vegetation, light transmittance into the waterbody must be determined. This is important as light transmittance changes with different hydrodynamic factors as presented in this study. Recently, studies by Ito and Kudoh (1997) and Svendsen et al. (2002) have characterized several parameters of the water conditions in Kongsfjorden and additionally some of the atmospheric factors. However, the former studies did not include solar UV radiation data. In coastal waters, UV-radiation and blue light are strongly attenuated due to dissolved organic material (Björn 1993), and depends on the input of dissolved organic matter (DOM) during the warmer season due to rainfall or input of melt water from snow layers and glaciers. A mean annual total run-off into the Kongsfjord was estimated to be about 1.4 x 10^9 m^3 with 90 % of the freshwater supply occurring within three summer months (Svendsen et al. 2002). Therefore, the underwater light regime must be determined during the course of the seasons to obtain a data base for modelling the spectral light distribution influence

during the year in relations to atmospheric studies.

The environmental light conditions are quite different in the coastal area compared to the open ocean. Penetration of light is largely determined by scattering and absorption of biological and inorganic material, with higher concentrations in coastal areas. Coastal waters show large temporal changes and regional differences in the concentration of dissolved and particulate matter influencing penetration of solar radiation into the water body. Thus, Jerlov (1976) classified marine waters into nine types of coastal and five types of oceanic waters depending on the respective transmittance characteristics.

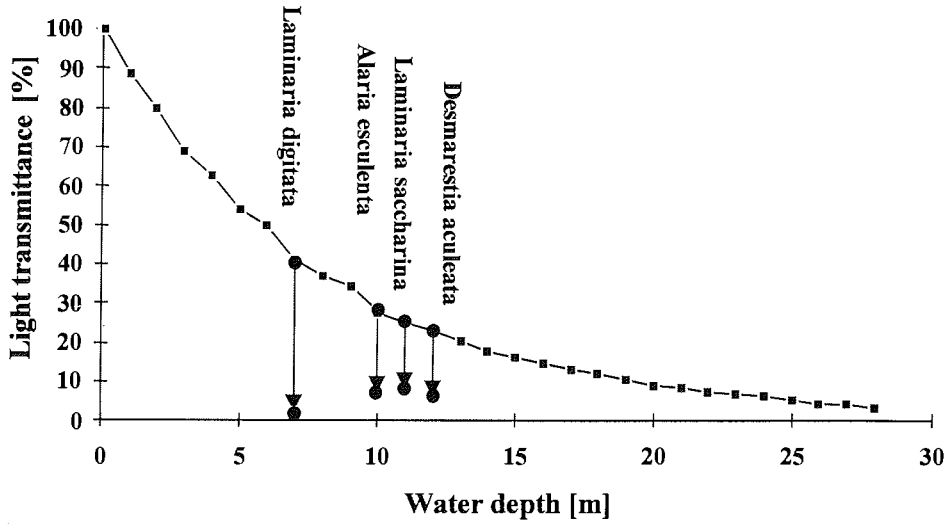

Fig. 2 Light transmittance in the water body of the Kongsfjord, as well as above and below the canopy consisting of different brown macroalgal species (dark circles). Light below the canopy was only 5 to 30 % of the light impinging on the algal canopy. Measurement was done in a clear water body K_d = 0.12 in June 1996 (Modified after Hanelt et al. 2003).

Marine macrophytes also form communities (kelp forests) with vegetation layers comparable to terrestrial forests with canopy species overtopping the understorey species (Lüning 1970, Dayton 1985). This has effects on photosynthetic performance and adaptation of the photosynthetic apparatus of the different species within kelp ecosystems. Irradiance of photosynthetically active radiation (PAR) is strongly attenuated by the kelp canopy as shown in Fig. 2, for light measurements above and below a canopy of different large kelp species in Kongsfjorden. In addition, the change in the light field includes not only a decrease in the photon irradiance but also changes in the light quality. Salles et al. (1996) found that below the canopy the spectrum was enriched in green and in far-red light, probably affecting photosynthesis as well as the photomorphogenetic development of the understorey. The result is that organisms living below the cover of the canopy are protected against high irradiances as well as harmful UV-irradiances, which can impinge on the top of the canopy during low tide on sunny days.

In spring low temperatures coincide with clear water condition, and the harmful UV wavelengths penetrate deeply into the water column. E.g. in the spring of

1997/98, the threshold irradiance with the potential to affect primary plant productivity negatively was still found at about 5-6 m depth. Under these conditions, the water body in spring was characterized as a Jerlov coastal water Type 1 (Fig. 3) (Hanelt et al. 2001). With increasing temperature in summer, snow layers and glacier ice melted, resulting in a high discharge of turbid fresh water into the fjord. During melt water input, a turbid fresh water layer was formed above the more dense sea water and in the inner basin of the Kongsfjord, a decline in the salinity of the local water mass occurs as a result of the melt water input (Svendsen et al. 2002). This caused a stratification in the optical features, salinity and temperature of the water body. Under these conditions, light attenuation was stronger than defined for a Jerlov coastal water Type 9. Solar radiation was strongly attenuated in the first meter of the water column. Consequently, organisms in deeper water were fully protected against harmful UVB radiation (Hanelt et al. 2001). Melt water input is only a phenomenon during the summer season. This applies to Arctic shorelines in a half-open fjord system where the water exchange with the clearer oceanic water is retarded. At open coastlines the melt water will be exchanged much faster with oceanic water which will diminish the observed turbidity effects on light penetration.

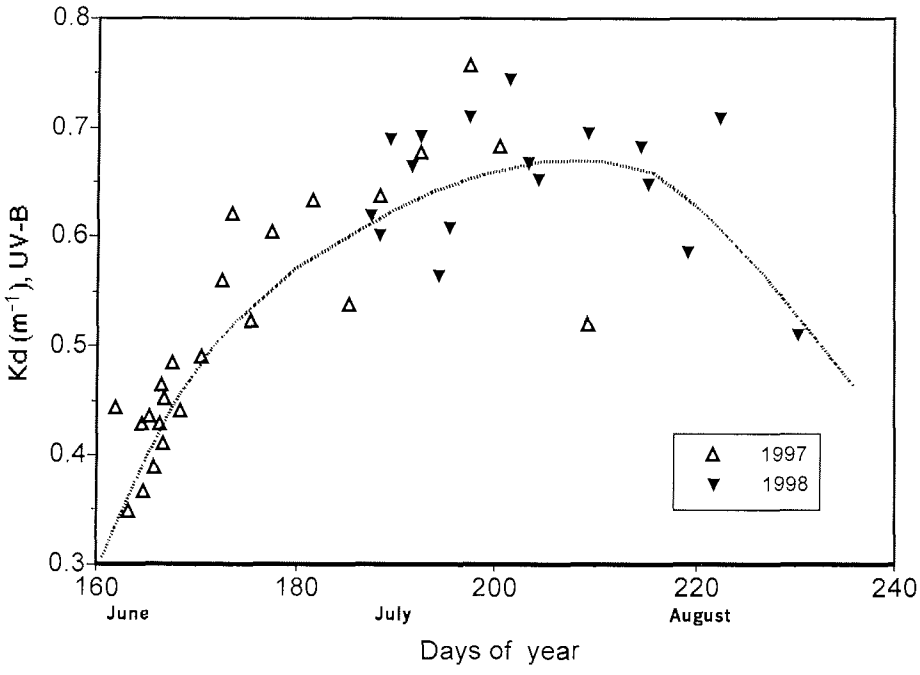

Fig.3 Average K_d of the UV-B radiation calculated from spectroradiometrical measurements in a depth range from 0 to 6 m for 1997 and 1998. In spring water has a high transparency (low K_d). Due to inflow of sediments with melt water, the water body becomes turbid in summer. In autumn, the transparency increases again, as the discharge of turbid melt water stops. (Modified after Hanelt et al. 2001)

In spite of the increased turbidity in the water column during summer (Fig. 3), intertidal organisms are still exposed to increased UVB during low tide and high sun position. Additionally in the surface water layer, turbidity decreases when rising tide causes an addition or exchange of the turbid surface water with clearer oceanic water, causing a transmittance pattern dependent also on the tidal cycle. Therefore, the combination of a stratified water column of different layers of turbidity, tidal level and the sun angle cause a peculiar pattern in the underwater light regime.

Continuous measurements of UV-B (280–320 nm) in air during summer (1998–2000) revealed a maximum radiation fluence of 52.6 kJ m^{-2} in June 1998, similar to a daily mean of irradiation of 0.61 W m^{-2} (Svendsen et al. 2002). The maximum irradiation on 24. June 1998 was about 1.2 W m^{-2} (Bischof et al. 1999). During several expeditions to Spitsbergen a maximal value of PAR (400-700 nm) of about 1300 µmol m^{-2} s^{-1} (~277 W m^{-2}) was recorded. A corresponding maximal irradiation of 19 W m^{-2} in the UVA range (320-400 nm) was measured (Bischof et al. 1998; Hanelt et al. 2001). A minimal vertical attenuation coefficient for downward irradiance (K_d) within the water body for photosynthetic active radiation (PAR) was 0.15 m^{-1} determined in surface water layer (0-4 m) on 8th June 1998 and a K_d of 0.12 m^{-1} in deep water (6-20 m) on the 1st September '96. This means, that under clear water conditions, the water body corresponds to a coastal water Type 1 after Jerlov (1976). The average K_d for UV-B was found to be in June 1997 low at about 0.35 m^{-1} (Hanelt et al. 2001) whereas Poll et al. (2002) determined a minimal K_d of 0.58 m^{-1} in June 2001 using a biological DNA dosimeter. However, maximal averaged K_d values for UV-B of about 0.8 m^{-1} (even 1.28 m^{-1}, Poll et al. (2002) were measured after strong discharge of turbid, sediment-rich melt water from the glaciers into the fjord. Then, attenuation was much higher than defined for the coastal water Type 9. Values above 0.8 generally occurred in the upper water layer, or below the ice cover, which is not typical for the whole water column. In contrast to the conditions in Arctic Kongsfjorden, vertical attenuation of downward irradiance is much lower in Antarctica. Figueroa (2002) determined the bio-optical water characteristics in the Gerlache and Bransfield Strait in Antarctic summer 1995/96. He found an averaged $K_{d\ (305nm)}$ ranging between 0.23 and 0.33 m^{-1} and for PAR between 0.15 and 0.31 m^{-1}, values, which demonstrate the more clear water conditions in the Antarctic ocean.

Due to a long cold winter in 1998, a 1 m thick ice layer persisted on the fjord until mid June. This is a rather rare event, as the ice cover and the pack ice usually drifts out of the fjord by April. An ice cover of about 1 m with an additional snow layer of about 30 cm resulted in a decrease of PAR to about 2.4 % of the irradiance measured in air. Without snow, PAR decreased to only about 8.5 %. Maximal fluence rate of PAR measured directly below the ice with a snow cover was about 6.5 µmol $m^{-2}s^{-1}$ (i.e. 1.4 W m^{-2}) on a sunny day at noon and UV-A was about 0.42 W m^{-2}. UV-B radiation below the ice was so low that it could not be determined.

Salinity and temperature

The seasonal variation in freshwater discharge creates a stable stratification in summer, the autumn cooling destabilizes the stratification and in winter it becomes weak or mixed. Atmospheric heating during summer and cooling during winter enhances this effect. The upper layer circulation in summer is confined to a shallow surface layer (Svendsen et al. 2002). In the inner basin, the salinity of the upper water layer can drop below 30 psu (practical salinity units; = ‰) within a few meters. Generally, the local water mass in spring has a salinity of about 34.5 psu. During melting processes in summer the salinity of the surface waters drops below 28 psu in the inner basin near the glaciers and to 30 psu in the middle of the fjord (Svendsen et al. 2002). The brackish water occupies several meters as upper layer, but its thickness decreases towards the open sea. The surface temperature can exceed 4 °C because of absorption of solar radiation.

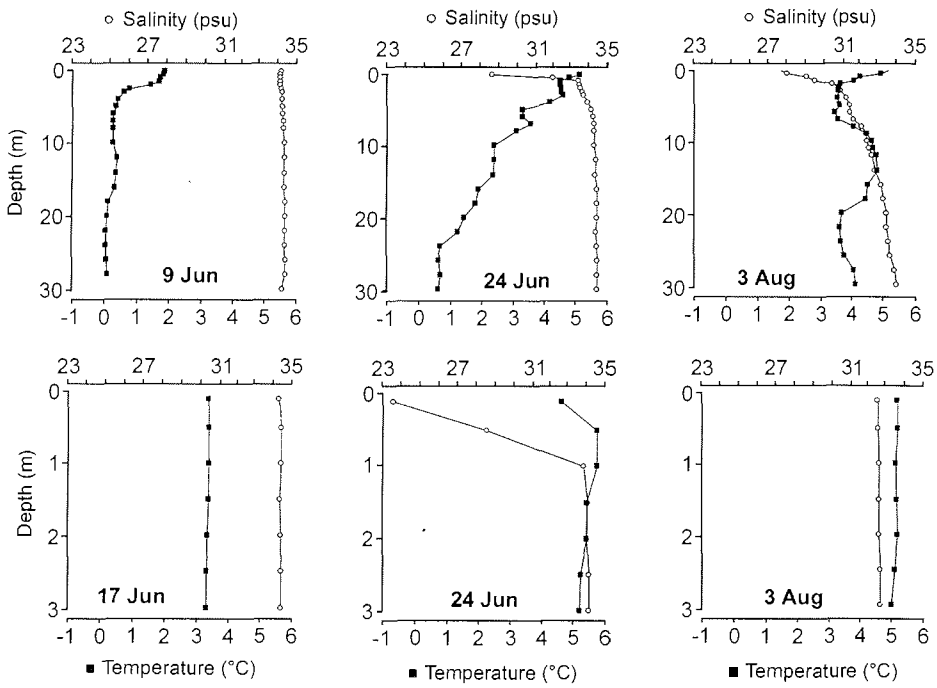

Fig. 4 Changes of temperature (■) and salinity (o) with depth on different days in summer 1997. Upper three graphs show the conditions in the deep water body of Hansneset, lower graphs in the shallow water body of Nansen Bay (Modified after Hanelt et al. 2001)

We measured the variation of water temperature and salinity at different depths during the investigation period in 1997 at two different locations: the shallow coast of the Nansen Bay and at the steep coast of Hansneset at the opposite side of the fjord (Fig. 4). On the 17th June, temperature was nearly constant at about 3.3 °C from the surface down to 3 m depth in the shallow water of the Nansen Bay. Temperature increased during summer and reached a maximum of 5.8 °C on the 24th June at about 1 m depth. In August, temperature had stabi-

lized around 5 °C from the surface down to 3 m depth. Highest salinity occurs in spring and early summer with 34.5 psu. Due to melt water, salinity decreased to 23.4 psu in the surface water and varied slightly around 34 psu in depths < 1 m. This indicates a stratification of the water body corresponding to the change in the water transparency mentioned above. The lowest salinity of 19.3 psu in the Nansen Bay was measured on 18th August 1997 due to a temporary discharge of a high amount of fresh water.

In the deep water body at Hansneset (Fig. 4), surface temperature increased strongly from 1.9 °C on 9th June to 4.9 °C at the beginning of August. The minimum of about 0 °C in spring was found at about 24 m depth. The temperature in deeper water rose slowly but also continuously, e.g. in 20 m depth from 0.1 °C at the beginning of June to 4.2 °C in the middle of August. Surface temperature fluctuation during summer was higher at the surf-exposed site of Hansneset than in the shallow water of the Nansen Bay due to the stronger mixing of cold deep water with warmer surface water at Hansneset. The salinity at the beginning of June was about 34.4 psu and was reduced by melt water to 27.7 psu in the beginning of August 1997. A change of salinity and temperature occurred at least down to 10 m depth, a reduction of the salinity in August was observed even down to 18 m (Hanelt et al. 2001).

During June 1997 relatively homogenous temperature and salinity prevailed in the whole 3 m water column of the Nansen Bay. Then, temperature increased and salinity decreased during summer at both places. Water temperature was about 4 °C at both sites, even down to 20 m depth at Hansneset. July and August were characterized by temporarily reduced salinity due to melt water discharge at higher air temperatures. Salinity in the Nansen Bay was less affected at 3 m than at Hansneset at a depth of 5 m, which is a result of the stronger water circulation at the wave exposed location. The pattern of changes in temperature and salinity are recurrent every year with increasing air temperature in summer. In the course of the summer season, salinity continuously decreased within the water body until air temperature became colder again and water temperature increased particularly in shallow water regions within the fjord (Hanelt et al. 2001, Svendsen et al. 2002).

In conclusion, a higher turbidity in the upper water layer, decreased salinity and increased water temperature are mainly caused by the freshwater discharge due to glacier ablation, snowmelt, summer rainfall and ice calving and thus, a direct seasonal effect of the increasing air temperature. The green house effect as well as the stratospheric ozone depletion may also further affect the abiotic factors in the underwater environment in Kongsfjorden. After the sea ice breaks up in spring, solar radiation penetrates deeply into the water body, just during a time when many algal species grow with maximum rates. Under these conditions, growth and metabolism of algae and other organisms might be affected by UV-radiation. Later in summer during low tide, only organisms in shallow water and in the eulittoral are affected by UV radiation but not in the mid and lower sublittoral. This is due to the high UV absorption within the upper turbid water layers. These communities, however, may experience a decrease of salinity. The temperature rise during summer might not be a big problem as most organisms have an Arctic cold-temperate distribution (Laudien et al., this issue; Wiencke et al, this issue) and can easily withstand slight temperature shifts.

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Seasonal development of structure and optical surface properties of fast ice in Kongsfjorden, Svalbard

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Introduction

This paper provides a brief overview on physical parameters and surface processes of Arctic fast ice throughout spring in Kongsfjorden, Svalbard, studied in the years 1997, 1998, 2002, and 2003. Kongsfjorden is an Arctic fjord on the western coast of Spitsbergen with seasonal fast ice in its inner part (Svendsen *et al.* 2002). The timing of its fast ice formation, snow and ice surface changes and ice disappearance has high influence on the local heat budget of the atmosphere-ice-ocean system, the salt budget in the fjord and the ecosystem. Fast ice formation and decay is closely related to the oceanic boundary conditions such as influence from the West Spitsbergen Current. Due to relatively warm water of Atlantic origin, fast ice forms late (usually not before December) compared with other Arctic locations, e.g., in the Canadian Arctic (e.g., Brown and Cote 1992). Kongsfjorden is usually free of fast ice from July to December. Despite Kongsfjorden fast ice differs in several aspects from other thermodynamically grown first-year ice in the Arctic and Antarctic, the fjord is well suitable for studies of small scale processes that affect sea ice and snow formation and decay.

Before 1997, information on the ice extent in Kongsfjorden is mainly availably in the form of data collected in the context of biological studies (Mehlum 1991; Lydersen and Gjertz 1986). Only recently, several sea-ice glaciological research projects were conducted by the Norwegian Polar Institute and the Alfred Wegener Institute, dealing with the physical properties of ice and snow, and with the development and decay of fast ice in the inner part of Kongsfjorden (Fig. 1). For the energy balance, the snow cover and upper ice layers are most important, because they affect albedo and solar radiation transmissivity most. In 1997, 1998, 2002, and 2003, detailed studies of optical surface properties like spectral reflectance and albedo, were performed as well as investigations of melt processes and superimposed ice formation (Gerland *et al.* 1999; Winther *et al.* 2001, 2004; Nicolaus *et al.* 2003; Hamre *et al.* accepted). The maximum mean total ice thickness in Kongsfjorden was observed to be ca. 0.9 m or less in all years. This includes a snow layer on top with maximum mean thickness of 0.23 m (Gerland *et al.* 1999; Nicolaus *et al.* 2003).

Here, the main results of these studies are summarized. We focus on the development of ice concentration in Kongsfjorden, snow and ice thickness, texture of snow and sea ice, salinity and temperature, and spectral surface reflectance and albedo during the transition from late winter to summer conditions.

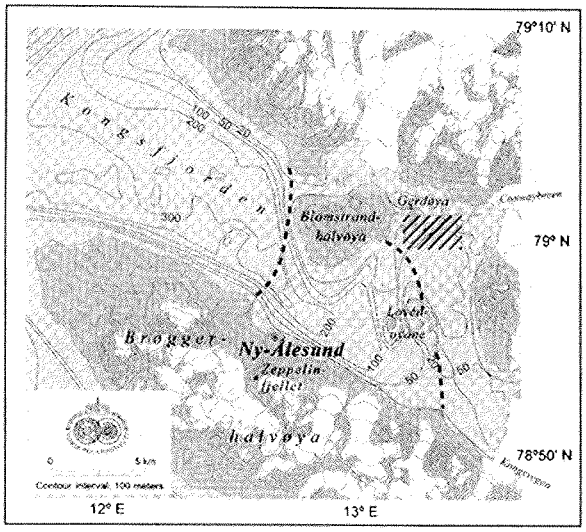
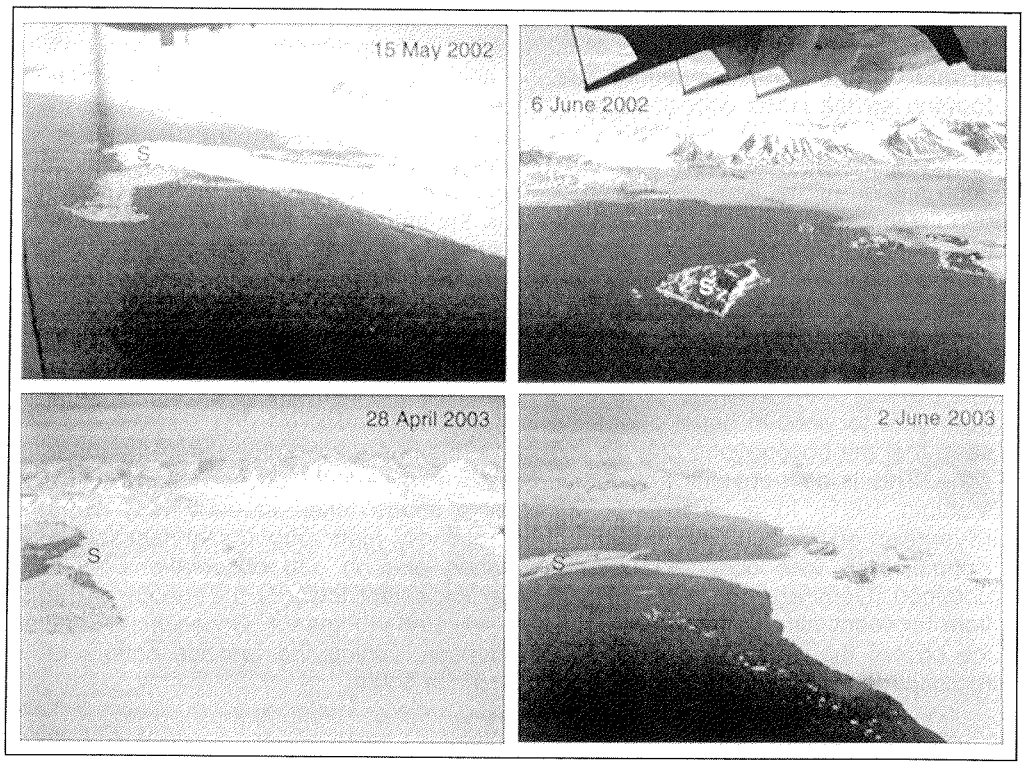


Fig. 1 (left): Map of Kongsfjorden with the research area indicated (marked rectangular box in the inner northern part of the fjord). Dotted lines show ice edge positions for years with more (left) or less fast ice (right) in the fjord. See further explanation in the text.

Fig. 2 (below): Aerial photographs (northward view) of the inner part of Kongsfjorden at different times of the year, before (left) and after (right) melt onset (see dates in the photographs). For orientation, the island "Storholmen" (the westernmost island of the "Lovénøyane") is marked with an "S". (photos: C. Haas, M. Nicolaus, 2002 & S. Gerland, 2003).



Furthermore, studies will be presented regarding formation of snow ice in winter and early spring, and superimposed ice after melt onset or during warm episodes in early spring.

Freeze up, maximum ice extent

No work has been published yet describing the onset of fast ice formation in Kongsfjorden. However, observations and knowledge of the hydrographic background conditions (Svendsen *et al.* 2002) give reason to assume that initial fast ice forms first in the inner, northern part of Kongsfjorden between December and early February. There, the water is shallower than in the transition zone and outer parts of the fjord, hence it is less influenced by Atlantic water, and it is protected from swell and waves. The islands Gerdøya, Lovénøyane and some smaller nameless islands form fixing points for fast ice formation. Ice core analysis from a core from the inner part of the fjord show granular ice layers in the upper part, and vertical columnar ice with very long crystals underneath, formed under calm conditions (Gerland *et al.* 1999). Typical thicknesses for the granular ice layer are 0.10-0.12 m and for the columnar ice 0.50-0.60 m. In addition to the fast ice, sea ice from other areas may be advected into Kongsfjorden. This "imported" sea ice may originate from drifting, broken-off fast ice from the nearby Krossfjorden, or first-year and multi-year ice from the area off the west coast of Spitsbergen, for example originating from the Arctic Ocean or the Barents Sea, drifting eventually with the West Spitsbergen Current northwards to Kongsfjorden. Throughout spring it is regularly observed that parts of the fast ice in Kongsfjorden break off and drift in and out the fjord, sometimes freezing again onto the original fast ice edge, forming an ice cover with a rough surface. Another contribution to ice types in Kongsfjorden is icebergs from the glaciers terminating in the fjord (e.g. Kongsvegen, Conwaybreen, see Fig. 1). The quantification of the areal fraction of such "imported" ice types is difficult. Icebergs are found embedded in the fast ice usually in shallow areas, often grounded, in the inner part of the fjord. Then, the areal fraction of those icebergs is small. In summer, when the fast ice disappeared, they are often the only forms of ice in the fjord. Sea ice from Krossfjorden may drift relatively easy into Kongsfjorden, because Krossfjorden is close to Kongsfjorden. No own observations of multi-year sea ice in Kongsfjorden exist, but there exists certainly the possibility that multi-year ice can be imported to Kongsfjorden.
The position of the ice edge, portions of drifting ice and icebergs varies significantly from year to year. In aerial photography from 2002 and 2003 (Fig. 2), it can be seen that the Lovénøyane play an important role in protecting the ice in the inner zone. This is also obvious from earlier observations (Lydersen and Gjertz 1986; Mehlum 1991). In a LANDSAT TM satellite image taken in early May 1998 (Svendsen *et al.* 2002), a situation with relatively high amounts of fast ice in Kongsfjorden was observed in spring, when also *in situ* observations were obtained (Gerland *et al.* 1999). Typical fast ice extent towards the mouth of the fjord for years with more or less fast ice is indicated in Figure 1. Occasionally, fast ice covers the entire fjord over shorter periods, joining the fast ice covers of Krossfjorden in the north and Forlandsundet in the south.

During winter and spring, before the onset of melt, there are also other ice formation processes than just congelation freezing. These are snow ice formation (Gerland *et al.* 1999), and occasionally early superimposed ice formation. Snow ice forms as a result of seawater flooding of the snow/ice-interface in the case of negative ice freeboard, and subsequent freezing of the seawater-saturated snow layer. Early superimposed ice forms during episodic warm spells, when surface snow metamorphoses or melts, and the melt water percolates downwards through the snow pack where it refreezes on colder layers, mainly ice lenses, or the snow/ice-interface. Rainfall during episodic spells in winter or early spring can also contribute to superimposed ice formation. The setting of Svalbard, where warm spells lead sometimes in winter to temperatures around or even above 0°C, enables for early superimposed ice formation. Corresponding conditions might be existent in Arctic/Sub-Arctic areas such as parts of the Barents Sea, but not in the major parts of the Arctic Ocean and more eastern Siberian shelf seas. A recent study with sampling of ice in inner Kongsfjorden in April 2003 (Gerland *et al.* unpubl.) revealed early formed snow ice and superimposed ice with a bulk layer thickness of 0.16 m. Superimposed ice and snow ice are general ice types both in the Arctic, Antarctic (e.g., Eicken *et al.* 1994), as well as in the Baltic Sea (e.g., Granskog *et al.* 2003). They increase the overall sea ice thickness significantly, and can therefore prolong the presence of the ice cover in summer, contributing to the total sea ice mass budget and mean surface albedo. Also on a small scale, they affect the physical and optical properties of snow and sea ice. Superimposed ice formation has been observed to happen in Kongsfjorden regularly around the time of the onset of melt (Gerland *et al.* 1999, see below).

Ice properties at the end of winter

In May, the fast ice reaches its maximum thickness of about 0.7 m with a snow layer of ca. 0.2 m thickness on top (e.g. Gerland *et al.* 1999). However, ice and snow thickness vary both locally and interannually. In addition to thickness determination by drilling, indirect measurements were applied using electromagnetic induction sounding in 1997 (Gerland *et al.* 1999), and 2003 (Fig. 3), when the total ice thickness was about 0.7 m.

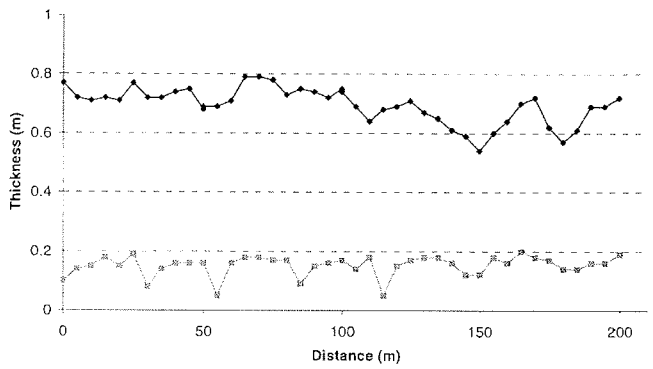


Fig. 3: Total ice and snow thickness along a 200-m profile south of Gerdøya, measured by means of electromagnetic profiling (total snow and ice thickness, upper curve) and snow-stake sounding (lower curve) at the end of April 2003. Although the ice appears level and undisturbed, it exhibits significant thickness variations over short distances.

As long as mean air temperatures stay below the freezing point of sea water, ice temperature vs. depth profiles exhibit negative gradients with near air temperatures at the surface and near seawater freezing temperatures at the bottom of the ice (Gerland *et al.* 1999). Due to the snow layer, most of the diurnal temperature variations do not reach deep into the ice in May, as monitoring revealed (Nicolaus *et al.* 2003, Gerland, unpubl. data). With no or only little formation of snow ice or superimposed ice layers, the salinity profiles resemble typical C-shape profiles with raised values at the top and bottom (Fig. 4a, March measurements, see also Gerland *et al.* 1999; Nicolaus *et al.* 2003), as known from other investigations of first year sea ice (Eicken 1992). Towards the onset of melt, salinity is decreasing in the upper parts of the sea ice, until the gradient eventually changes direction and salinities increase with depth (Fig. 4a, May and June measurements, see also Gerland *et al.* 1999; Nicolaus *et al.* 2003). However, in 2003, with extensive early superimposed ice and snow ice formation, salinity profiles with stronger variations in the surface layers were observed. Surface snow undergoes metamorphosis throughout spring with eventual grain growth, rounding and increased transparency for solar radiation. The change in snow crystal size, grain shape, liquid water content and thickness affects the surface albedo, resulting in a continuous decrease of albedo for 2002 (days 141-149) and 2003 (entire observation period, Fig. 5a). Fresh snowfall may lead to intermediate short-term increases of albedo.
Textural analyses reveal a crystal stratigraphy typical for fast ice. An ice core obtained in inner Kongsfjorden on 18 May 1997, consisted of a mixed layer of granular ice and ice with small columns in the uppermost 0.11 m, underlain by a transition zone of 20 mm thickness with larger ice columns (Gerland *et al.* 1999). Below, columnar ice with large vertical crystals (Fig. 4b) extended down to the bottom of the ice. There, the skeletal layer was inhabited by large amounts of ice algae, giving the lowermost 40 mm of the ice a brownish appearance. Analyses of melted samples from spring 1998 revealed that diatoms, such as *Nitzschia* spp., dominate (Hop *et al.* 2002).

Melt processes and ice decay

In the first weeks after melt-onset, the total ice thickness was observed to change only little (Gerland *et al.* 1999). However, superimposed ice formation at the surface and melting at the ice bottom obviously progress simultaneously, resulting in a principle change in the composition of the ice. Further, melting processes alter the textural and mechanical properties of the ice substantially because porosity increases by internal melting. This reduces the mechanical stability (fracture toughness) of the ice. The increased sea ice porosity was reported by Gerland *et al.* (1999) in a thin section of a core taken on 18 June 1997 (Fig. 4c). Sea water and brine-filled pore space in sea ice functions also as a habitat for ice algae.

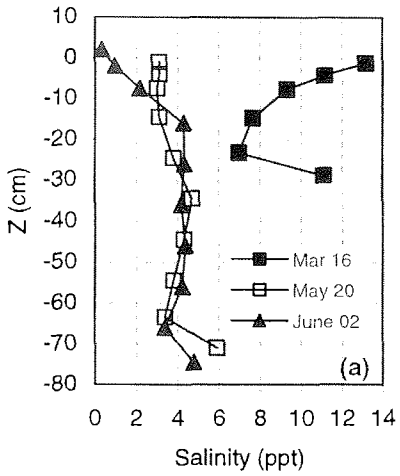
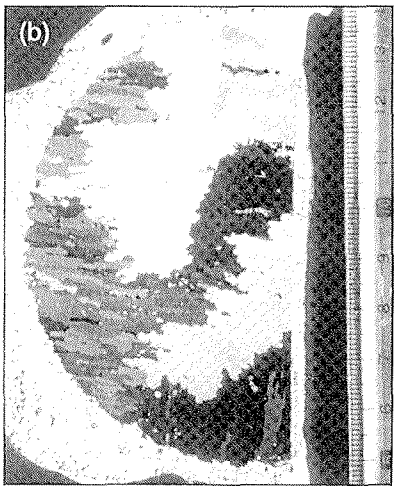
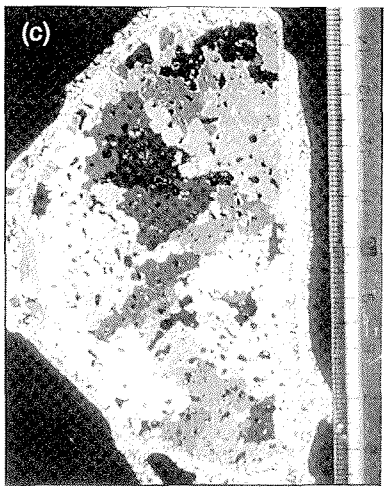


Fig. 4: (**a**, above) Typical ice salinity profiles obtained in 2002 at different stages of ice development. The May and June profiles show the complicated stratigraphy due to the presence of snow ice and superimposed ice (from Nicolaus *et al.* 2003). (**b**, below left) Horizontal thin section showing large crystals of columnar ice between crossed polarizers from 18 May 1997 at Dyrevika, and (**c**, below right), a corresponding thin section from 18 June 1997 from a core obtained near Gerdøya (from Gerland *et al.* 1999). Here, the enhanced porosity after the onset of melt is apparent. (b & c from Gerland *et al.* 1999).





Ice core samples, obtained in 1998 (Gerland *et al.* 1999) and 2002 (Nicolaus *et al.* 2003), show superimposed ice at the surface formed after the onset of melt. In a thick section from 30 May 2002, Nicolaus *et al.* (2003) showed a typical layering of superimposed ice below a layer of metamorphic snow and on top of highly porous sea ice, as it exists during each melt season. Along with earlier measurements in the same year, it was concluded that 0.23 m of snow cover were transformed into 50-60 mm of superimposed ice.

The changes of surface characteristics result in a strong albedo decrease with time, once melt has started (Figs. 5a&b). Detailed *in situ* measurements of spectral

surface reflectance and bulk snow and ice transmittance resolved reflectance and albedo decreases in different wavelength ranges according to snow metamorphosis and snow layer thinning as well as the role of the snow layer in attenuating solar radiation (Fig. 5b, see also Gerland *et al.* 1999; Winther *et al.* 2004). In 2002, mean surface albedo (running daily averages, calculated from global and reflected shortwave radiation) decreased strongly about 3 days after the onset of melt (day 147) from a level of above 0.75 down to 0.38 while the remaining surface snow melted (Fig. 5a). After all snow had melted, a dark clear superimposed ice surface remained. Only two days later, this surface began to deteriorate, resulting in a layer of coarse ice grains on the surface, leading to an albedo increase to values of 0.51. In 2003, no strong melt event was observed. Instead, the snow remained intact over the observation period. Consequently, the albedo did not drop as strongly as in the previous year (Fig. 5a).
Compared to typical multi-year sea ice, where the presence of large melt ponds dominates surface albedo in summer, the albedo of the investigated fast ice cover on Kongsfjorden is controlled by snow thickness, snow grain sizes, and the formation and decay of superimposed ice. The latter processes are based on wetting and melting of the snow cover, but here the surface consists of a water saturated layer of rotten snow and ice with puddles of variable size. The relatively flat surface topography, allows only weak lateral melt water flow, so that no larger and deeper melt ponds can develop (exception: around icebergs). However, detailed information on the fast ice properties in Kongsfjorden in the late stage of melting is sparse.

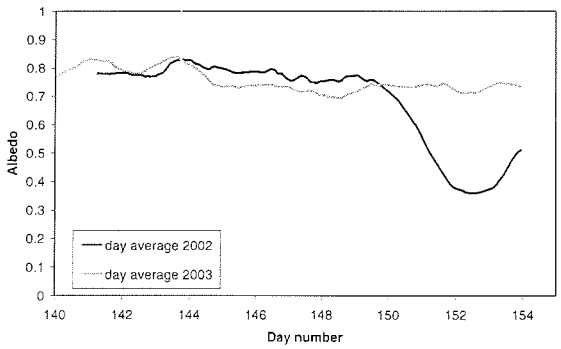


Fig. 5a: Time series of mean fast ice surface albedo for 2 weeks in 2002 and 2003 (running daily averages, calculated from shortwave global and reflected radiation).

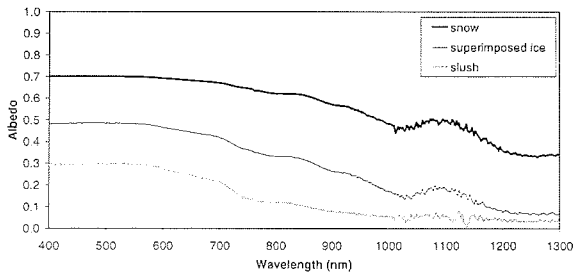


Fig. 5b: Spectral surface albedo over snow, superimposed ice, and slush on 4 June 2003. The two surfaces with reduced albedo were provided by removing the surface snow and superimposed ice layers manually.

Conclusions

The glaciological investigations of fast ice formation, development, and decay in Kongsfjorden have improved our understanding of some key processes relevant for sea ice and climate. This shows that Kongsfjorden is an excellent model case to study a number of processes relevant for large regions of Arctic and Antarctic. Process studies in this large-scale, open-air "laboratory" have the advantage that the ice can be accessed relatively easily and that climate data are continuously recorded at nearby research stations in Ny-Ålesund. Future studies will focus with more detail on the controlling parameters for fast ice development, the timing of formation, surface changes, melt onset and open water situation, as well as on quantitative descriptions and modelling of fast ice processes that change the energy and mass balance. Further, the Kongsfjorden fast ice as a research object in itself, and its coupling to the ocean and atmosphere, has led to the start of a long-term sea ice monitoring project at the Norwegian Polar Institute in 2003, including regular ice thickness drillings and daily visual observations from Zeppelinfjellet, a mountain near Ny-Ålesund (Fig. 1). Snow and ice properties in Kongsfjorden are also very relevant for studies of the Arctic ecosystem (Hop *et al.* 2002). Sea ice is a habitat for various biota (ice algae and ice underside fauna, seals, polar bears), and it influences indirectly other habitats, such as the seafloor with benthic flora and fauna, or by giving foxes access to the bird nesting places on islands in Kongsfjorden (Parker and Mehlum 1991 Mehlum 1991; Lydersen and Gjertz 1986). A marine laboratory is currently under construction in Ny-Ålesund which will further improve the infrastructure for interdisciplinary work at Kongsfjorden.

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Technetium-99 in Arctic marine algae from Kongsfjorden, Svalbard

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Introduction

Concerns regarding the levels and behaviour of radionuclide contaminants within the Arctic are key themes within current environmental pollution issues, due in part to the large number of actual and potential sources of nuclear contamination that exist in the Arctic and to the particular vulnerability of Arctic ecosystems to nuclear contamination (Wright et al., 1997). Technetium-99 (^{99}Tc; half-life 213,000 years) in the Arctic marine environment is present principally through the long-range oceanic transport of controlled discharges of radioactive effluents from the nuclear reprocessing plant at Sellafield in the UK, with minor contributions from Cap la Hague in France and global fallout. ^{99}Tc from Sellafield is transported from the Irish Sea into the Norwegian Coastal Current, via the North Sea and subsequently to the west coast of Svalbard in the West Spitsbergen Current (WSC). Following elevated discharges of ^{99}Tc from the Enhanced Actinide Removal Plant (EARP) at Sellafield in 1994, increased levels of ^{99}Tc were observed in sea water and marine biota off the Norwegian Coast (Brown et al., 1999; Rudjord et al., 2001). Further oceanic transport of EARP associated ^{99}Tc to Svalbard waters has been observed, with levels of ^{99}Tc in the WSC off the Western coast of Svalbard increasing five fold over the period 1994 (pre-EARP) to 2000 (Kershaw et al., 1999, 2003; Gerland et al., 2003).

Marine algae are often employed as sentinel biological indicators of pollution in coastal regions due to their widespread distribution and sessile nature (e.g. Ostapczuk et al., 1997; Favero and Frigo, 2002). With regard to radionuclide contamination, marine algae have been shown to accumulate a variety of radionuclides (e.g. Nicholas et al., 1983; Carlson and Holm, 1992; Rissanen et al., 1999) and in particular, several species of brown algae have been shown to accumulate ^{99}Tc, in the form of the pertechnetate ion (TcO_4^-), to relatively high levels (e.g. Pentreath et al., 1980; Masson et al., 1981), with concentration factors (biota/sea water activity ratio) of the order of 1×10^5 (IAEA, 1985). In the Svalbard area, Holm et al. (1984) determined activity concentrations of ^{99}Tc in *Fucus* spp. collected in 1980 and 1981 of between 8 – 23 Bq/kg (d.w.) and in *Laminaria* spp. and *Alaria esculenta* of between 0.5 – 2.7 Bq/kg (d.w.) and 0.9 –

2.4 Bq/kg (d.w.), respectively. More recently in 1999, ^{99}Tc activity concentrations in fronds and stipes of *Laminaria hyperborea* from Svalbard were recorded at 2.8 and 8.3 Bq/kg (d.w.), respectively (Rudjord et al., 2001).

In the Arctic, where sea water ^{99}Tc activity concentrations are typically low, the ability of marine algae to accumulate ^{99}Tc provides an enhanced signal of levels of this radionuclide within the Arctic marine environment. Furthermore, given the importance of many marine algae in benthic food webs and in some cases their direct economic importance to man, there is a need to understand their interactions with long-lived radionuclides such as ^{99}Tc.

Materials and Methods

A range of marine algal samples were collected from Kongsfjorden, Svalbard in 2000 from the intertidal zone at low tide, from shallow near shore waters and from greater depths. In 2001 and 2002, further collection of key fucoid and kelp species from Kongsfjorden was performed. Due to analytical limitations and the size of the target species available, samples consisted of either individual specimens (*Laminaria* spp and *Alaria esculenta*) or bulked samples of the same species (all other species), with sample sizes ranging from 100 g to 500 g wet weight. All samples were rinsed in the water they were taken from to remove adhering sand, animals or detritus and then placed in ziplock polyethylene bags before being frozen. On return to the laboratory, samples were dried at 105 °C to constant weight, before being homogenised in a stainless steel blender.

^{99}Tc, a pure beta emitter, is analysed using a radiochemical separation and concentration procedure (modified from Chen et al., 2003) before counting using a gas flow proportional beta counter. 10 - 20 g aliquots of dried homogenised material were digested using concentrated H_2SO_4 and HNO_3. ^{99m}Tc, added prior to the acid digestion is used as a chemical yield tracer. Once digested, the resulting solution is passed over an ion-exchange resin (BIO-RAD AG1-X4, 100 - 200 mesh) to concentrate ^{99}Tc. ^{99}Tc is then removed from the ion-exchange resin and separated from other radionuclides that may be present using combinations of co-precipitation and solvent extraction. Purified ^{99}Tc is subsequently electroplated onto stainless steel planchettes before counting on a low background anti-coincidence gas flow proportional beta counter (Risø, Denmark) calibrated with traceable ^{99}Tc sources. Chemical yield is determined via the gamma emissions of ^{99m}Tc. Recoveries are typically of the order of 70 – 85%, detection limits being of the order of 0.5 Bq/kg d.w., depending on the size of the sample used and the chemical recovery. Samples are analysed subject to internal NRPA QA/QC procedures involving blank correction, split samples, repeat analyses and participation in national and international intercomparisons. Results presented for all marine algae are based on dry weight.

Figure 1. *Fucus distichus* on the shore at Ny-Ålesund, Kongsfjorden

Results and Discussion

In 2000, the highest activity concentration of ^{99}Tc was observed in the fucoid seaweed *F. distichus* (Table 1), while lower values were observed in *Laminaria* spp. and *A. esculenta*. Of the algae collected from deeper waters, the brown alga, *Desmarestia aculeata*, showed a ^{99}Tc activity concentration of 13.5 Bq/kg, similar to the Laminariales (kelps) collected from the intertidal and near shore environment, while the red alga *Palmaria palmata,* showed only very low levels of ^{99}Tc accumulation (0.25 Bq/kg), as has been reported for various red algae (e.g. Masson et al., 1981; Topcuoglu and Fowler, 1984; Bonotto et al., 1988). In 2001 and 2002, similar ^{99}Tc activity concentrations were observed in *F. distichus* as was observed in 2000. The activity concentrations of ^{99}Tc in *L. digitata and A. esculenta* collected in 2001 were similar to those reported in *L. digitata* collected in Isfjorden, Svalbard in 1999 (Rudjord et al., 2001) and of the kelp species collected in this study in 2000. In contrast, the ^{99}Tc activity concentration in a sample of *L. digitata* collected in 2002 was several times higher and similar to those in *F. distichus*.

Species	2000 ^{99}Tc (Bq/kg)	2001 ^{99}Tc (Bq/kg)	2002 ^{99}Tc (Bq/kg)
Fucus distichus	34.3 ±3.3	42.9 ±11.2[a]	28.6 ±4.1[b]
Laminaria digitata	8.9 ±1.4[b]	7.1 ±0.7	28.9 ±2.8
Laminaria saccharina	2.8 ±0.3	4.4 ±0.4	-
Alaria esculenta	8.2 ±0.8	13.2 ±1.3	-
Desmarestia aculeata	13.5 ±1.3	-	-
Palmaria palmata	0.25 ±0.03	-	-

Table 1. ^{99}Tc activity concentrations (Bq/kg d.w.) in marine algae collected from Kongsfjorden in 2000, 2001 and 2002. For all species n=1, except (a), where n=5 and (b), where n=2 and average values are given. For year 2000 data see also Gerland et al. (2002)

The variation in the ability of algal species to bioaccumulate ^{99}Tc and in particular, the marked contrast between red and brown algal species is difficult to explain. One possible reason may be the presence of phlorotannins in brown algae. These phenolic compounds are confined to special cellular compartments, the physodes (Schoenwaelder, 2002), and play an important role in cell wall formation. Moreover, they are believed to function as a chemical defence against herbivory and UV radiation, and have been shown to be induced by these factors (Pavia and Brock 2000; Clayton and Wiencke, this issue). Additionally, these phenolic compounds are able to chelate inorganic metal ions

such as Cu, Pb, Ni, Zn, Co, Cd and Ca (Ragan and Glombitza, 1986; Döpfner et al., 1990), and so may be involved in cellular detoxification. Therefore, it is possible that ^{99}Tc may have an affinity to these compounds, which do not occur in red algae. Furthermore, green algae, which also lack phlorotannins, have been shown to have similarly low ^{99}Tc bioaccumulation abilities as red algae (Topcuoglu and Fowler, 1984).

Topcuoglu and Fowler (1984) demonstrated higher ^{99}Tc uptake in the younger, actively growing laterals of the brown alga *Sargassum vulgare*, compared to the older main axis. Moreover, they showed that heat-killed individuals of this species did not accumulate ^{99}Tc and that elevated temperatures and illumination enhanced the accumulation rates. This suggests that algal metabolism plays an important role in ^{99}Tc uptake and may explain the high activity concentrations of ^{99}Tc in *F. distichus* compared to the other brown algae in this study. Several studies (Stocker and Holdheide, 1938; Latala, 1990; Johanson and Snoeijs, 2002) have shown that photosynthetic rates in *Fucus* species from the North Atlantic to Arctic are ten fold higher than species of the genus *Laminaria*. Differences in photosynthetic rates between species may then correlate to differences in the degree of ^{99}Tc bioaccumulation, although this has yet to be verified.

As a whole, the ^{99}Tc activity concentrations in *F. distichus*, *Laminaria* spp. and *A. esculenta* observed in this study were all higher than those in 1980 and 1981 for these marine algae in the Svalbard area (Holm et al., 1984). Assuming a transfer rate of ^{99}Tc from the principal source of this radionuclide (i.e. Sellafield) to Svalbard in the region of 4 to 5 years (Kershaw et al., 2003), the observed ^{99}Tc activity concentrations in marine algae in this study would reflect the increased EARP associated ^{99}Tc discharges of ca. 550 TBq in total from Sellafield between 1994 and 1998. For comparison and assuming the identical principal source and transit time, the lower 1980 and 1981 ^{99}Tc activity concentrations in marine algae reported by Holm et al. (1984), would have resulted from a period of lower discharges during the 1970's of an estimated 40 TBq/a (Gray et al., 1995).

Compared to contemporary reported ^{99}Tc activity concentrations in marine algae from other locations along the transport route of the EARP associated discharges, the activity concentrations in marine algae in Kongsfjorden can generally be considered to be low (Figure 2). For example, activity concentrations of ^{99}Tc in *F. distichus* from Kongsfjorden in 2001 were between 1.8 and 9 fold lower than those reported in *F. vesiculosus* collected in the same year from mainland Norway and Northern Scotland and 364 fold lower than that in *F. vesiculosus* collected directly outside Sellafield (MAFF and SEPA, 2001; Gäfvert et al., 2003). Furthermore, activity concentrations of ^{99}Tc in *L. digitata* collected from Kongsfjorden in 2001 were 4.6 times lower than those in *L. hyperborea* from the same year in Southern Norway (Gäfvert et al., 2003). The lower activity concentrations of ^{99}Tc observed in marine algae in Kongsfjorden compared to locations along the transport route of this radionuclide is a

reflection of the concomitant gradient in levels of ^{99}Tc in the surrounding sea water, primarily due to the progressive dilution of the EARP associated ^{99}Tc signal through the ingress of North Atlantic Water and coastal run-off with distance from the radionuclide's source.

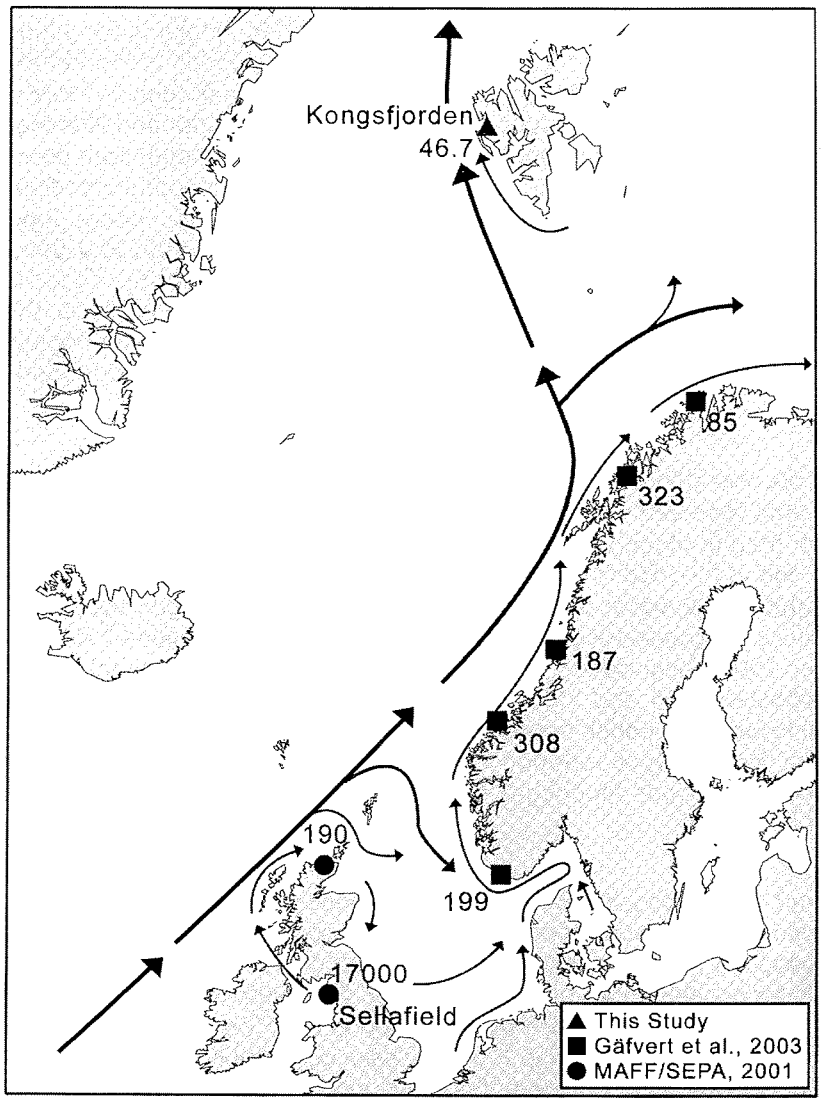

Figure 2. Activity concentrations of ^{99}Tc (Bq/kg d.w.) in *Fucus* spp. in 2001 between Sellafield and Kongsfjorden, Svalbard and the major current systems involved in the transport of this radionuclide.

Concentration factors (CF) are defined as the ratio of the concentration of a radionuclide in the biota of concern to the surrounding sea water (Bq kg^{-1} dry weight biota / Bq l^{-1} seawater) and since the inferred CFs represent snapshot and not equilibrium values, care must be taken in their use. However, a CF range of 7×10^2 to 1.9×10^5 for the marine algae in this study is in good agreement with the IAEA recommended range for macroalgae of 5×10^2 to 1×10^5 (IAEA, 1985). CFs for *F. distichus* were similar and, in general, an order of magnitude higher than for the other brown algae and two orders of magnitude higher than the red alga *P. palmata*. The CFs for ^{99}Tc in *F. distichus* collected from Kongsfjorden in 2001 and 2002 are in good agreement with a previously reported range for *F. vesiculosus* of 1.5×10^5 to 2.6×10^5 for Northern Norwegian waters from the period 1998 to 2001 (Kolstad and Lind, 2002). Likewise, the average ^{99}Tc CF for *L. digitata* collected from Kongsfjorden in 2000 was of the same order of magnitude as a CF of 1.4×10^4 for *L. digitata* collected in 2001 from Southern Norway (Kolstad and Lind, 2002). All *Laminaria* spp. samples showed similar CFs, with the exception of the *L. digitata* sample collected in 2002, which showed a ^{99}Tc CF of 1.2×10^5, reflecting the higher ^{99}Tc activity concentration measured in this sample.

Species	2000 ^{99}Tc CF	2001 ^{99}Tc CF	2002 ^{99}Tc CF
Fucus distichus	137000	156000[a]	168000[b]
Laminaria digitata	42000[b]	20000	120000
Laminaria saccharina	11000	12000	-
Alaria esculenta	39000	37000	-
Desmarestia aculeata	54000	-	-
Palmaria palmata	700	-	-

Table 2. ^{99}Tc concentration factors (d.w.) for marine algae collected from Kongsfjorden in 2000, 2001 and 2002. For all species n=1, except (a), where n=5 and (b), where n=2 and average values are given.

Conclusion

Over the sampling period, average ^{99}Tc activity concentrations were highest in the fucoid algae *F. distichus* (28.6 – 42.9 Bq/kg) and in general were an order of magnitude higher than the brown algae *Laminaria* spp, *A. esculenta* and *D. aculeata* and two orders of magnitude higher than in the red alga *P. palmata*. Concentration factors for all marine algae in this study were in the range of 7×10^2 to 1.9×10^5. Further work should be undertaken to elucidate ^{99}Tc uptake mechanisms in different marine algae, in order to understand the observed range in concentration factors in brown, red and green algae.

^{99}Tc activity concentrations in marine algae in Kongsfjorden during the period 2000 to 2002 probably reflect the elevated EARP associated discharges from Sellafield in the mid to late 1990's and may provide an enhanced signal of the low levels of this radionuclide in the Arctic marine environment. That detectable levels of ^{99}Tc in marine algae from Kongsfjorden were observed illustrates the role of ocean currents in the long-range transport of radionuclide contamination and highlights the vulnerability of the wider Arctic marine environment to any future contamination scenario.

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The authors wish to express their gratitude to the divers of the Koldewey Research Station of the Alfred Wegener Institute in Ny-Ålesund, for their assistance in the collection of marine algae used in this study and to thank the staff of the Norwegian Polar Institute Sverdrup station, Ny-Ålesund and the Governor of Svalbard in Longyearbyen.

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2. STRUCTURE AND FUNCTION OF THE ECOSYSTEM

Snow algae from north-western Spitsbergen (Svalbard)

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1. Introduction

The basis for our research activities on snow algae in north-western Spitsbergen was laid out during several expeditions between 1995 and 1998 under the long-term project Koldewey 07 (KOL 07). At that time the research team was still part of the Institute of Biology at Humboldt University Berlin and early work mainly focussed on locating snow algal fields along the north-western coast of Spitsbergen (Fig. 1) and field observations (see Müller *et al.* 1998a, 1998b). In 1999 a doctoral thesis was started and subsequent work concentrated on the establishment of a culture collection of snow algae at the Berlin laboratory (***CCCryo***) to enable *in vitro* studies of clonal isolates regarding the systematics, phylogeny and physiological character of snow algae (Leya et *al.* 2001, 2004). Further expeditions within the KOL 07 project were conducted in 1999, 2000 and 2002 to expand the culture collection and gather further environmental data.

Snow algae are freshwater microalgae. They have adapted their cell metabolism to their extreme habitat - snow and glacier fields in polar and alpine regions of the earth. They are extremophiles and/or extremotolerants, coping with the different extreme environmental conditions they are exposed to better than their putative ancestors, mesophilic freshwater algae. During a short vegetational period in summer the substrate temperature ranges at around 0 °C, occasional freezing occurs, usually only few nutrients are available, and, depending on the latitude of the snow fields, the algal populations may be exposed to high solar radiation. During winter the situation is very different. In polar regions total darkness prevails for three months, and the unavailability of liquid water due to constant subzero temperatures restricts photosynthesis and metabolism.

Most well known is the phenomenon of Red Snow which is the result of the formation of resting stages of Chlorophyceae living on snow (Fig. 2d-f and h). These cysts accumulate a range of carotinoids with astaxanthin and its derivatives being the predominant ones. These red coloured secondary pigments are stored in the cytoplasm masking the central and often reduced chloroplast. Though the red resting stages are responsible for the macroscopically visible and most well known Red Snow, Green Snow or Violet Snow can also be observed. The mass development of actively proliferating cells and the predominating chlorophylls (Fig. 2a-b) or other cytoplasmatic pigments such as iron tannins (Fig. c and g) in the latter case account for this.

The environmental factor having the most obvious impact on snow algae in their extreme habitat is temperature. During the vegetational period between early June and end September, air temperatures in north-western Spitsbergen in general do not rise above +10 °C, only occasionally extremes up to +18 °C can be reached (Leya 2004). Snow algal cells have to cope with a more stable, however much lower substrate temperature of about 0 °C throughout the summer months.

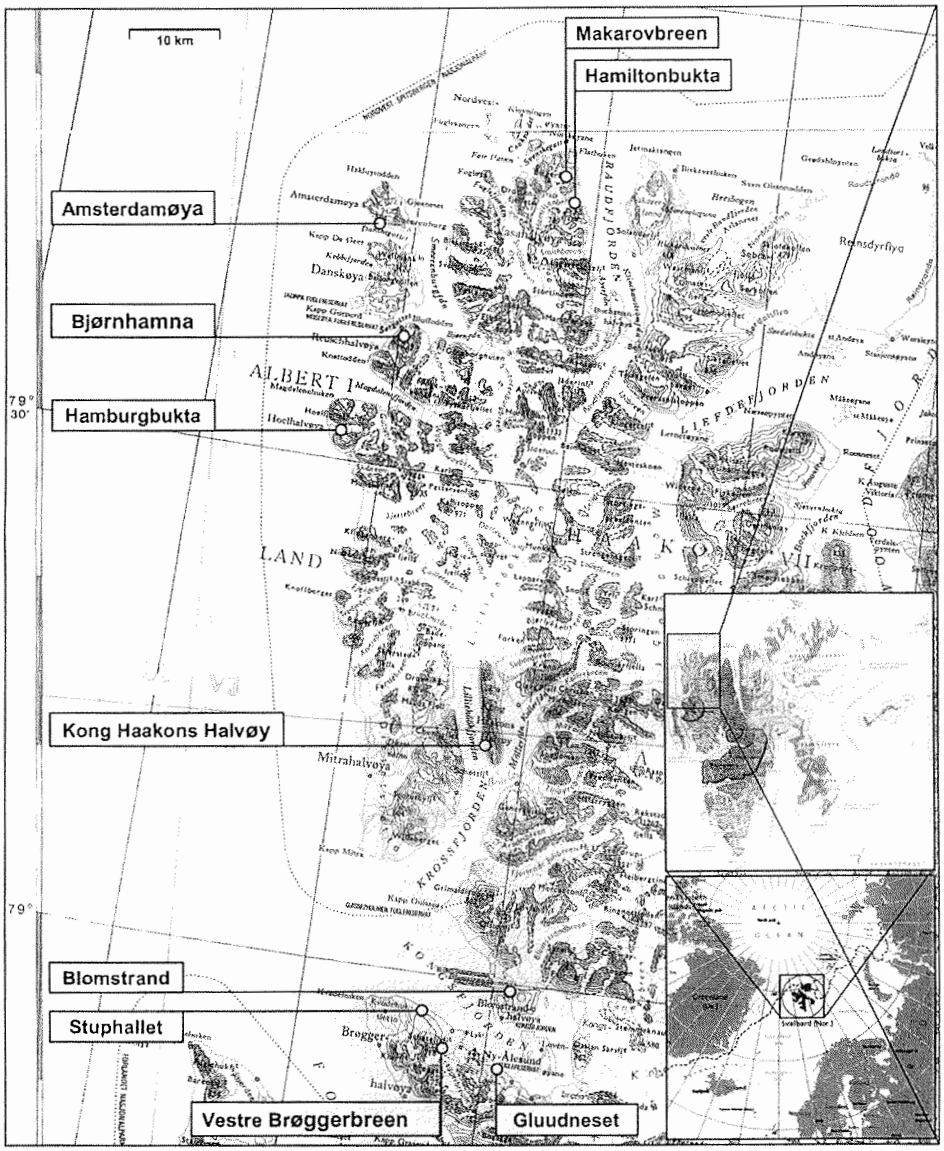

Fig. 1: Map of the north-western coast of Spitsbergen (Svalbard) with the locations where snow algal fields have been sampled during the expeditions between 1995 and 2002. A detailed description including geographical data can be found in Leya (2004). (Map modified on the basis of: Spitzbergen, Nordre Del. Blad 3, Norsk Polarinstitutt, Oslo 1982).

During the rest of the year, as soon as an insulating and persistent snow cover has formed, temperatures stabilise at around -7 °C whilst the air cools down to a mean of -15 °C with extremes as low as -35 °C (Leya 2004). These temperature profiles makes the climate in north-western Spitsbergen a northern tundra one, not a high arctic one, which can be found further to the east and north. This local pattern is a result of the northernmost extensions of the warm Gulf Stream, the North Atlantic Drift.

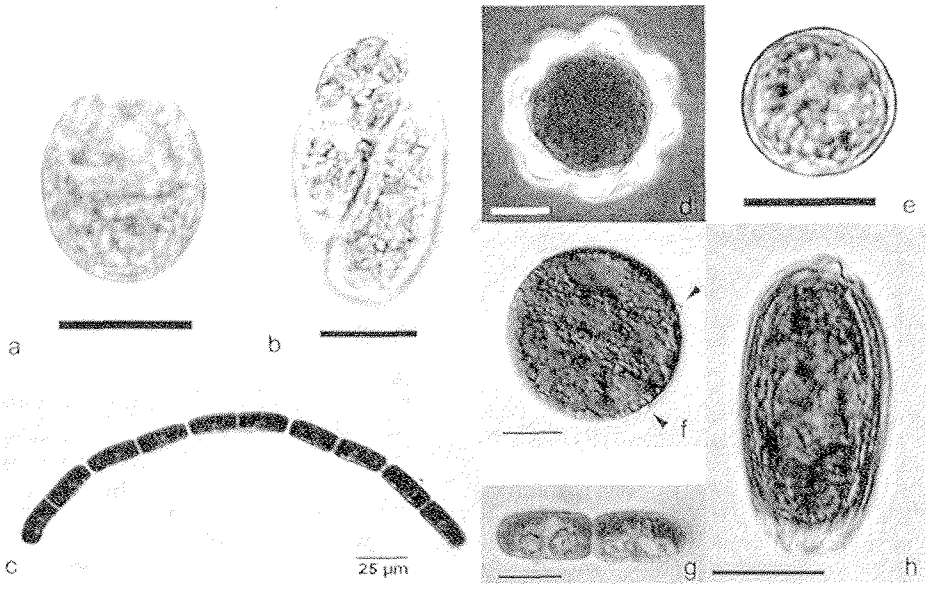

Fig. 2: Examples of microalgae found on snow and glacier habitats. Actively proliferating cells of the Chlorophyceae account for Green Snow: a) *Chlamydomonas* sp.-strain CCCryo 002b-99, b) *Chloromonas nivalis* strain CCCryo 005-99. Resting stages of the Chlorophyceae responsible for Red Snow: d) hypnozygote of cf. *Chlamydomonas nivalis*, e) cyst "small-orange", f) cyst "warty" showing wart-like cell wall protrusions (arrowheads), h) hypnozygote of *Chloromonas nivalis*. Vegetative cells of the Zygnematophyceae (desmids) responsible for Violet Snow on glacier fields: c) *Ancylonema nordenskiöldii* and g) *Mesotaenium berggrenii*. Scales = 10 µm.

Temperature key values T_{max} and T_{opt} characterising snow algae

The temperature the algae are exposed to is one key parameter in the life of snow algae. However, it is inadequate to assess strains to the group of snow algae, solely because they have been isolated from "cold substrates". As we have shown in screenings where the maximum (T_{max}) and optimum (T_{opt}) temperatures for growth have been detected, a number of strains isolated from tundra soils, rock surfaces and even snow in Spitsbergen still show growth at temperatures well above +20 °C, and thus can be defined as mesophiles[1] (see also Fig. 3a). The time-saving methodology for screening a high number of strains at several temperature levels between +2 and +23 °C in one experiment is de-

[1] e.g. the strain SAG 26.86/UTEX 1969 was isolated from snow in the Cascade Mountains in Oregon (U.S.A.) and originally was designated as *Chlamydomonas nivalis*. In 1988 H. Ettl re-identified this strain as the mesophilic *C. augustae* (U. Schlösser, pers. comm.).

scribed in Leya (2004). To date snow algae are classified into two groups: obligate cryophiles (psychrophiles) and non-obligate cryophiles. Hoham & Duval (2001) evaluated several publications on temperature studies of snow algae and concluded that for obligate cryophiles both temperature key values lie below +10 °C; for non-obligate cryophiles T_{max} and T_{opt} do not exceed the threshold of +20 °C. Accordingly, mesophilic algae still survive temperatures above +20 °C. It has to be noted that these limits are fairly subjective depending on the assay protocol. A satisfying definition for cryophily under this aspect has not been found yet. We concluded that it is more important to state the temperature key values for T_{max} and especially T_{opt} of a strain than classifying it as a obligate or non-obligate cryophile or even just calling it a snow alga.

Material & Methods

All material and methods applied in this project, and the results presented in this synoptical article are described in detail in the dissertation by Leya (2004).

2. The Culture Collection of Cryophilic Algae (CCCryo)

Our field collections of snow samples during KOL 07 expeditions were the source for single cell isolates of snow algae, with the year 1999 being the starting point. To date the culture collection holds 161 clonal strains of microalgae in approximately 51 species/varieties from 19 genera. 88 % of these strains originate from polar regions (mainly Spitsbergen), 3 % are from other regions of our earth and 9 % were purchased from other culture collections for comparative studies. Assays screening for T_{opt} and T_{max} revealed that from the strains isolated from Spitsbergen, approximately two-thirds could be classified as cryophiles with T_{max}-values between +5 and +20 °C. Of these, 21 strains were categorised as obligate cryophilic not tolerating temperatures above +10 °C. Although the remaining strains were isolated from snow fields or adjoining tundra vegetation, they proved to be mesophiles showing maximum and/or optimum growth well above +20 °C. Figure 3 shows the different genera accounting for mesophilic (a), non-obligate cryophilic (b), and obligate cryophilic (c) taxa among the strains isolated from Spitsbergen. Whilst the mesophiles are represented by typical soil algal genera, the obligate cryophilic snow algae are solely made up by the rather planktonic genera *Chlamydomonas* and *Chloromonas*[2]. A complete list of the strains held at our collection including morphological descriptions, their specific temperature key values and culture data, can be found in Leya (2004).

[2] Note: The genera *Chlamydomonas* and *Chloromonas* represent two groups artificially separated on the basis of the occurrence or non-occurrence of a pyrenoid. Recent phylogenetic studies by Pröschold *et al.* (2001) using ssu rDNA sequence data relocate various strains within this group of the Chlorophyceae. According to that study most snow algal isolates will be assigned to the genus *Chloromonas*.

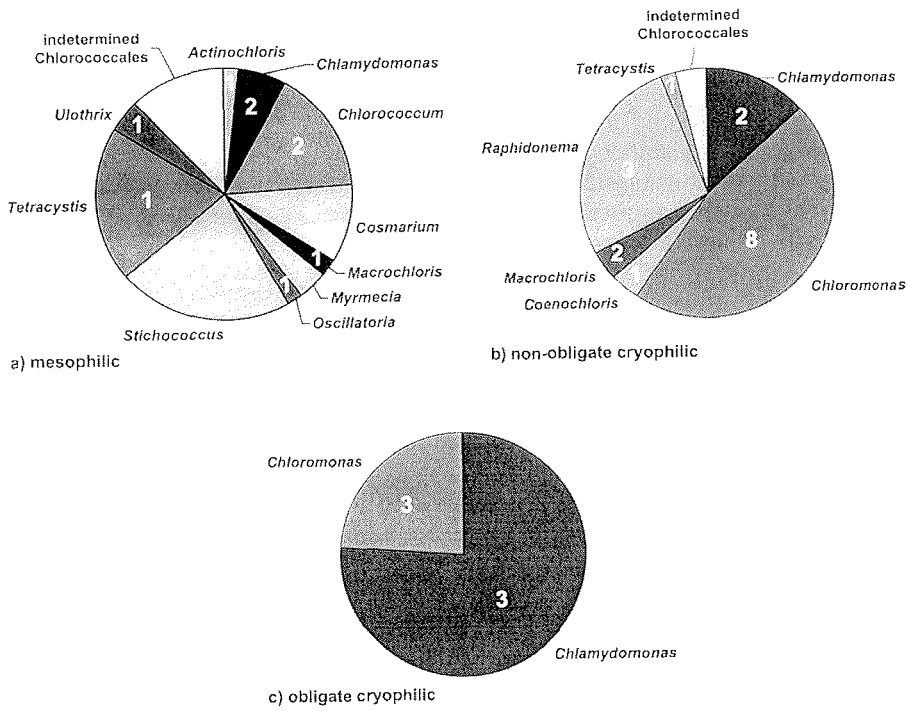

Fig. 3: Summary of the strains held in our culture collection ***CCCryo*** with regard to the temperature demands on genus level: a) mesophiles, b) non-obligate cryophiles, c) obligate cryophiles. Segment size reflects the proportion of strains relative to the strains held at ***CCCryo***, white numbers in the segments give the number of species of the genus cultured at our collection.

3. The phylogeny of snow algae from north-western Spitsbergen

The analysis of the small subunit rDNA (ssu rDNA) sequence data of several snow algal strains combined with those of other algal strains proved that the cryophilic character had developed several times during evolution in various taxonomic groups. A phylogenetic tree including the major groups of the Viridiplantae is depicted in Figure 4. Furthermore, as mesophilic taxa are positioned basal to those clades comprising cryophilic strains, it can be derived that cryophily is a relatively young character. Its evolutionary origin presumably dates back to the first glaciations of our earth's polar regions approximately 20 million years ago. The asterisks in Figure 4 mark those strains isolated from "cold substrates" (e.g. mountain soil, cold peat, snow, ice), and it is well recognised that this environmental character is not decisive for one single "cold unicell" clade within the genus *Chloromonas* as proposed by Buchheim (1997).

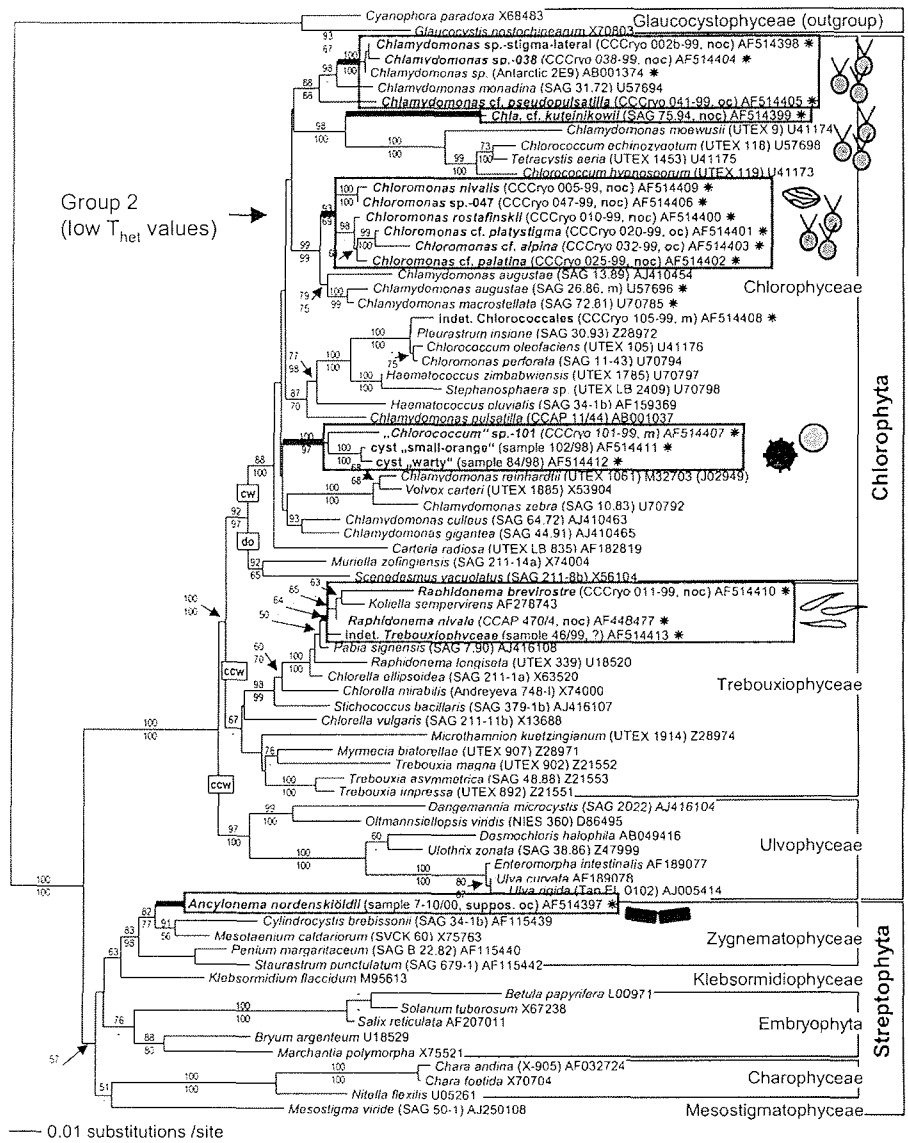

Fig. 4: Molecular phylogeny of the major group of Viridiplantae based on ssu rDNA sequence data. Two taxa of the Glaucocystophyceae were used as the outgroup to 75 taxa of Viridiplantae. The 18 strains of which the ssu rDNA sequences were determined in this study are printed in **bold**. Information stated: presently valid name (strain no. of the culture collection or sample no. respectively, cryophilic indicator according to our investigations [**oc** = obligate cryophile, **noc** = non-obligate cryophile, **m** = mesophile]) GenBank-accession no. of the National Center for Biotechnology Information (NCBI). * = taxa/strains isolated from cold habitat. The tree is based on the neighbour joining method using the evolutionary model GTR+I+G (proportion of invariable sites I = 0,34, estimated shape parameter of the gamma distribution G = 0,52). Bootstrap values (> 50 %) are stated for the neighbour joining method (2000 repl., above branches) and the weighted maximum parsimony method (500 repl., below branches). Labels referring to the position of the flagellar basal bodies: do = directly opposing, cw = clockwise, ccw = counterclockwise. Boxes indicate snow algal clades from Spitsbergen and show their polyphyletic evolution. Group 2 = comp. label in Figure 5).

4. The heterogeneous nucleation temperature T_{het}

A life at the edge of the freezing point of water requires biochemical adaptations to maintain metabolism and prevent cell death. During their short vegetational period in summer, snow algae are often and repeatedly exposed to subzero temperatures due to day/night shifts or during unstable weather conditions. The freezing substrate has two effects on the cells: (1) desiccation and (2) the danger of intracellular ice crystal formation. Desiccation is a result of two processes: (a) due to a rising concentration of solutes in the freezing mixture of snow, ice and melt water the cells experience an increasing osmotic pressure which results in the efflux of water from the cell to the environment by diffusion, and (b) the vapour pressure of the frozen substrate is considerably lower than in the snow algal cells, as the cytoplasm still remains liquid due to supercooling, and consequently the cells desiccate.

Morphological adaptations to this stress are realised by the formation of thick-walled (hypnoblasts) or gelatinous (gloeocysts, palmella) resting stages, both preventing the loss of water. The formation of destructive ice crystals primarily can be prevented by supercooling of the cytoplasm, which means the cytoplasm remains liquid though it has been cooled below its melting point (= freezing point). The temperature at which a solution finally freezes after supercooling due to the existence of a sufficient number of ice nuclei is called the heterogeneous nucleation temperature (T_{het}). We suspect that snow algae are able to produce freeze protectants to retard intracellular ice crystal formation. Antifreeze proteins (AFPs) can have such an effect by masking potential ice nuclei and thus inhibiting further growth (for a comprehensive review see Wang 2000). To identify potential snow algal strains, we observed single cells during a controlled freeze protocol on a cryomicroscopic set-up. Figure 5 shows the cumulative proportions of frozen cells at specific T_{het} values of different algal strains. The value for T_{het} when 50 % of the cells freeze was taken as the key value T_{het50}. It proved that only a specific group of snow algae (group 2) had considerably lower T_{het50}-values than other algal strains tested (group 1). Interestingly, next to mesophilic taxa, such as *Chlamydomonas reinhardtii* (strain CCCryo 152b-01) which does not tolerate substrate temperatures below +10 °C, some non-obligate cryophilic (002b-99) and even some obligate cryophilic (050-99, 073-99) snow algal strains also belong to group 1. Though these strains are obviously unable to prevent ice crystal formation they might have other adaptations to withstand stress from freezing, e.g. they might produce osmotically active but physiologically ineffective substances, such as polyols, sugars or amino acids to prevent water loss.

But what is so special about those non-obligate cryophilic snow algal strains in group 2? A comparison with our phylogenetic data (Fig. 4) revealed that all those strains with low T_{het} values belong to one clade. This evolutionary line comprises the cryophilic *Chloromonas* spp.. Consequently, when screening snow algal strains for antifreeze substances, it would be sensible to survey taxa or strains of this clade first.

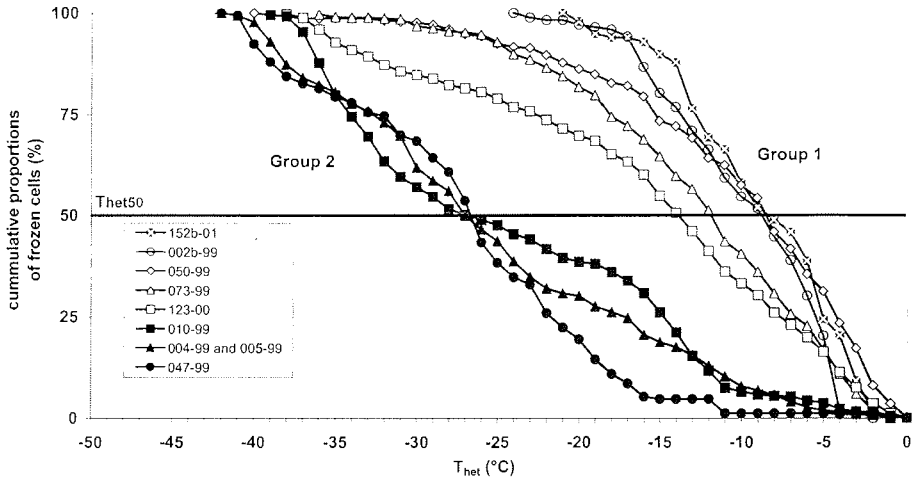

Fig. 5: Cumulative proportions of the number of frozen cells of different algal strains after supercooling to the heterogeneous nucleation temperature T_{het}. T_{het50} is regarded as the key value, i.e. the temperature when 50 % of the cells freeze.

5. Conclusions and outlook

We have established a comprehensive clonal collection of cryophilic microalgae (***CCCryo***) which is outstanding in Germany and one of a few worldwide. Our field studies, laboratory experiments and phylogenetic analyses show that these extremophiles are a much more diverse group as previously thought. From our results we also gain support that some strains have developed an enzyme kit with lowered activity temperatures between +2 and +15 °C ("coldzymes/extremozymes"). Others seem to produce antifreeze substances, and yet another group is able to synthesize astaxanthin and other carotinoids giving them an effective tool to scavenge radicals during reduced metabolism (i.e. as resting stages). Our future projects aim to identify these proteins (proteome analyses), substances and mechanisms using molecular methods (transcriptional analyses) and transfer this knowledge to applications in biotechnology.

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Species composition and zonation of marine benthic macroalgae at Hansneset in Kongsfjorden, Svalbard

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Introduction

In 1991, when the Koldewey Station in Ny-Ålesund was opened, very little was known about the species composition of marine benthic macroalgae from Spitsbergen, including Kongsfjorden. Apart from the classic work of Kjellman (1883) there have only been studies by Svendsen (1959) on macroalgae from Isfjorden and by Florczyk & Latala (1989) on the phytobenthos in Hornsund. Catalogues of marine algae from Svalbard have been published by Vinogradova (1995a) and Hansen & Jenneborg (1996). The only study that included collections from Kongsfjorden was conducted by Hansen & Haugen (1989) and concerned intertidal communities on rocky shores on north-west Spitsbergen. Most studies have involved collections of algae in the intertidal or by dredging, and the diving studies have targeted the macrofauna associated with macroalgae (Lippert et al. 2001). The invertebrates associated with macroalgae include about 100 species, dominated by bryozoa and amphipods. The general zonation pattern and the depth distribution of individual species of macroalgae remain to be properly described. The aim of our studies was to use SCUBA diving to provide basic data on the species composition and zonation of marine benthic macroalgae at a site typical of the middle region of Kongsfjorden.

Materials and methods

A steep, hard-bottom location was selected on a medium exposed, rocky island close to Hansneset on the western shore of Blomstrandhalvøya, which is currently an island because of the recent glacial retreat (78°55'N, 11°59'E, Fig. 1). The declining rocky substrate has small fields of pebbles and stones and slopes at an angle averaging about 40 °. The light, temperature and salinity regimes vary with the seasons as described in detail by Hanelt et al. (2001; this issue) and Svendsen et al. (2002). The 1 % water depth for photosynthetically active radiation is about 18 m in spring and 7 m in summer. During midsummer the salinity decreases down to 28.5 psu, whereas the temperature increases up to 5 °C in the upper metre of the water column.

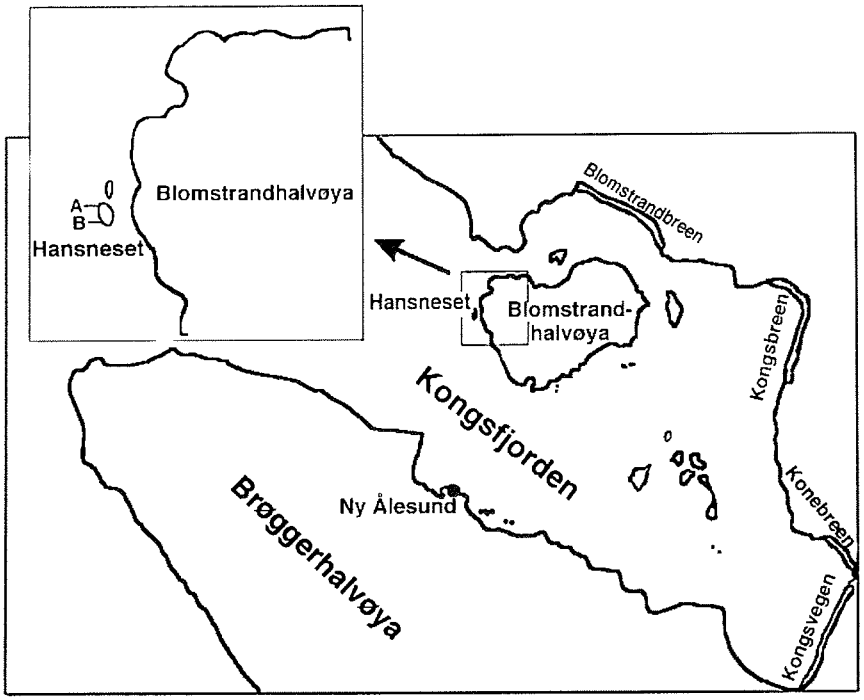

Fig. 1 Map of the study area and location of the two transects

The studies were performed during three sampling periods. The first collection was made between late May and mid July 1996 at transect A (Fig. 1). The second and third collections were made along transect B in August and September of the years 1996 and 1998. Both transects were set at right angles to the shroreline in a westerly direction and they were about 25 m apart. Sampling at A involved qualitative collections at different depths along a 50 m line from the shore. Sampling at B involved quantitative collections from within 50x50 cm quadrates starting at the low water line and repeated in duplicate at 1.5, 2.5, 5.0, 10, 15, 20, 25 and 30 m depth. Fresh weight of the macroalgae was determined directly after sampling and blotting with tissue paper. Identification of species was based on descriptions by Taylor (1966), Kornmann & Sahling (1977) and Vinogradova (1995b). For phytogeographical classification, the system of Lüning (1990) was used. Sources for geographical distribution are South & Tittley (1986) and Guiry & Nic Dhonncha (2004).

Results

Overall, 30 species were collected, including 7 green, 14 brown and 7 red algae (Table 1). Of these, only two species can be classified as Arctic species, *Laminaria solidungula* and *Devaleraea ramentacea*. Two species, *Spongomorpha*

centralis and *Ptilota gunneri*, have an exclusively cold temperate distribution, just extending to Spitsbergen. Most of the species (17) occur in the Arctic to cold temperate region, whereas 7 species extend from the Arctic to the warm temperate region. The flora of Kongsfjorden occupies an intermediate position

Table 1: Species collected at Hansneset, Kongsfjorden, their life-form types (a: annual, pp: pseudoperennial, p: perennial), depth zonation and distributional centre. Depth zonation of rare species is indicated as follows: US: upper sublittoral, LS: lower sublittoral. Phytogeographic regions are marked as follows: a: Arctic region, c: cold temperate region, w: warm temperate region

Class Order	Species	Life-form	Depth Zonation (m)	Distri-bution
Chlorophyta				
Codiolales	*Acrosiphonia flagellata* Kjellman	pp	0.5-4.5	ac
	Acrosiphonia incurva Kjellman	pp	0.5-4.5	ac
	Acrosiphonia sonderi (Kützing) Kornmann	pp	0.5-4.5	ac
	Spongomorpha aeruginosa (Linnaeus) van den Hoek	pp	0.5-4.5	acw
	Spongomorpha centralis (Lyngbye) Kützing	pp	0.5-4.5	c
	Urospora penicilliformis (Roth) Areschoug	pp	0.0-4.5	acw
Ulvales	*Monostroma obscurum* (Kützing) J. Agardh	a		acw
Phaeophyta				
Ectocarpales	*Pylaiella littoralis* (Linnaeus) Kjellman	a	0.0-2.5	acw
Sphacelariales	*Sphacelaria plumosa* Lyngbye	pp	0.5-10.5	ac
Dictyosiphonales	*Dictyosiphon foeniculaceus* (Hudson) Greville	a	0.5-15.5	ac
Chordariales	*Chordaria flagelliformis* (O.F. Müller) C. Agardh	a	0.0-5.5	ac
	Elachista fucicola (Velley) Areschoug	a	0.0-1.5	acw
Desmarestiales	*Desmarestia aculeata* (Linnaeus) J.V. Lamouroux	p	7.5-20.5	acw
	Desmarestia viridis (O.F. Müller) J.V. Lamouroux	a	3.5-15.5	ac
Laminariales	*Alaria esculenta* (Linnaeus) Greville	p	1.5-13.5	ac
	Chorda filum (Linnaeus) Stackhouse	a	US	acw
	Laminaria digitata (Hudson) J.V. Lamouroux	p	1.5-13.5	ac
	Laminaria saccharina (Linnaeus) J.V. Lamouroux	p	1.5-16.5	ac
	Laminaria solidungula J. Agardh	p	LS	a
	Saccorhiza dermatodea (De La Pylaie) J. Agardh	a	0.5-13.5	ac
Fucales	*Fucus distichus* Linnaeus	p	0.5-5.5	ac
Rhodophyta				
Palmariales	*Devaleraea ramentacea* (Linnaeus) Guiry	pp	0.5-7.5	a
	Palmaria palmata (Linnaeus) Kuntze	pp	US, MS	ac
Gigartinales	*Callophyllis cristata* (C. Agardh) Kützing	p	4.5-16.5	ac
	Coccotylus truncatus (Pallas) M.J. Wynne & J.N. Heine	pp	LS	ac
Ceramiales	*Odonthalia dentata* (Linnaeus) Lyngybe	p	LS	ac
	Phycodrys rubens (Linnaeus) Batters	pp	5.5-30.0	ac
	Ptilota gunneri P.C Silva, Maggs & L.M. Irvine	p	4.5-30.0	c

between the marine floras of East Greenland and northern Norway. In East Greenland the number of Arctic species is increased by 12 (Lund 1959b), whereas in northern Norway the number of Arctic species is the same as in Kongsfjorden. However, the number of cold temperate species (46) is considerably higher (Jaasund 1965). The composition of life-form types in Kongsfjorden reflects the fact that West-Spitsbergen is influenced by both Arctic and Atlantic water masses (Svendsen et al. 2002). Similar biogeographical relationships have been found for invertebrates in Kongsfjorden (Lippert et al. 2001; Hop et al. 2002; Laudien et al. this issue).

The zonation of the macroalgal community at our study site shows four algal belts: the littoral, the upper sublittoral down to about 3 to 5 m depth, the mid sublittoral between 3 and 8 to 15 m depth, and the lower sublittoral down to about 30 m depth.

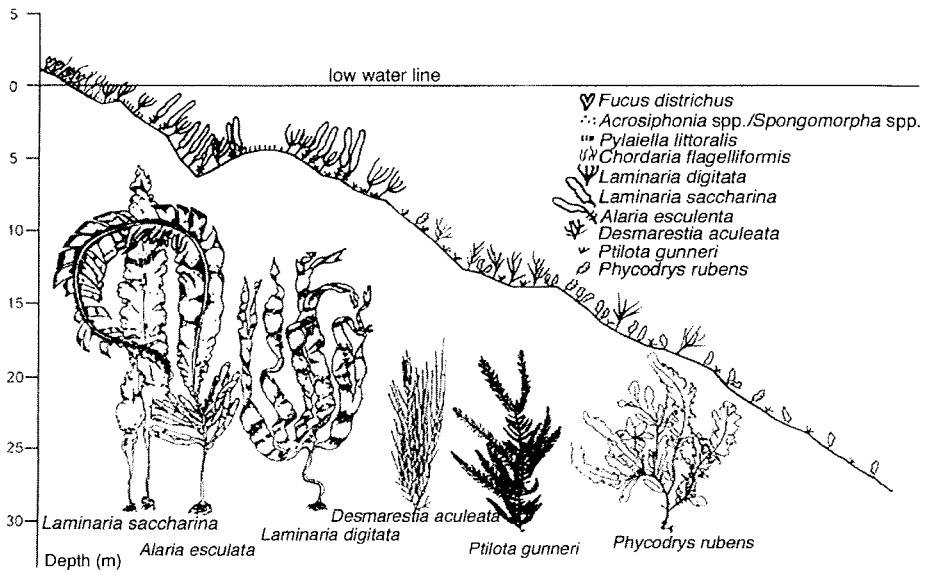

Fig. 2. Zonation of dominant macroalgae at Hansneset (Blomstrand, Kongsfjorden)

The upper sublittoral is characterised by the brown algae *Fucus distichus* with the epiphyte *Elachista fucicola*, *Pylaiella littoralis*, *Chordaria flagelliformis*, *Saccorhiza dermatodea*, the green algae *Urospora penicilliformis* and the morphologically similar species of the genera *Acrosiphonia* and *Spongomorpha*. The most important red alga in this belt is the endemic Arctic species *Devaleraea ramentacea*.

The key species in the mid sublittoral are the brown algae *Alaria esculenta*, *Laminaria digitata* and *L. saccharina*. The red algae *Callophyllis cristata* and the brown algae *Desmarestia viridis*, *Sphacelaria plumosa* and *D. aculeata* occur as undergrowth species in the mid sublittoral, the latter sometimes forming a separate belt between the mid- and low sublittoral.

The lower sublittoral is characterised, besides crustose red algae, by the red algae *Phycodrys rubens* and *Ptilota gunneri*. Both species also grow as undergrowth species in the mid sublittoral.

The brown algae *Dictyosiphon foeniculaceus* and the endemic Arctic *Laminaria solidungula*, the green alga *Monostroma obscurum* as well as the red alga *Palmaria palmata* are rare species at our study site. The species with highest biomass per m^2 are *Alaria esculenta*, *Laminaria digitata* and *L. saccharina*, followed by *Fucus distichus* and species of the genera *Acrosiphonia* and *Spongomorpha*. The maximum wet biomass in the upper sublittoral is 4.5 kg m^{-2}, in the mid sublittoral 6.5 kg m^{-2} and in the lower sublittoral 0.9 kg m^{-2}.

Discussion

The zonation pattern described above is comparable to that described for Isfjorden (Svendsen 1959) and other Arctic locations, e. g. for the region of the Nuvuk Islands in the north-eastern Hudson Bay (Keats et al. 1989) and for East Greenland (Lund 1959a, b). It is principally determined by the physical conditions, in particular by ice scour and the radiation conditions (e.g. Welch et al. 1992; Wiencke et al. 2000). Drifting sea ice or icebergs have a strong abrasive effect on the macroalgal community in the littoral and upper sublittoral. Strategies to avoid abrasive effects of this mechanical stress in the upper zones are colonization of small crevices and cracks in the rocks (*Fucus distichus*) or in small rock pools. In glacial fjords, such as Kongsfjorden, locations sheltered from the outward moving ice stream (e.g. Hansneset) generally have more macroalgae in the upper zones. Annual (e.g. *Urospora penicilliformis*, *Pylaiella littoralis*, *Saccorhiza dermatodea*) or pseudoperennial species (e.g. *Devaleraea ramentacea*) have clear advantages over perennial species. *Acrosiphonia* spp. and *Spongomorpha* spp. survive the winter by rhizoidal cushions and develop new filaments in spring. *Devaleraea ramentacea* is pseudoperennial with a high regeneration capacity. New thallus parts are formed on the frond basis in each growth season. The sporophyte of *Saccorhiza dermatodea* is annual with a high growth rate, which helps to out-compete other species. Its microscopic gametophyte is not negatively affected by ice scouring, which rather helps with the further propagation of the species through fragmentation and dispersal of gametophytic filaments. Both species typically grow on ice scoured patches as already pointed out by Keats et al. (1985).

In addition to the mechanical stress caused by drifting ice, the upper littoral (above the high water line) is for a large part of the year covered by a strong ice foot. This ice foot can be a protection against mechanical stress for resistant species that can withstand freezing. On the other hand it makes colonisation of

the upper littoral by perennial species physically impossible (Svendsen 1959; Keats et al. 1989).

The radiation regime influences the depth distribution of species in two ways. In the upper sublittoral, both UV radiation and photosynthetically active radiation (PAR) may be too high for sensitive species, allowing only the presence of the more tolerant species (Hanelt et al. 1997; Aguilera et al. 1999; Bischof et al. 2002; Wiencke et al. 2000; Karsten et al. 2001; Bischof et al. this issue). In contrast, PAR is limiting in the lower sublittoral and the depth distribution limit of individual species is determined by the balance between photosynthesis and respiration, the so-called metabolic carbon balance (Gómez et al. 1997).

The results described here are part of a larger study at Hansneset and other parts of Kongsfjorden. The future papers will include also the microscopic species, which are presently under investigation. Moreover, data on algal biomass and coverage will be provided.

Acknowledgements

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Trophic interactions between macroalgae and herbivores from Kongsfjorden (Svalbard)

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Introduction

The sublittoral hardbottom zones of Kongsfjorden are characterized and dominated by a dense macroalgal community mainly consisting of *Laminariales* (kelps) in the upper parts and low-light acclimated red algal species in depths below 18m. These algal communities produce high amounts of biomass (6.5 kg wet mass m^{-2}; Wiencke et al., this issue), which represent an important trophic contribution to the shallow water ecosystem as a potential food source, either as fresh algae or as particulate organic matter (POM). A large fraction of macroalgal primary production is released as POM into the benthic/pelagic food web (Hawkins et al. 1992, Hay & Steinberg 1992) and consumers such as filter feeders will benefit from it. In contrast, it has been suggested that the fraction of macroalgal production consumed directly as fresh algae by herbivores is small (Hawkins et al. 1992). The ecological importance of living macroalgae as food for invertebrates in the shallow water benthic ecosystem is little understood, particularly in the Arctic region.

Therefore, the present study investigates for the first time trophic interactions between invertebrates and macroalgae in Kongsfjorden with special emphasis on defense mechanisms against grazing. The main questions to be addressed - are there herbivorous animals feeding on fresh macroalgae and do they show specialisations or preferences for certain algal taxa?

To answer this question diving investigations were undertaken to map the macroalgal community and the associated invertebrate fauna, followed by a characterisation of mobile animals with respect to their feeding-behaviour. Some herbivorous invertebrates could be found and were used in bioassays to evaluate preferential feeding behaviour within the grazers.

Materials & methods

To map the macroalgal community and associated invertebrate fauna diving investigations were undertaken at many dive sites throughout the Kongsfjorden area. If possible, each dive site was visually evaluated by diving along

horizontal transects at 0, 5, 10, 15, 20, 25 and 30 m over a distance of 60 m each. All macroalgal species were recorded, depth range for every species was estimated and samples of every algal species were taken at mean typical depth. Sampled specimens were wrapped underwater with fine-meshed nets to catch the associated volatile animals for later laboratory studies.
All potential herbivorous meso- and macrograzers were collected by hand or with nets pulled over the algae and kept in the laboratory together with a mixture of 4 species of macroalgae: *Palmaria palmata, Monostroma arcticum, Desmarestia aculeata* and *Laminaria saccharina* in order to study their feeding preferences.
All living animal and algal specimens used for the experiments in the laboratory were collected between June and August 2002 by SCUBA diving in the Kongsfjorden area in water depths down to 30 m. In the laboratory they were maintained in running seawater at a temperature of 4-5°C.

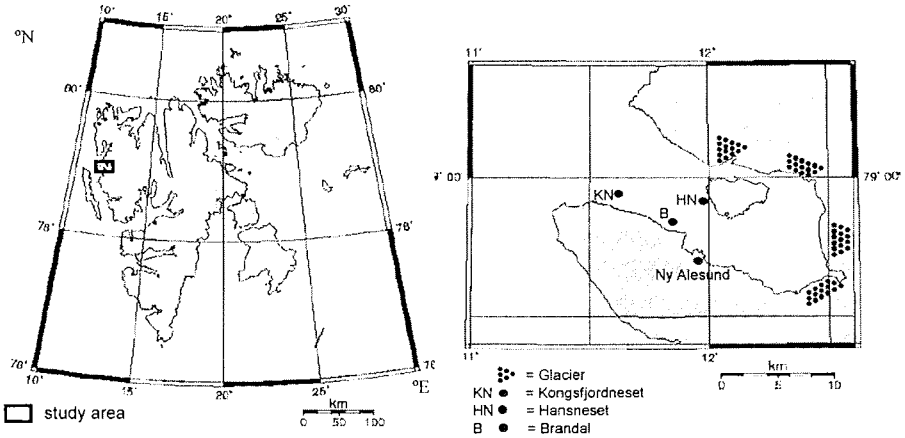

Fig. 1: Map of Svalbard (left) and Kongsfjorden area (right) with sampling and study sites

The amphipod *Gammarellus homari* was collected at the dive site Hansneset (Fig.1) between 2 and 6 m depth. A pool of about 120 individuals could be maintained in a flow-through seawater tank at a temperature of about 4-5°C for the period of June to August 2002. The amphipods were fed with the red alga *Devaleraea ramentacea* and did not show any changes in terms of vitality or feeding-behaviour through this time period (unpublished data).

The echinoid sea urchin *Strongylocentrotus droebrachiensis* was collected at the dive site Kongsfjordneset (Fig.1) at water depths between 12 and 15 m on barren ground and maintained in a flow-trough seawater tank at a temperature of about 4-5°C.

In preparation for the feeding experiments 20 individuals were starved for two days prior testing. First trials in the laboratory of keeping them together like the amphipods in a pool of 80-120 individuals per m^{-2} failed because they exhibited cannibalistic behaviour after several days. To prevent this the animals were collected freshly from the field two days before the experiment. So every individual was used only once to avoid these unwanted side-effects caused by stress.

The 4 algal species tested were collected at the dive sites Hansneset (*Laminaria saccharina* at 12 m; *Devaleraea ramentacea* at 4 m), Kongsfjord-neset (*Alaria esculenta* at 12 m) and Brandal (*Palmaria palmata* at 14 m). In the laboratory the plant material was kept at 4-5°C in running seawater before use in the experiments.
Non-choice feeding experiments were carried out with the herbivorous animals in the laboratory. For every test 20 animals were placed in beakers (1.5 l for the sea urchins, 0.2 l for the amphipods) filled with sea water at a temperature of 4-5°C and allowed to feed on a freshly cut piece of treatment alga (~1g for the sea urchins, 0.1g for the amphipods) for 24 hours.
To estimate autonomous weight change of the algal species during the tests, 20 equal-sized pieces of the same alga were put in beakers without the herbivore as control alga. Algal pieces were weighed before and after testing. To estimate the eaten amount of each treatment alga, consumption was calculated per individual within 24 hours using the following formula:

$$\text{Consumption} = [(T0 \times Cf/C0) - Tf]$$

(T: treatment alga, C: control alga, 0: before testing; f: after 24h; Cf/C0: mean factor of autonomous weight change for the 20 control algae)

Results

38 species of benthic macroalgae were identified (Table 1), as well as about 100 invertebrate taxa living in association with these plants, most belonging to the sessile epifauna (Lippert et al. 2001). The depth distribution of some of the algal species found in this study here is described by Wiencke et al. (this issue).

Table 2 shows the names of the associated volatile invertebrate species. After several days of observation in the field, feeding in the laboratory and by using taxonomic literature most invertebrate species were identified as non-herbivorous animals or at least as not feeding on living macroalgae.

Table 1: Macroalgal species present in the inner and outer basin of the Kongsfjorden

Phaeophyceae	Rhodophyceae	Chlorophyceae
Alaria esculenta	*Bangia atropurpurea*	*Acrosiphonia* spec.
Chorda filum	*Callophyllis stellata*	*Chaetomorpha linum*
Chorda tomentosa	*Ceramium strictum*	*Chaetomorpha melagonium*
Chordaria flagelliformis	*Coccotylus truncatus*	*Monostroma arcticum*
Desmarestia aculeata	*Devaleraea ramentacea*	*Prasiola crispa*
Desmarestia viridis	*Kallymenia microphylla*	
Dictyosiphon foeniculaceus	*Odonthalia dentata*	
Ectocarpus siliculosus	*Palmaria palmata*	
Elachista fucicola	*Phycodrys rubens*	
Fucus distichus	*Phycodrys* spec.	
Laminaria digitata	*Polysiphonia urceolata*	
Laminaria saccharina	*Porphyra umbilicalis*	
Laminaria solidungula	*Ptilota gunneri*	
Pylaiella littoralis	*Rhodomela lycopodioides*	
Saccorhiza dermatodea	*Scagelia* spec.	
Scytosiphon lomentaria		
Sphacelaria plumosa		
Stictyosiphon tortilis		

Table 2: Volatile invertebrate species found on or in short distance to the macroalgae and proposed feeding strategy

Phylum	Group	Species	Food / Feeding Strategy
Mollusca	Polyplacophora	*Tonicella* spec.	herbivorous, encrusting red algae
	Gastropoda	*Margarites helicinus*	herbivorous, biofilms
		Buccinum undatum	carnivorous, scavenger
		Onchidoris spec.	carnivorous, bryozoans, barnacles
		Facelina bostoniensis	carnivorous, hydroid polyps
		Dendronotus frondosus	carnivorous, hydroid polyps
Annelida	Polychaeta	*Nereis* spec.	carnivorous, predator
Crustacea	Amphipoda	*Gammarellus homari*	herbivorous, macroalgae
		Anonyx nugax	carnivorous, scavenger
		Onisimus spec.	omnivorous, plancton
		Caprella spec.	carnivorous, predator
	Decapoda	*Sclerocrangon boreas*	carnivorous, predator
		Hyas araneus	omnivorous, scavenger
Echinodermata	Echinoidea	*Strongylocentrotus droebrachiensis*	herbivorous/ macroalgae
	Asteroidea	*Pteraster pulvillus*	detritivorous/carnivorous
		Henricia spec.	detritivorous/carnivorous
		Crossaster papposus	detritivorous/carnivorous
		Asterias rubens	detritivorous/carnivorous
		Hippasteria phrygiana	detritivorous/carnivorous

The most abundant animals were the amphipod species *Caprella spec, Onisimus spec, Anonyx nugax* and *G. homari*, the gastropod *Margarites helicinus* and the echinoid sea urchin *S. droebrachiensis* contributing more than 95% to all animals counted. All other taxa were found only occasionally. The amphipod *G. homari* was found in the field only in the inner basal parts of the host alga *Devaleraea ramentacea* from 2 to 5 m.

The echinoid *S. droebrachiensis* was found in the field only in the outer basin of Kongsfjorden on hardsubstrate between 8 and 18 m.

Finally, only two conspicuous herbivorous species feeding on living macroalgae could be identified and differentiated from non-herbivorous animals, the amphipod *G. homari* and the echinoid *S. droebrachiensis* (see table 2). Both taxa were chosen as model grazers and bioassays were designed according to the trophic requirements of those two species (see chapter Materials & Methods).

Amphipod bioassay

In the feeding experiments the amphipod *G. homari* preferred the red algae *Palmaria palmata* and *Devaleraea ramentacea*, with mean daily consumptions of 50 mg per individual (Fig. 2). The mean consumption of kelps was lower (about 20mg per day; see Fig. 2), and in the case of *Alaria esculenta* the stipe seems to be preferred compared to the blade.

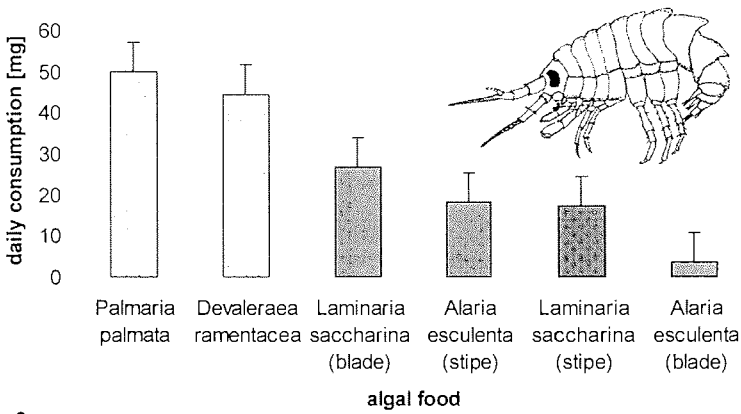

Figure 2:
Macroalgal feeding experiments with the amphipod *Gammarellus homari* under controlled laboratory conditions. Pre-weighed thallus pieces of the different plants were offered for 24h as food items and the mean daily consumption (mean± SE) per individual calculated (n=20); algae are arranged in the order of decreasing mean consumption (closed bars: brown algae, open bars: red algae).

Sea urchin bioassay

In the feeding experiments the sea urchins showed a preference for the brown alga *Laminaria saccharina* and the red alga *Palmaria palmata* (mean consumption of 400 and 340 mg; Fig. 3). In contrast, the red alga *Devaleraea ramentacea* and the brown alga *Alaria esculenta* were consumed only in lower

amounts of about 210 mg per day. Within the kelp-like species there was no difference between the consumed amounts of blades or stipes as in the case of the experiments with *G. homari.*

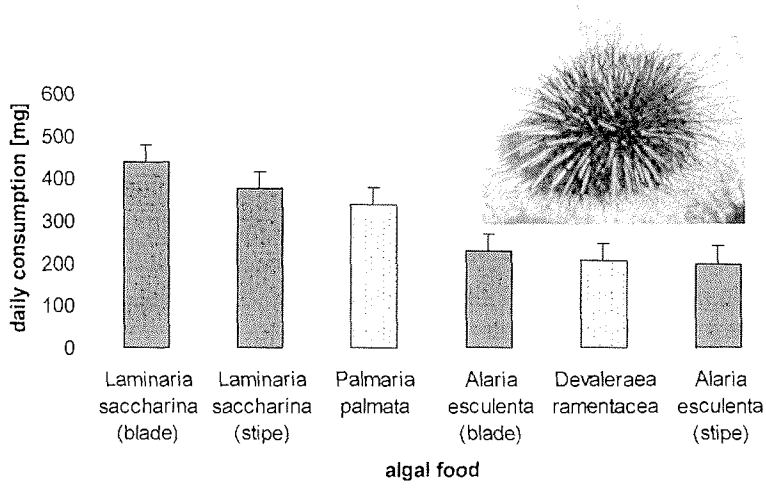

Figure 3:
Macroalgal feeding experiments with the sea urchin *Strongylocentrotus droebrachiensis* under controlled laboratory conditions. Pre-weighed thalli pieces of the different plants were offered for 24h as food items and the mean daily consumption (mean± SE) was calculated per individual (n=20); algae are arranged in the order of decreasing mean consumption (closed bars: brown algae, open bars: red algae).

Discussion

In temperate regions herbivorous amphipods often show a nocturnal activity (Brawley 1992). Due to constant daylight in the Arctic summer and the wide absence of predators like fish in Kongsfjorden (H. Wessels, personal observation) it can be concluded that herbivorous amphipods in Kongsfjorden are active throughout the day. Additionally volatile mesograzers like amphipods are believed to live on the algal species they feed upon (Brawley 1992). So in the field the host algae serve as refuge as well as food which forces the amphipods to specialize on macroalgal species suitable for both purposes. In the case of *G. homari*, this is fulfilled to a high extent by the red alga *Devaleraea ramentacea.*
The explanation for the high consumption of *Palmaria palmata* in the laboratory and the absence of *G. homari* associated with this species in the field could be the good palatability, as well as the low suitability as refuge because of the leathery and thick blades and the low ramification (Norderhaug 2004). The very low consumption of the blades of *Alaria esculenta* (4mg) might be a result of the

fact that the blades of this kelp are much thinner and less leathery than in other kelps and therefore provide a lower nutritional quality and higher amounts of UV-absorbing phlorotannins (Steinberg 1984), which are reported to deter herbivores than the stipes (Steinberg 1984, Toth & Pavia 2002, Clayton 2002 pers. comm., Lüder 2004 pers. comm.).
The second grazer in this study, the green sea urchin *S. droebrachiensis* is a macrograzer believed to be a generalist (Hawkins et al. 1992). Macrograzers are animals much larger than amphipods or isopods. Macroalgae serve macrograzers normally as food source only, and not as refuge (Hawkins et al. 1992). Therefore we hypothesize that in the case of the sea urchin palatability and availability of algae are the driving forces for food selection which would lead to generalistic grazing in chemically unprotected algal communities. Experiments with a larger set of algal species, which will be published in a following publication, support this hypothesis.
Finally there was no clear preference for certain taxa or for morphologically similar algae as observed in the case of *G. homari*. Moreover, differences between the consumed algal species were much smaller, and a more generalistic feeding was obvious. This could also be confirmed by feeding-experiments with homogenated algal extracts embedded in alginate. In these experiments differences in consumption between the provided algal species were even lower as in the experiments with fresh algae (Wessels et al. unpubl.).

Hence, with only two herbivorous species present the pressure of herbivore competition and feeding pressure on macroalgae in Kongsfjorden can be called low. So there seems to be a lack of competition-driven pressure for specialization on actually unpalatable macroalgae like as for the tropics (Cronin 1997, Bolser & Hay 1996). If future experiments verify the hypothesized minor role of chemical defense in Kongsfjorden, the reported general correlation between feeding pressure and chemical defense (Bakus 1974, Bertness 1981, Steinberg 1992, Cronin 1997) could be confirmed. Similar results were reported concerning the low rate of chemical defense against predation in benthic invertebrates of Kongsfjorden (Lippert, this issue).
However, the possible presence of chemical anti-herbivory has to be further evaluated in future experiments, in which the macroalgae will be homogenized, embedded in artificial foodpellets and offered to both main grazers again. If under such an experimental design the presented differences in algal preference diminish, this would indicate that morphology of Arctic macroalgae has a great impact on anti-grazing mechanisms (Brawley 1992).
Furthermore, the impact of the mesograzer *G. homari* on the macroalgal community can be considered to be low. Although this amphipod is the only regularly occurring grazer, it typically exhibits a low abundance. The only algal species which may be affected by *G. homari* is *Devaleraea ramentacea*. In the

field the amphipod clearly prefers this red alga as habitat. But for the kelp-dominated areas *G. homari* does not play a significant role in terms of a "top-down"-regulation.

In contrast, the impact of *S. droebrachiensis* on the macroalgal communities in Kongsfjorden is high similar as described by Pearce & Scheibling (1990). Mass developments and invasions of sea urchins are often prevented by sea urchin-predators such as sea otters or walruses (Estes & Steinberg 1988, Konar 2000). However these controlling animals are absent in Kongsfjorden. Consequently, completely bare stripped areas so-called „barren grounds" or "white spots" (Arnold 1976, Chapman 1981, Schiel 1982, Dean et al. 1984, Harrold & Pearse 1987, Watanabe & Harrold 1991) may develop, where all macroalgal species typically growing on these hard substrata are strongly affected or may even disappear. In addition, this confirms the classification of *S. droebrachiensis* as generalist. In contrast to the amphipod *G. homari*, these sea urchins carry out strong "top-down" control of macroalgal communities in Kongsfjorden.

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Palatability and chemical ecology of abundant marine invertebrates from Kongsfjorden (Svalbard)

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Introduction

The development of chemical defences in marine organisms of tropical and Antarctic regions is supposed to be mainly driven by the influence of intense biological interactions, such as predation and competition, due to a high biodiversity. In tropical regions mainly predation and herbivory by fishes are thought to have substantially influenced the selection for noxious and toxic chemical compounds in many sessile or slow moving invertebrates and macroalgae (Bakus and Green 1974; Green 1977; Hay and Fenical 1988; Hay and Steinberg 1992). Similarly Antarctic benthic communities are suggested to be structured mainly by biological factors favoured by the stable physical conditions below the zone of ice scour (Dayton et al. 1974). Of particular importance in Antarctic waters is the widespread occurence of spongivory by sea stars (McClintock 1994). The incidence of toxicity in sponges is similar to that in tropical and much higher than in temperate species (McClintock 1987). In contrast, at northern high latitudes the selective pressure for chemical defences has been generally proposed to be less important. Physical factors such as wave action, ice gouging (Gutt et al. 1996), and the influence of glaciers and river runoffs, adding high loads of inorganic material and freshwater mainly to

inner-fjord locations (Wlodarska-Kowalczuk et al. 1998) are supposed to be the major forces structuring Arctic benthic communities. Additionally, it is suggested that the relatively young evolutionary history of the Arctic Ocean contributes to a low biodiversity due to a relatively short period for adaptation and speciation (Gray 2001).

However, only few data exist on predator-prey relationships among lower trophic levels in Arctic waters, especially within the less abundant hard bottom communities. Large demersal fishes are not common (Dayton et al. 1994), and investigations by Thorson (1957) and Gulliksen (1979) found predation on Arctic benthic communities in general to be sparse. The selective pressure for chemical defences against predators may therefore be assumed to be poorly developed in benthic invertebrates of northern high latitudes. On the other hand, predation is just one factor exerting selective pressure on marine invertebrates. Many invertebrate species in temperate, tropical and Antarctic regions have, among others, chemical defences against the formation of microbial, and possibly deleterious, surface films. Total bacterial numbers in cold waters are proposed to be generally low compared to temperate and tropical latitudes, and it could be hypothesized that organisms would invest less resources into antimicrobial defence when the threat of bacterial colonization is lower. Water column bacterial numbers alone of course do not necessarily reflect the selective pressure exerted on marine invertebrates, and locally microbial cell density can be increased, for example, at Arctic inner fjord locations due to a high amount of particulate organic carbon at glacial meltwater outflows (Jankowska and Wlodarska-Kowalczuk personal communication). Consequently, the selective pressure for the development of chemical defences against deterimental surface colonizing or pathogenic micoorganisms in polar regions is not necessarily lower than in higher latitudes.

Therefore, the overall aim of this study was to assess for the first time chemical defences against predation and microbial colonization in 17 abundant sessile or slow moving invertebrates from an Arctic environment. Screening across a wide range of systematic groups (sponges, actinians, octocorals, nudibranchs, ascidians, bryozoans) is expected to give a good indication of the distribution and abundance of chemical defences in the study area.

Material and methods

Invertebrate Sampling

Samples were collected at four different sites in Kongsfjorden (Hansneset, a cave close to Hansneset, Kongsfjordneset and Prins Heinrich Islands). Seventeen abundant sessile invertebrate species representative for the different sites were collected from their natural habitat by SCUBA diving during the summers of 1999, 2000 and 2001. For experiments assessing the palatability of invertebrate tissue, samples were shock frozen in liquid nitrogen and stored at –28°C until use. Specimens for later extraction were shock frozen, lyophilized and as well stored at –28°C. Voucher specimens were preserved in 5% formaldehyde-seawater solution for later taxonomic identification. Species identification showed that the collection of *Styela* spp. consists of a mixture of the two morphologically extremely similar species *Styela rustica* and *Styela gelatinosa*.

In situ palatability assays

Ten of the investigated species were assayed for their palatability *in situ* to determine if invertebrate tissue would be consumed by naturally occurring consumers. One invertebrate species per experiment (test tissue), and in parallel fish as a control, were offered to naturally occuring predators in the field. Test tissue from several individuals of the respective species and the control tissue were cut into 1 cm^3 cubes and attached to 40 cm long pieces of buoyant rope using labelled safety pins. Three tissue pieces per rope were attached equidistantly. For one experiment, a total of 18 test food pieces and 18 control pieces were distributed randomly among 12 ropes. These ropes were placed at 4 m depth in the Komgsfjord by tying them to metal bars and were regularly checked to describe active consumers. After 24 hours numbers of pieces of each food type remaining were counted and the difference in consumption between test and control food was compared by Fisher's exact test (Sokal and Rohlf, 1981).

Extraction procedure, fractionation and isolation

To obtain crude extracts, a known weight of freeze-dried tissue from each species was ground by mortar and pestle, and extracted repeatedly in 1:1 methanol:dichloromethane, or subsequently in methanol, 1:1 methanol:dichloromethane, and dichloromethane. The partial extracts were combined and concentrated under reduced pressure by rotary evaporation at 40°C. Crude extracts were transferred into pre-weighted vials, evaporated to dryness under nitrogen or vacuum, and weighted. The extract yield per g DW freeze dried tissue is referred to as the natural concentration and was used to calculate the amount of extract used in feeding assays (see below). All crude extracts were stored at –28°C until use in feeding experiments.

Preparation of artificial food

Crude extracts were incorporated into artificial food and tested for potential feeding deterrent effects by offering them to a general predator in laboratory assays (see below). Artificial food pellets were prepared with alginic acid using freeze-dried and finely powdered fish as a feeding stimulant. Crude extract was added in a small volume of solvent at natural concentrations to the powdered fish and then the solvent was removed from the mixture by rotary evaporation until dryness. For control food the fish was treated with an equivalent amount of solvent only. The feeding stimulant (control) or the feeding stimulant coated with an invertebrate extract to be tested (test) was added to a liquid mixture of alginic acid and seawater. The mixture was spread out in a Petri-dish, hardened with $CaCl_2$ solution and pellets of 6 mm in diameter were cut using a cork borer. Pellets containing organic crude extracts are hereafter referred to as test pellets. Pellets without extract are referred to as control pellets.

Feding assay with amphipods

Laboratory feeding experiments were performed with the abundant amphipod species *Anonyx nugax* from Kongsfjorden. Numerous individuals of this species were caught by traps baited with fish and kept in laboratory aquaria at Ny Ålesund research station. For feeding experiments, in each of eight one liter plastic beakers 20 amphipods were placed. Each beaker held one test pellet and one control pellet. To determine changes in pellet mass caused by water

uptake or loss of material during the experiment, an equal number of beakers without amphipods were prepared as controls, each containing one test and one control pellet. All pellets were gently blotted and weighed before the experiments. Amphipods were removed when about half of the pellets in the experimental beakers had been consumed, but latest after 8 hours. After the experiment, all test and control pellets were blotted again and weighed to determine mass changes during the experiments. Crude extracts of seventeen species were tested for palatability in this assay. Data were analysed according to Peterson and Renaud (1989) (α=0.05).

Antimicrobial effects of secondary metabolites

Potential antibacterial activity of crude extracts was tested in the agar disc-diffusion assay (Acar 1980) against five sympatric bacterial strains, isolated from the vicinity of the investigated species. The bacterial isolates used were phylogenetically diverse and represented typical cultivable members of the marine microbial community in cold seas (Lippert et al. 2003). Bacterial strains were grown in liquid medium at 4°C for at least 7 days prior to the experiments. Inocula of each strain were spread on separate agar plates using a sterile glass rod to provide a uniform film of the test bacteria. Crude extracts were dissolved in aliquots of the extraction solvent to give natural concentrations and were applied onto each side of a sterile paper disc. Discs were then placed in a previously sterilized drying oven at 30°C and solvents were allowed to evaporate. Control discs were prepared in the same manner with solvent only. Up to six extract discs and one solvent control disc per plate were placed on the surface of agar plates previously seeded with individual bacterial strains. After five days of incubation, zones without bacterial growth were recorded. According to extract availability one to five replicates per extract were tested. Solvent control discs were never observed to inhibit bacterial growth.

Results

In situ assays for palatability were performed with ten species, exposing tissue to natural consumer assemblages. From diving observations it is suggested that these assemblages mainly consisted of amphipods. With the exception of the

ascidian *Styela* spp., all invertebrates were consumed significantly less compared to the control food offered simultaneously (Fig. 1). Six of the offered species were not preyed on at all while the control food was eaten to at least 29 % in all experiments.

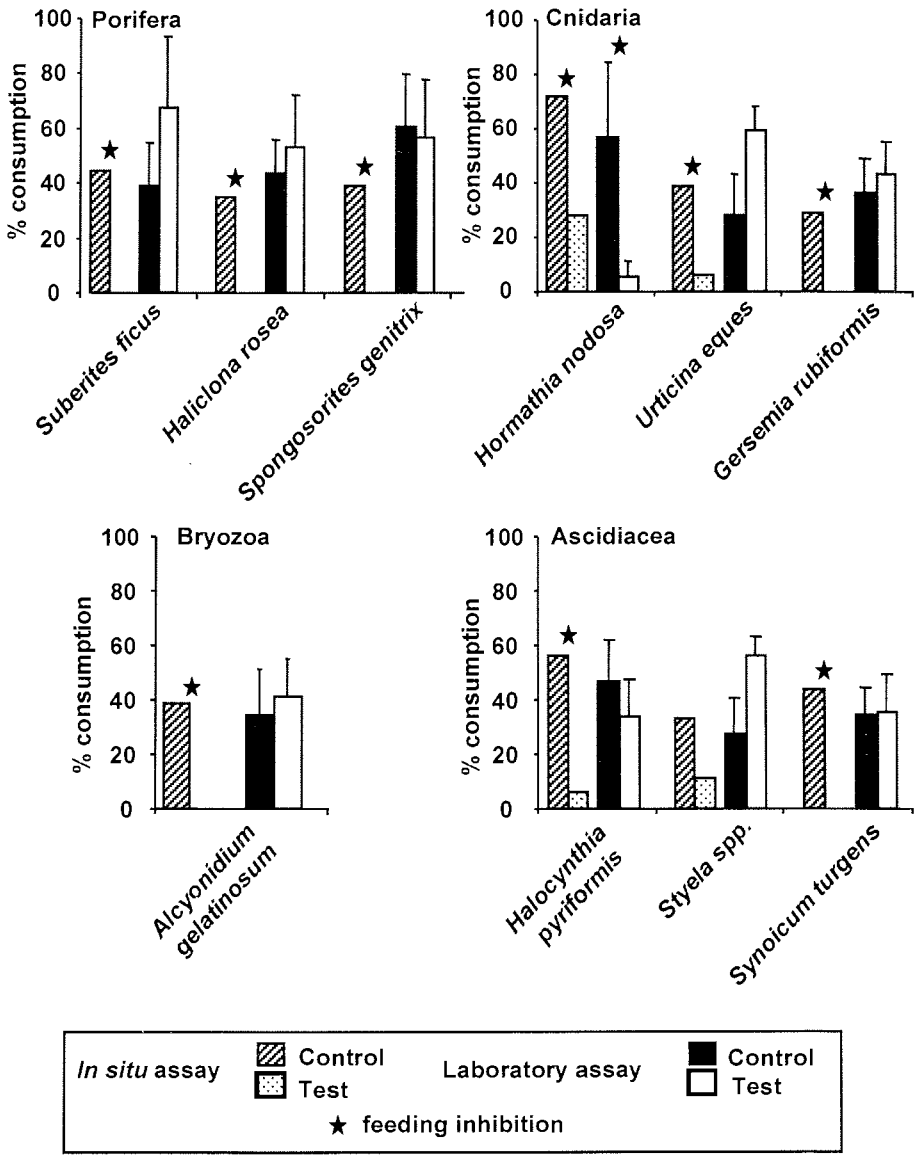

Figure 1: Invertebrates tested in parallel in *in situ* assay with natural assemblages of predators and laboratory assay with the amphipod *Anonyx nugax*. Results of *in situ* experiments are given in percent pieces test and control tissue consumed. Results of laboratory experiments are shown in percentage (mean ± SD) consumption on test and control pellets. Significant lower consumption (α<0.05) on test food is indicated by asterisks.

Results of laboratory assays with crude extracts of 17 species using the amphipod *Anonyx nugax* as a consumer are given in Figs. 1 and 2. Crude extracts of two species, the actinian *Hormathia nodosa* (Fig. 1) and the sponge *Haliclona viscosa* (Fig. 2), significantly deterred feeding ($P \leq 0.0001$), The remaining ten species had no significant inhibiting effect on the consumption by amphipods at natural extract concentrations.

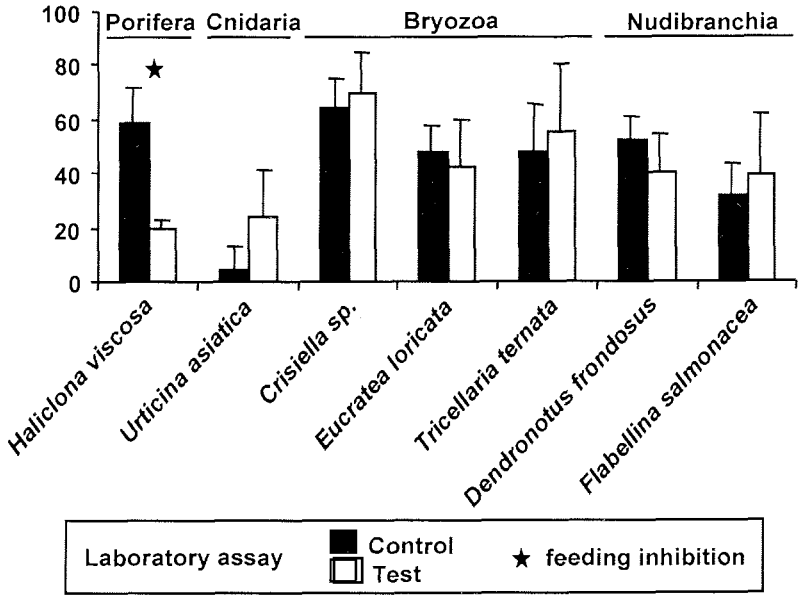

Figure 2: Invertebrates tested in the laboratory assay with the amphipod *Anonyx nugax*. Percentage (mean ± SD) of test and control pellets consumed in palatability assays are given. Asterisks indicate significant inhibition of feeding by crude extracts ($\alpha < 0.05$, Student's t-test).

Antimicrobial effects at natural extract concentrations were found in 6 out of 17 species tested against five strains of sympatric bacteria (Fig. 3). The extract of the sponge *Haliclona viscosa* had the strongest antimicrobial activity in terms of the number of strains inhibited. Only this sponge inhibited the growth of all five test bacteria, while the soft coral *Gersemia rubiformis*, the bryozoan *Alcyonidium gelatinosum* and the nudibranch *Flabellina salmonacea* inhibited two strains, the bryozoan *Crisiella* sp. and the ascidian *Halocynthia pyriformis* both inhibited only one strain.

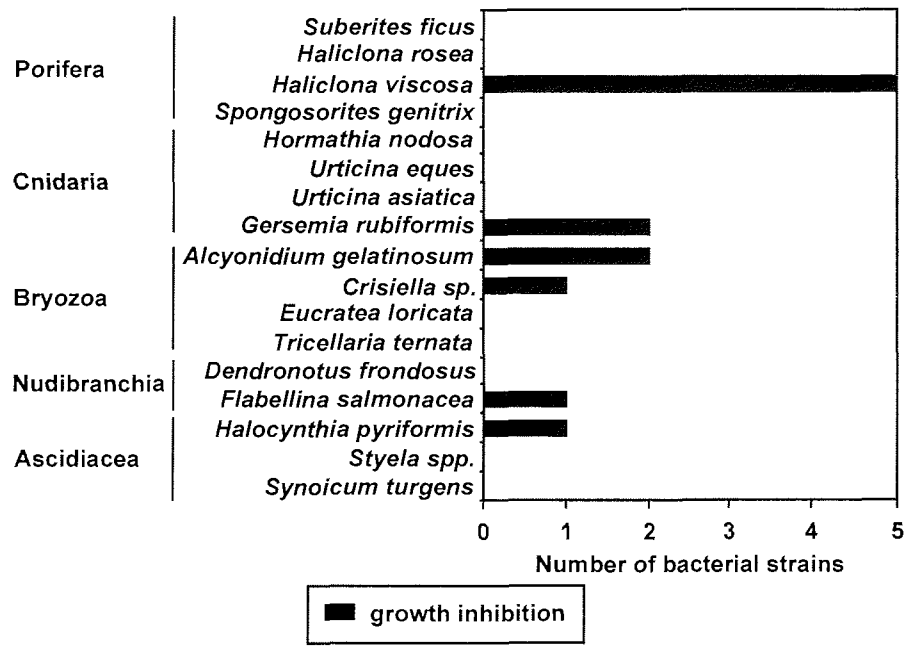

Figure 3: Inhibition of bacterial growth by extracts of 17 invertebrate species, tested against five sympatric bacterial strains.

Discussion

In the *in situ* palatability assays, testing tissue of ten species, nine invertebrates were significantly rejected by naturally occurring predators. In contrast, feeding experiments testing crude extracts of the same ten species showed that only one, the actinian *Hormathia nodosa*, significantly inhibited consumption by the amphipod *Anonyx nugax* (Fig. 1). These data indicate that a high percentage of species from Kongsfjorden (90%) is defended against predation, but that the incidence of invertebrates with chemical defence is low (10%). If chemical defence is not a common way of the investigated invertebrates to protect against predation, other defensive strategies, such as structural defences or a poor nutritional quality, must be responsible for the low palatability of the offered tissue. However, Lippert and Iken (2003) did not find an obvious correlation between consumption and nutritional quality of benthic invertebrates from Kongsfjorden, while structural defences have not been studied in detail yet.

In total, crude extracts of seventeen species were tested against the amphipod *Anonyx nugax* in laboratory feeding assays (Figs. 1 and 2). Only two species (12%), the actinian *Hormathia nodosa* and the sponge *Haliclona viscosa* deterred feeding. Similarly to the low incidence of chemical defence against predators, only a small portion of invertebrates was chemically defended against bacteria (35% of 17 species tested). Thus, the results from antifeeding and antibacterial experiments of the present study indicate a rather low percentage of chemically defended invertebrates compared to tropical (Pawlik et al. 1995; Newbold et al. 1999) and Antarctic regions (McClintock 1987; McClintock and Gauthier 1992), and it is also in the lower range of that found in temperate waters (*fide* Bakus and Green 1974).

Predation is suggested to be low in the Arctic (Thorson 1957; Gulliksen 1979) and may thus not be a strong selective force to drive the evolution of chemical defences in marine organisms from high northern latitudes. Although the effect of predators can be high in infaunal communities (Ambrose 1984, 1986), predator-prey relationships within the less abundant hard bottom communities are badly studied in Arctic waters. The *in situ* experiments in the present study were performed to examine and quantify the feeding response of a natural assemblage of predators towards tissues of benthic invertebrates. They also may give limited insight into predation intensity in the study area. Regular feeding on the control food (29-72% consumption) documented the presence of natural assemblages of predators. However, controls were never eaten entirely. This could indicate that natural assemblages of consumers had been saturated after a limited feeding period, probably due to their generally relatively low abundance in the middle and inner Kongsfjorden. Regular controls of the running experiments suggest that mostly amphipods were feeding on the offered food. It is unlikely that fish species were feeding on the experiments since there have been very few observations of fish during over 200 SCUBA dives in Kongsfjorden between spring and late summer. However, fishes cannot be excluded completely as consumers in the *in situ* experiments, because some fish species are reported from the shallow water (<30 m) with mainly pleuronectids feeding on epibenthic fauna (Hop et al. 2002). According to Hop et al. (2002) and personal underwater observations starfish are the most

conspicuous carnivorous invertebrates in Kongsfjorden beside prosobranch snails. While carnivorous starfish like *Solaster endeca* and *Crossaster papposus* are common in the outer Kongsfjorden, suspension feeding *Henricia sanguinolenta* and detritivorous *Poraniomorpha hispida* were found in the inner fjord (references in Jangoux 1982; personal observation). Overall, down to 30 m water depth predation seems to play a minor role, at least in the inner part of the fjord. The selective pressure for chemical defences against predators might therefore be assumed to be low.

While the low incidence of chemical defences against predation in the Kongsfjord might be explained by a low selective pressure, i.e. low abundance of predators, the question remains why also the incidence of antimicrobial activity is low compared to other regions, for example the Antarctic (McClintock and Gauthier 1992). Cold adapted microorganisms have been described in similar presence and abundance from Antarctic and Arctic waters (Zajaczkowska and Zajaczkowski 1989; Zdanowski 1995; Knoblauch et al. 1999), and there is no evidence to assume strong differences in bacterial pressure between both polar regions. Comparisons of the number of bacterial strains that could be cultured from invertebrate surfaces showed that bacteria colonized the investigated species to different degrees (Lippert et al. 2003). These observations suggest that there may be mechanisms other than secondary metabolites responsible for antifouling properties of some of the invertebrates from Kongsfjorden, for example, mechanical or physical defences like tissue sloughing (Barthel and Wolfrath 1989), mucus secretion (Krupp 1985) or surface acidity (Hirose et al. 2001). Additionaly, latitudinal comparisons are sometimes difficult, due to the variety of methods used and an uneven distribution of knowledge on chemical defences among the taxonomic groups. While extensive literature exists on sponges (McClintock 1987; Newbold et al. 1999) and gorgonian corals (Van Alstyne and Paul 1992; Jensen et al. 1996), little is known about bryozoans (Walls et al. 1993), and even less about actinians.

However, although the presented data support the initial assumption of a low incidence of chemical defence in Arctic waters, it has to be kept in mind that Kongsfjorden is a single location and may not be representative for other Arctic

regions. Kongsfjorden, although located at high latitude, is under the influence of the relatively warm West Spitsbergen Current (Svendsen et al. 2002), and therefore has to be regarded as a sub-Arctic rather than a high Arctic fjord (Hop et al. 2002). In addition, Kongsfjorden is strongly influenced by glacial activity. High sedimentation rates, freshwater influence and ice scouring are known to have a considerable structuring effect on the fauna especially of inner fjord locations (Wlodarska-Kowalczuk et al. 1998). The biological dynamics in Kongsfjorden might be representative for western Spitsbergen fjords (Hop et al. 2002), but environmental conditions may be very different at other locations in high northern latitudes that are more under the influence of Arctic water masses, such as the northern and the eastern shores of Spitsbergen. More chemical ecology studies at different sites in the high northern hemisphere are necessary to draw general conclusions on the significance of chemical defences in Arctic waters.

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The sponge community of a semi-submerged cave in Kongsfjorden, Svalbard

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Introduction

Despite decades of sampling biological material from Svalbard including both continental shelf areas and fjords, little is known about the sponge communities of Kongsfjorden (Hop *et al.*, 2002). A research report for the Directorate for Nature Management (Trondheim, Norway) on the distribution of marine benthic macro-organisms from areas of Svalbard (including Bear Island) revealed in total 260 different species of sponges (Gulliksen *et al.*, 1999). This report presented an inventory of data (species records) collected from published (since The Norwegian North-Atlantic Expedition in 1876-1878) and unpublished sources. Although most of the sampling, especially in earlier investigations, was carried out with surface-operated sampling gear (for example dredges, sledges, trawls and grabs), also diving inventories from the University of Tromsø, starting in 1972, have been incorporated in parts into the database (Gulliksen *et al.*, 1999). Based on results from an additional study on the megabenthic communities in the waters around Svalbard, another three species could be added to the total record number of sponges (Piepenburg *et al.*, 1996). So far, the number of sponge species for Kongsfjorden in the literature until the year 2000 only covered seven records, as follows: *Artemisina arcigera*, *Halichondria labiata*, *Halichondria panicea*, *Isodictya* cf. *palmata*, *Lissodendoryx indistincta*, *Reniera heterofibrosa*, and *Stylaxia variabilis* (Gulliksen *et al.*, 1999).

However, recent studies with focus on the macrofauna associated with macroalgae and the chemical ecology and palatability of marine invertebrates in Kongsfjorden provided new insights into sponge communities (Lippert *et al.*, 2001; Lippert, 2003; Lippert *et al.*, 2003; Volk & Köck, 2003).

In addition, the present contribution emphasizes the sponge community in a distinctive habitat, a semi-submerged cave in Kongsfjorden.

Material and Methods

The invertebrate community of the cave was video-monitored and selected organisms have been photographed prior to sampling. Sponges investigated in

this study were collected in August 2002 by SCUBA diving off the coast of Blomstrandhalvøya, near Hansneset, Kongsfjorden, in a semi-submerged cave at a depth of 2-4 m. Portions of sponges were collected by cutting tissue with a sharp knife, leaving the remaining sponge intact for re-growth. Tissue samples for chemical investigations with focus on natural products isolation were immediately frozen and stored at –20 °C until used for extractions, while tissue samples for subsequent microbial studies in collaboration with Dr. Gunnar Gerdts from the Biologische Anstalt Helgoland, Germany, were immediately flash frozen using liquid nitrogen. Sponges were identified on the basis of spicule and tissue preparations at the Zoölogisch Museum, Universiteit van Amsterdam, The Netherlands (Hooper & Van Soest, 2002). Voucher specimens are deposited at the Porifera collection in Amsterdam.

Results and Discussion

The cave is located at the northwestern coast stretch of Blomstrandhalvøya, not far from the spit Hansneset (78°59'50" N, 12°01'50" E). Detailed maps of Kongsfjorden area could be obtained from two recent reviews (Svendsen *et al.*, 2002; Hop *et al.*, 2002). According to Riedl, the cave shows features of the type of a grotto (Riedl, 1966). It is a sea-cave, which finishes up in a blind valley. The entrance of the cave is situated in the waterline, so that the swell can run unhindered in and results in breakers for the most part at the back. The extent of the cave is 14 m in width at the entrance and 22 m in length from the middle of the start, respectively. During high tide, the height at the cave entrance is 4 m from the water surface to the ceiling and arches up to 7 m in the center of the cave. In cross-section, the cave width proceeds from the entrance to the back rather regular and reaches 16 m only in the central part of the cave. Water depth at the beginning is 6 m in maximum, which gently descends to 3 m at the back of the cave.

The community composition within the cave habitat is largely determined by light conditions and therefore results in a typical gradient of settlement. Whereas the entrance zone of the cave is characterized by a belt of brown macroalgae including the endemic Arctic species *Laminaria solidungula*, followed by individuals of the red algae *Phycodrys rubens* and *Ptilota plumosa*, the back of the cave harbours an extensive invertebrate community on hard bottom substratum. This invertebrate community predominately consists of the often large and conspicuous octocoral *Gersemia rubiformis* and the sponges *Spongosorites genitrix* and *Suberites ficus*, scattered with individuals of the ascidian *Halocynthia pyriformis* and the sea anemone *Urticina eques* (Moen & Svensen, 2003).

In addition to the sponge species mentioned above, individuals of *Halichondria panicea*, *Haliclona excelsa*, *Sycandra utriculus* and *Sycon ciliatum* have been successfully identified (see Table 1).

Table 1. Sponge species collected in the semi-submerged cave at Blomstrandhalvøya, near Hansneset. For each sample, the registration number in the Porifera collection at the Zoological Museum Amsterdam (ZMA POR.), field number, date of collection and depth are given.

Species	ZMA POR.	Field Number	Date	Depth
Sycon ciliatum	17263	MAK 02/03	01.08.2002	4 m
Suberites ficus	17265	MAK 02/06	05.08.2002	2 m
Spongosorites genitrix	17266	MAK 02/07	05.08.2002	3 m
Spongosorites genitrix	17267	MAK 02/08	05.08.2002	3 m
Halichondria panicea	17268	MAK 02/09	05.08.2002	3 m
Spongosorites genitrix	17269	MAK 02/10	05.08.2002	4 m
Sycandra utriculus	17270	MAK 02/11	06.08.2002	5 m
Suberites ficus	17271	MAK 02/12	06.08.2002	3 m
Suberites ficus	17272	MAK 02/13	06.08.2002	3 m
Haliclona excelsa	17273	MAK 02/17	07.08.2002	3 m
Haliclona excelsa	17274	MAK 02/20	12.08.2002	3 m
Spongosorites genitrix	17275	MAK 02/21	12.08.2002	3 m
Spongosorites genitrix	17276	MAK 02/22	12.08.2002	3 m

Summarizing the results of the present and two recent studies (Lippert, 2003; Lippert *et al.*, 2003), seven new records of sponge species for Kongsfjorden could be contributed to the previous check-list (Gulliksen *et al.*, 1999): *Haliclona excelsa*, *Haliclona rosea*, *Haliclona viscosa*, *Spongosorites genitrix*, *Suberites ficus*, *Sycandra utriculus*, *Sycon ciliatum*. While *Suberites ficus* is only a new record for Kongsfjorden, the other sponge species represent new records for the entire Svalbard archipelago.

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Soft bottom community structure and diversity in Kongsfjorden (Svalbard)

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Introduction

Marine diversity is currently one of the most studied topics in ecology especially under the frame of global and regional environmental changes. In the marine realm biodiversity declines from the tropics to the Arctic (Thorson 1957, Brattegard & Holthe 1997, Roy *et al.* 1996, 1998, Gray 2001). Habitat and environmental heterogeneity generally favour biodiversity; additionally, intermediate physical disturbance enhances heterogeneity (Connell 1978, Connell & Keough 1985, Zacharias & Roff 2001). Shallow water systems are particularly interesting, because they are affected by environmental changes first and act as small-scale laboratories (Dayton 1990, Arntz *et al.* 1997). In marine polar habitats a common structuring disturbance is ice scouring (Dayton 1990, Gutt 1991, Dowdeswell & Forsberg 1992, Dayton *et al.* 1994, Gutt *et al.* 1996, Conlan *et al.* 1998, Sahade *et al.* 1998, Gutt & Piepenburg 2003). Hereby the benthic community is affected differently varying with latitude, depth, local current regimes, substrates, geography and site exposure resulting in a high variability both on spatial and temporal scales.

In northwest Spitsbergen five tidewater glaciers calve icebergs (incl. bergy bits *sensu* Armstrong *et al.* 1966) into the Arctic glacial Kongsfjorden (Liestøl 1988, Dowdeswell & Forsberg 1992). When icebergs contact the sea floor, scouring and associated sediment reworking takes place, which has been recognized as strongly affecting the local benthic fauna distribution and diversity (e.g., Holte *et al.* 1996, Wlodarska *et al.* 1996). The benthic soft bottom fauna inhabiting such dynamic areas has been described from a number of glacial or glaciofluvial fjords of Spitsbergen (e.g., Gromisz 1983, Gulliksen *et al.* 1984, Kendall-Aschan 1993, Wlodarska *et al.* 1996, Holte *et al.* 1996, Wlodarska-Kowalczuk *et al.* 1998). However, data on depths shallower than 25m are scarce and only cover Hornsund and Skoddebukta (Gromisz 1983, Wlodarska *et al.* 1996). Community analyses from Kongsfjorden start at 50m depth (Wlodarska-Kowalczuk *et al.* 1998).

The present study encompasses six different depth zones of a soft-sediment biotope and compares the macrobenthic communities for taxonomic and zoogeographical composition, biomass and diversity as well as feeding modes of dominant species. Variations in faunal associations are detected by cluster analysis of similarity from abundance and biomass data. Assuming that diversity is affected by iceberg scouring and in accordance with the 'intermediate disturbance hypothesis' (Connell 1978) depth zones affected by moderate iceberg scouring should show enhanced heterogeneity. In contrast areas of high scouring frequencies should host pioneer, physically controlled macrofaunal assemblages whereas more mature, less diverse communities should dominate areas of low disturbance frequency.

Material and methods

Study area

The study area, Brandal (78°58.53'N, 11°51.35'E), is situated in the inner part of the Arctic glacial Kongsfjorden on the western coast of Spitsbergen. It is located on the northeastern fringe of the Brøgger Peninsula, which forms the southern coast of Kongsjord. The latter is 20km long, its width varies from 4km to 10km at the mouth between Kvadehuken and Kapp Guissez. Maximum depth is close to 350m, and the outer part of the fjord connects directly with the North Atlantic Ocean via the Kongsfjord-Renna trough (Bluhm *et al.* 2001, Jørgensen & Gulliksen 2001, Svendsen *et al.* 2002).

The range of the semidiurnal tides is from 1.5 to 2m with weak currents. Mean sea surface temperature is just above 0°C, but can rise to 6°C in summer, while the temperature at 20m is 3.6°C (Bluhm *et al.* 2001). During summer the 34 psu isohaline may reach 5m depth. A review of the physical environment was presented in Svendsen *et al.* (2002; see also Hanelt *et al.;* this issue), the marine ecosystem is reviewed in Hop *et al.* (2002).

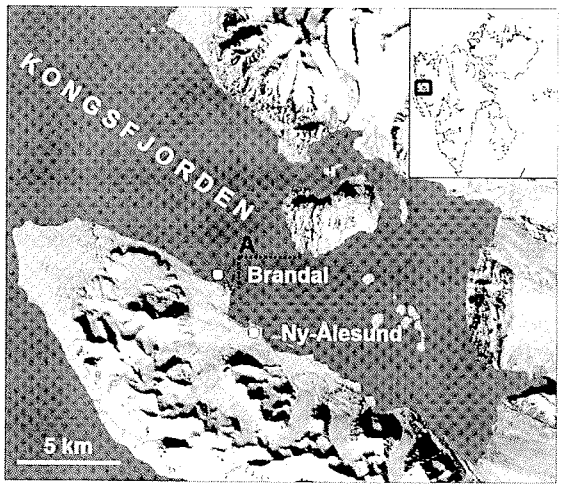


Fig. 1: Landsat TM image of Kongsfjord on Spitsbergen, (Svalbard archipelago) (modified from Svendsen *et al.* 2002). The study site Brandal, the village Ny-Ålesund and transect A of Dowdeswell and Forsberg (1992, see Discussion) are indicated.

Brandal (Fig. 1) is a soft-sediment habitat. The bottom inclines only gradually within the first 50m from the beach, followed by a steeper slope. Sediments are composed of a sand-clay mixture and are apparently well aerated. Occasionally ice-rafted stones overgrown by macrofauna and macroalgae (e.g. *Laminaria digitata*, *Palmaria palmata*) can be found.

Macrofauna

Macrozoobenthos was sampled in five replicates along six transects (5m, 10m, 15m, 20m, 25m, 30m) by pressing a corer of 20cm in diameter 20cm deep into the substrate. The enclosed sediment was sucked with an airlift system consisting of a tube (6cm in diameter, 80cm long with a n-end at the upper end), a compressed-air injection device coupled to a dive tank and a connected 0.5mm mesh retaining bag. All remaining material was sorted in the laboratory and animals preserved in 70% ethanol.

Thereafter all macrofaunal organisms were sorted using a binocular microscope, identified and counted. Biomass was estimated from a preserved subsample by weighing after blotting on filter paper, including valves of shelled organisms. Thereafter sub-samples were dried to constant mass at 60°C, weighed again and ignited in a muffle furnace at 500°C for 24h in order to es-

timate ash free dry mass (AFDM). Percentages of animals in the total faunal abundances were calculated for the five different depth zones separately. Shannon-Wiener diversity indices (H', Log e) were calculated for abundance values for each sample. Multivariate analysis was applied using the *PRIMER v5* package (Clarke & Gorley 2001). Data were square root transformed and Bray-Curtis similarities calculated. Classification (using group average linking) of samples was performed and groups of samples distinguished based on the resultant dendrogram. Statistical differences were analysed by means of an analysis of similarity (one-way ANOSIM, 95% confidence interval, Clarke & Gorley 2001). Species with the highest frequency (>75%) and significant dominance (>1%) within a group were identified as characteristic of that group using SIMPER (Clarke & Gorley 2001).

Results

Figure 2 shows that the number of cores taken was sufficient to detect >90% of the soft bottom fauna as the species-accumulation curves (*sensu* Gray 2001) flattened out at three to four cores. Annelids made up 79%, molluscs 11%, crustaceans 8%, echinoderms 1%, others (including priapulids, sipunculids, anthozoans and ascidians) made up less than 1% of 45 species and the additional 18 families not identified further. Regarding the number of individuals, annelids made up 84% of the fauna, molluscs 10%, crustaceans 3%, echinoderms 1% and others <2%. All taxa and their biomasses are listed in Table 1. Eleven species inhabited the complete depth range. The majority of all individuals of amphipods (80%) occurred at 5m. The bivalve *Cyrtodaria siliqua* was only collected at the shallow transect. In contrast the bivalve *Ciliatocardium ciliatum*, the gastropod *Oenopota* sp. and the polychaetes *Amphitrite cirrata* and *Orbinia* sp. were only present at 30m. At 5m the dominant species were: *Crassicorophium crassicorne* (32%) and *Spio armata* (26%). *Scoloplos armiger* (11-22%) and *Dipolydora quadrilobata* (14-31%) dominated all other depth zones, *Euchone analis* 10m and 15m (10%, 14%), *Spio armata* 10m, 20m-30m (11-14%) and *Chaetozone setosa* 20m-30m (11-14%). Five species were classified as Arctic species, 34 as Arctic-boreal, and 20 as cosmopolitans, 3 taxa were not classified. At all depths the zoogeographical species composition was very similar, with around 8% Arctic representatives, 58% Arctic-boreal, and 34% cosmopolitans. Comparable biogeographical relationships have been found for macroalgae of Kongsfjorden (Wiencke *et al.*; this issue).

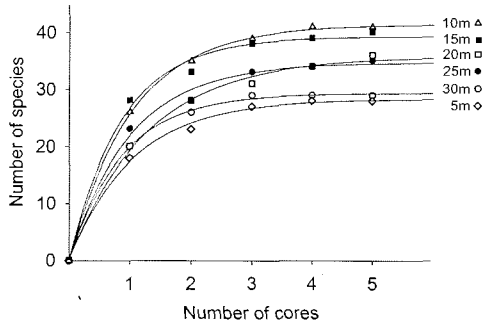
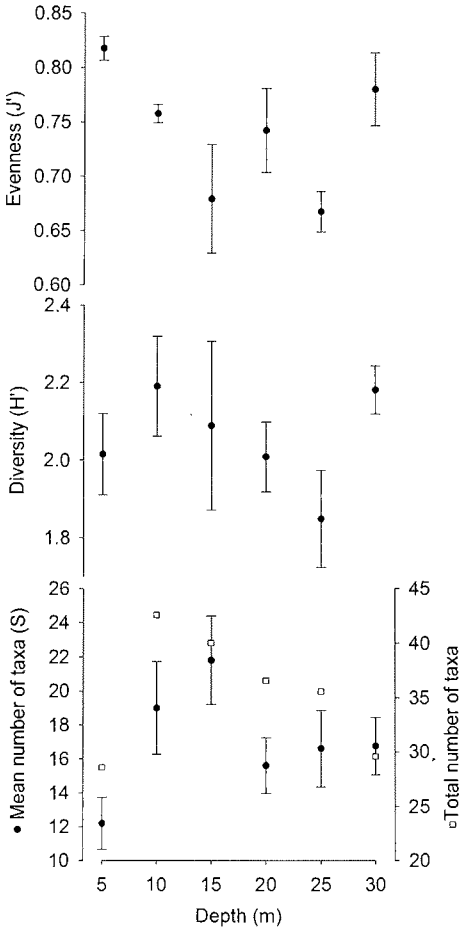


Fig. 2: Species-accumulation curves of six depths (5-30m) showing that curves flatten out at three to four cores.

The total mean faunal abundance was 6296 ind. m^{-2}. The lowest value was recorded at the shallowest transect with 2260 ind. m^{-2} (28 species), followed by the deepest transect with 5443 ind. m^{-2} (29 species), intermittant depths showed higher abundances and species richness (10m: 5969 ind. m^{-2}, 42 species; 15m: 8802 ind. m^{-2}, 41 species; 20m: 6781 ind. m^{-2}, 36 species; 25m: 8521 ind. m^{-2}, 35 species) (Figure 3, Table 1). Significant differences in species richness were detected between the 5m and the 10 and 15m station, respectively (ANOVA $p<0.05$). The diversity ranged between 1.85 (0.28 SE) at 25m and 2.19 (0.29 SE) at 10m, overall diversity was 2.06 (0.12 SE). Highest evenness was found at 5m (0.82 ± 0.01 SE) and lowest at 25m (0.67 ± 0.02 SE). Biomass ranged between 3.5 g m^{-2} (5m) and 25.0 g m^{-2} (15m) AFDM.



Cluster analysis both of abundance and biomass data showed that the samples from 5m depth differed greatly from the rest. The latter formed two subgroups: the medium depth stations (10m-20m) and the deeper stations (25m, 30m) (Fig. 4, dendrogram for biomasses looks similar, not shown).

Fig. 4: Dendrogram resulting from cluster analysis of Bray-Curtis similarities using abundance data of soft bottom macrobenthos. Depth zones sharing a letter do not differ significantly (one-way ANOSIM, $p<0.05$).

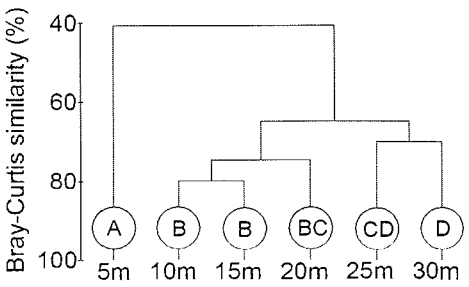


Table 1 Mean abundance (A, ind. m^{-2}) and biomass (B, g AFDM m^{-2}), n.d. = not determined

Taxon	5m		10m		15m		20m		25m		30m	
	A	B	A	B	A	B	A	B	A	B	A	B
PRIAPULIDA												
Priapulus caudatus[1]			21	0.085	10	0.042	21	0.085	21	0.085		
SIPUNCULIDA												
Sipunculida indet.					10	n.d.						
ANTHOZOA												
Edwardsia fusca[1]			63	8.682	10	1.447	10	1.447				
MOLLUSCA												
Astarte borealis									10	3.104		
Astarte sulcata[1]	21	0.075	21	0.075	31	0.113	10	0.038				
Axinopsida orbiculata[1]	10	0.003	10	0.003	271	0.073	10	0.003	302	0.081	326	0.087
Chaetoderma nitidilum[1]									10	n.d.		
Ciliatocardium ciliatum											13	n.d.
Crenella decussata[1]	21	0.001	313	0.148	448	0.196	73	0.051	156	0.077	273	0.129
Cryptonatica affinis[1]			10	0.012	21	0.024						
Cylichna cf *arctica*[1]	52	0.060	21	0.024	73	0.083	52	0.060	10	0.012	26	0.030
Cyrtodaria siliqua[2]	52	0.040										
Hiatella rugosa[2]	42	0.635	52	0.005	135	0.040	115	0.203			39	0.005
Liocyma fluctuosa[1]	73	0.378	10	0.054	10	0.054	10	0.054				
Macoma sp.			31	0.035	31	0.035	21	0.023	52	0.058	13	0.014
Montacuta sp.[1]	94	0.011					42	0.029			78	0.008
Oenopota simplex[1]	42	0.048	21	0.024	10	0.012	10	0.012				
Oenopota sp.											26	0.030
Polinices pallidus									10	0.012	13	0.015
Serripes groenlandicus[1]			31	0.882	42	1.176	10	0.294	31	0.882		
Thracia septentrionalis[2]	10	0.002	10	0.002	10	0.002			10	0.002	13	0.003
POLYCHAETA												
Ampharete cf. *baltica*[2]					42	0.045	31	0.034	229	0.249	378	0.410
Amphitrite cirrata											13	1.679
Apistobranchus tullbergi[1]					10	n.d.			10	n.d.	65	n.d.
Brada villosa[1]			52	0.117	21	0.298	10	0.006	31	0.005		
Chaetozone setosa	10	0.004	354	0.151	531	0.351	635	0.572	781	0.234	469	0.141
Chone sp.[1]					10	0.073	42	0.291	52	0.363		
Dipolydora quadrilobata[1]	73	0.012	1188	0.198	2344	0.390	1906	0.317	3583	0.596	2018	0.336
Eteone spetsbergensis[1]			10	0.127								
Eteone flava[1]			94	0.247	198	0.668	198	0.623	135	0.397	117	0.344

Table 1 continued

Taxon	5m, A	B	10m, A	B	15m, A	B	20m, A	B	25m, A	B	30m, A	B
Euchone analis[1]	167	0.435	1031	1.278	1000	1.039	365	0.540	167	0.208	117	0.146
Glycera capitata							10	n.d.				
Lumbrineris sp.	10	0.133	73	0.933	83	1.066	31	0.400	146	1.865	130	1.665
Maldanidae 1 indet.*									31	0.299		
Maldanidae 2 indet.			177	1.692	417	3.982	271	2.588	188	1.792	65	0.622
Marenzelleria wireni[2]	10	0.017	10	0.017								
Ophelia limacina	10	n.d.	52	n.d.	42	n.d.			21	n.d.		
Ophelina sp.	10	n.d.	83	n.d.	63	n.d.	10	n.d.	73	n.d.	39	n.d.
Orbinia sp.[2]											13	0.259
Paraonidae indet.									10	n.d.		
Phyllodoce groenlandica[1]			52	1.935	42	1.781	10	0.393				
Polynoinae indet.			31	0.098	21	0.065					13	0.041
Praxillella praetermissa[1]	10	n.d.	31	n.d.			21	n.d.				
Scalibregma inflatum	21	0.121	21	0.121			10	0.060	10	0.060	39	0.227
Scoloplos armiger	83	0.138	1271	2.097	1927	1.985	1906	2.217	1167	0.612	534	0.280
Sigalionidae indet.					21	0.344	10	0.172			13	0.215
Spio armata[1]	365	0.317	385	0.335	323	0.281	750	0.652	1083	0.942	508	0.442
Spio filicornis[1]			10	0.028	10	0.028	21	0.056	42	0.111		
Travisia forbesii[1]	115	0.699	156	0.783	167	0.349	63	0.082	42	0.019	13	0.006
CRUSTACEA												
Anonyx nugax[1]	63	0.167	10	0.066					10	0.066	26	0.165
Crassicorophium crassicorne[1]	729	0.079	10	0.001								
Onisimus edwardsi[1]	31	0.009			10	0.003	10	0.003	21	0.006		
Paroediceros lynceus[1]	42	0.066	10	0.016								
Protomedia sp.[1]	42	0.013	10	0.003			21	0.006			52	0.016
Priscilla armata[2]	52	0.011	10	0.002								
Monoculodes sp.									31	n.d.		
Ischyrocerus megalops			10	0.003	21	0.007						
Synidothea nodulosa[1]			21	0.048	31	0.071			21	0.048		
Sclerocrangon boreas									10	1.65		
ECHINODERMATA												
Chiridota laevis			31	1.936	104	6.442	21	1.290				
Holothurioidea indet.					10	n.d.						
Ophiura robusta			135	0.801	208	1.232	31	0.185	10	0.062		
ASCIDIACEA												
Pelonaia corrugata			21	0.830	31	1.246	10	0.415				

[1] taxon not reported for Kongsfjord or [2] for Svalbard according to Gulliksen *et al.* (1999)

*most likely *Praxillella praetermissa* (A. Bick, Univ. Rostock, pers. comm.)

Discussion

The species list presented includes 63 taxa, of which 30 were not reported for Kongsfjorden and seven not for Svalbard yet. The remaining 32 taxa make up a rather low proportion (16%) of the entire benthic Kongsfjorden macroinvertebrates summarised by Gulliksen *et al.* (1999) and comprising almost 200 invertebrates. Both the analyses of only a single biotope (sand-clay bottom 5m-30m deep), and the small sample area do not permit the presentation of a complete description of the soft bottom benthos of Kongsfjorden. However, the aim of this study was to reveal differences in the diversity between depth zones differently impacted by ice-scouring. Only some of the abundant species found in the present study were also reported from a study conducted on deeper soft bottom macrofauna at Kongsfjorden (50-70m, Wlodarska-Kowalczuk *et al.* 1998). In both surveys the bivalve *Axinopsida orbiculata* was present in lower abundances. The values of *Chaetozone setosa* and *Paraonidae* indet. at 25m (781 and 10 ind. m^{-2}) are in good accordance with the value of 739 and 11 ind. m^{-2} respectively found by Wlodarska-Kowalczuk *et al.* (1998). Our values of *Eteone flava* and *Lumbrineris* sp. exceed the abundance of *Eteone longa* and *Lumbrineris fragilis* given by the deeper study. The bivalves *Macoma* sp. and *Liocyma fluctuosa* were found in the present study, although they were absent in the previous survey of Kongsfjorden, but found in Julibukta, Skoddebukta and Bettybukta (Wlodarska-Kowalczuk *et al.* 1998). This could be due to the distance of the sample location to the glacier front as the present study area was located approximately 8.5 nautical miles (nm) from the front, while the previous Kongsfjorden study was carried out up to 1nm from the glacier. In contrast the three other fjords were sampled up to 2nm, 1.9nm and 2.5nm, respectively from the front. Svendsen *et al.* (2002) measured the highest flux of particulate inorganic matter (PIM, 800 g m^{-2} d^{-1}) in front of the Kongsbreen glacier. The value successively declined with distance and was lower than 20g m^{-2} d^{-1} at 5.5nm from the front. Inorganic material is particularly stressful to suspension feeders, affecting their feeding by clogging of filtering organs (e.g., Moore 1977). Therefore sedimentation can have a significant effect on the distribution of these bivalves. Likewise the polychaetes *Ophelina* sp. and *Maldanidae* 1 indet. (only parts available, most likely belonging to *Praxillella praetermissa*, A. Bick, pers. comm.) were found in the present study and in fjords sampled in maximal distances ranging between 1.7 and 4nm from the glacier front (Wlodarska-Kowalczuk *et al.* 1998). Similar patterns were found for benthic decapod fauna in front of the South Patagonian Icefield (Mutschke & Gorny 1999). Accordingly, in Potter Cove (King George Island, South Shetlands) the benthic communities are dominated by ascidians, which are able to flush their filtration unit by contraction and therefore substitute sponges not being able to clean their filtering chambers (Sahade *et al.* 1998).

Polychaete worms and molluscs dominated the fauna, both in number of species (28 and 18) and individuals (4544 and 820 ind. m^{-2}). Crustaceans occurred only in lower numbers (10 species, 78 ind. m^{-2}). While the same proportions for annelids and molluscs were found by Wlodarska-Kowalczuk *et al.* (1998), they observed a lower percentage of crustaceans (annelids : molluscs : crustaceans = 8 : 5 : 1 as opposed to 8 : 5 : 2.9 in the present study). Similarly Holte *et al.* (1996) found low proportions of crustaceans in Gronfjord and Adventfjord and Görlich *et al.* (1987) in glacier-impacted parts of Hornsund. As the identified crustaceans are highly mobile organisms the results

of the previous fjord studies may be underestimations in presence and number as all samples were taken by grab-sampling. The SCUBA operated airlift may be a more adequate method for quantitative sampling of these species. Other methods like dredge sampling or underwater photography have also shown abundant populations of motile crustaceans and ophiuroid species (Syvitsky *et al.* 1989, Wlodarska *et al.* 1996) and support our results. The dominant species were surface detritivorous and suspensivorous polychaetes (*Dipolydora quadrilobata*, *Spio armata*, *Euchone analis*), the subsurface detritivorous polychaete *Scoloplos armiger* and the surface detritivorous and carnivorous amphipod *Crassicorophium crassicorne*. The previous study of Wlodarska-Kowalczuk *et al.* (1998) carried out closer to the glacier front (up to 1nm) and therefore in the area of higher impacts derived from sedimentation of PIM revealed that approx. 50% of the soft-sediment fauna was deposit feeding and sub-surface detritivorous, while this proportion declined in the present study (36%) and suspensivorous species increased from 14% (Wlodarska-Kowalczuk *et al.* 1998) to 27%. These findings agree well with the general trend of increasing dominance of deposit feeding infauna with a decreasing distance from the glacier front and increasing level of glacier activity (Farrow *et al.* 1983, Syvitsky *et al.* 1989, Holte *et al.* 1996, Wlodarska *et al.* 1996). These findings again can be explained with the higher load of PIM towards the glacier front and the unfavourable conditions for filter feeders. Our biomass values ranged between 51 and 248 g m^{-2} wet mass and 3.5 and 25.0 g m^{-2} AFDM, respectively. Kowalczuk *et al.* (1998) observed for their two Kongsfjorden samples 6 and 11 g m^{-2} wet formalin masses. These values are significantly lower than the present ones, which again can be explained by the different impacts of sedimentation on the communities sampled. Low faunal biomass near the glacier fronts has also been related to the scarcity of food available to subsurface detritivorous species as a consequence of low levels of primary production and the dilution of organic matter in the substrate by high sedimentation (Görlich *et al.* 1987). Furthermore, the different sampling technique (van Veen grab in their case) and the low sample number are also mentioned by Kowalczuk *et al.* (1998) to possibly result in some underestimation. Compared to hard-bottom areas from Kongsfjorden our biomass values are about one order lower than values (380-2300 g m^{-2} wet mass) estimated by Jørgensen and Gulliksen (2001). This is due to the relatively small size of soft-bottom fauna.

Table 2 Ranges of Shannon index (H', Log e), from different glacial or glaciofluvial Spitsbergen bays at sampling depths ranging from 2 to 80m, modified from Wlodarska-Kowalczuk *et al.* 1998 (1´ Wlodarska-Kowalczuk *et al.* 1998, 2 Kendall-Aschan 1993, 3 Gromisz 1983, 4 Wlodarska *et al.* 1996, 5 Gulliksen *et al.* 1984, 6 Holte *et al.* 1996).

Site	Depth	H'
Kongsfjord (present study)	5-30	1.85-2.19
Kongsfjord (1)	50-70	1.49
Skoddebukta (1)	30-75	1.49-2.54
Yoldiabukta (1)	57-75	1.26-1.48
Julibukta (1)	30-50	2.22-2.30
Ekmanfjord (1)	30-55	2.22-2.31
Tempelfjord (1)	40-80	1.85-2.01
Bettybukta (1)	40-80	0.43-2.11
Sassenfjord (2)	30-95	2.6-2.9
Hornsund at Hyrnebreen (3)	5-53	0.7-1.38[a]
Hornsund at Storbreen (3)	18-37	1.2-2.07[a]
Skoddebukta (4)	2-60	0.38-2.49
Van Mijenfjord (5)	25-75	2-2.5[a]
Raudfjord (5)	25-75	2.7-3.2[a]
Adventfjord (6)	26-52	1.38-1.79

[a]Values taken from charts

Shannon diversity ranged between 1.85 and 2.19 with lower diversity at shallow depth and highest diversity at 10m. Our values are somewhat higher than the previous estimates (Kowalczuk *et al.* 1998), but corre-

spond well with results published from different Spitsbergen glacial or glaciofluvial bays (Table 2). Variations in diversity of similar habitats have been related again to differences in inorganic sedimentation levels (Kendall & Aschan 1993, Wlodarska *et al.* 1996). The differences in diversity along the depth range of the present study, where transects were located very close to each other (total distance between the 5m and 30m transects <100m) and differences in sedimentation level should have been negligible, must have another reason. Obviously the differences are related to water depth. Analysing the biodiversity of soft-bottom fauna from the Norwegian continental shelf Ellingsen (2002) found that species richness (for all 508 species pooled) was not correlated with depth or median grain size. However, the frequency and extent of disturbance due to iceberg scouring (which is related to water depth) might explain the observed differences in diversity: Iceberg depth can be calculated from observations of freeboard and given assumptions concerning the density and shape of the iceberg. Dowdeswell and Forsberg (1992) found that the frequency of icebergs along their transect A (Fig. 1) with a freeboard high enough to scour the ground at 5m was 17%, while 4% could ground at 10-15m depth and only 0.5% could scour below 21m (value taken from their Fig. 3). Thus, it is more probable that shallower areas are disturbed by scouring than deeper zones.

The 'intermediate disturbance hypothesis' (Connell 1978) may explain the observed species richness (Fig. 5). In situations where disturbance is minimal, species richness (SR_S *sensu* Gray 2000) is reduced because of competitive exclusion between species, which can explain the lower total number of taxa at 30m (29 species). With an increasing level or frequency of scouring — more icebergs ground in shallower areas, since the majority of icebergs is smaller — competition is relaxed, resulting in increasing species richness (intermittant depth zones: 35-42 species). At higher or more frequent levels of disturbance species start to be eliminated by stress (5m: 28 species) so that diversity falls again. Thus, it is at intermediate levels of scouring activity that species richness is highest. In the Antarctic icebergs are much bigger and therefore scouring impact reaches areas up to 400m depths. The results are however comparable: in the Antarctic habitat many different succession stages can be found in the same areas (Gutt & Piepenburg 2003) resulting in a very high diversity, but on a wider scale.

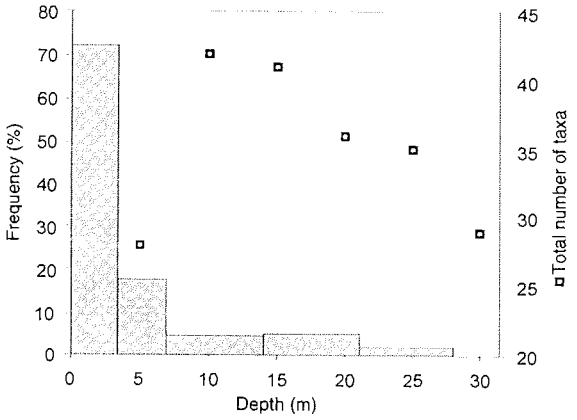


Fig. 5: Iceberg depth-frequencies (gray bars, calculated from Dowdeswell and Forsberg 1992, Fig. 3) and total number of soft-bottom taxa (•) from Brandal.

However, since our data is restricted to a small area within Kongsfjorden, future studies on a wider area are needed, including the direct quantification of disturbance resulting from scouring of icebergs from tidewater glaciers in Kongsfjorden, before generality can be attached to our findings.

Conclusions

63 macrobenthic taxa were found in the soft bottom habitat of Kongsfjorden (Svalbard), 30 of which had not been reported for Kongsfjorden and seven not for Svalbard before. Suspensivorous or surface and sub-surface detritivorous polychaetes and deposit-feeding amphipods were dominant. Only eleven of 45 species and an additional 18 families inhabited the complete depth range (the polychaetes *Dipolydora quadrilobata*, *Chaetozone setosa*, *Euchone analis*, *Lumbrineris* sp., *Ophelina* sp., *Scoloplos armiger*, *Spio armata, Travisia forbesii*, the bivalves *Axinopsida orbiculata* and *Crenella decussata,* and the opistobranch *Cylichna* cf *arctica*). Similarity clustering of samples showed a significant difference between the shallow station (5m) and the rest. The latter formed two subgroups, the medium depth stations (10m, 15m, 20m) and the deeper stations (25m, 30m). The biomass ranged from 3.5 to 25.0 g ash free dry mass m^{-2} and Shannon diversity was 2.06 (0.12 SE). Observed differences in diversity together with information on ice-scouring support the 'intermediate disturbance hypothesis'.

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Macro-epibenthic communities and diversity of Arctic Kongsforden, Svalbard, in relation to depth and substrate

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Introduction

Low and relatively constant temperatures, seasonal pulses of primary production, changes in ice cover, siltation and salinity are the main characteristics of polar marine ecosystems. Shallow coastal systems are especially affected by ice as a disruptive factor, which has a huge effect in structuring the established benthic communities, both in the Antarctic (Dayton 1990, Clarke 1996a, Grebmeier & Barry 1991, Sahade *et al.* 1998, Gutt 2001, Gutt & Piepenburg 2003) and the Arctic (Laudien *et al.*, this issue). Despite these similarities the two polar ecosystems differ considerably in their evolutionary histories. While the Antarctic has been evolving isolated the last 20 million years, the Arctic Holocene history only reaches back some 10 thousand years (Clarke & Crame 1992, Dunton 1992, Dayton *et al.* 1994). These differences are reflected in the recent biota with the Arctic being impoverished in respect to the Antarctic in terms of species richness, diversity and abundance of organisms (Poore & Wilson 1993, Rex *et al.* 1993, Clarke 1996a,b, Arntz *et al.* 1997, Gray 2002). However, at small-scale these differences are not evident and several Arctic benthic communities exhibiting high abundance and diversity have been described (e.g., Grebmeier *et al.* 1989, Kendall 1996, Sejr *et al.* 2000). The major difficulties in comparing both polar ecosystems are due to the use of different methods, depth ranges, scales, and analytical procedures.

Photo-transects allow the quantitative analysis of communities, providing information about the habitat, abundances, percentage cover, species associations of epibenthic assemblages and a fast data acquisition in the field. Although this method underestimates abundances of small, cryptic and highly mobile individuals (Barthel *et al.* 1991, Roberts *et al.* 1994, Jørgensen & Gulliksen 2001), it has been successfully used to define polar benthic assemblages (Dayton *et al.* 1974, Barthel *et al.* 1991, Barthel & Gutt 1992, Jørgensen & Gulliksen 2001, Teixído *et al.* 2002).

The present study is a preliminary analysis of epi-macrobenthic community structures in relation to substrate types and depth in the Arctic Kongsfjorden. The application of the same methods (photo-transects), environmental variables (depth and substrate) and analytical procedures as in Antarctic Potter Cove (Sahade 1999) will allow to further establish valid comparisons between both polar benthic systems.

Material and methods

Study area

The study area was the Arctic glacial Kongsfjorden located on the western coast of Spitsbergen (79°N, 12°E). The fjord is 20km long and 4 to 10km wide and has a maximum depth close to 350m. It is directly connected to the North Atlantic Ocean via the Kongsfjord-Renna trough (Bluhm *et al.* 2001, Jørgensen & Gulliksen 2001, Svendsen *et al.* 2002). For a detailed review of environmental characteristics and a description of the marine ecosystem see Svendsen *et al.* (2002) and Hop *et al.* (2002). Three stations with different substrate types were selected, Prins Heinrichøya (S1), mainly composed of a sand-clay mixture with occasional ice-rafted stones, Hansneset (S2), rocky bottoms interspersed with sediment pools and Kongsfjordneset (S3) pure bedrock (Fig. 1).

Sampling

Sampling was carried out during the boreal summer 2001 by means of photo-transects taken by SCUBA-divers with a Nikonos V camera, a 15-mm lens and a Nikonos SB-104 strobe, mounted on an aluminium frame (50 x 50cm) at particular depth profiles 15, 20, 25 and 30m (Kühne 1992, Sahade *et al.* 1998). Percentage cover of the main taxonomic groups was obtained from the slides taken at each of the three stations. Thereafter data were analysed to access diversity and community structure. In order to compare diversity patterns for both the different stations and the depth gradient, K-dominance curves were plotted. Multivariate analysis as classification (clustering) and ordination MDS (non-metric multidimensional analysis) followed to evaluate community structures using *PRIMER v5* (Clarke & Gorley 2001).

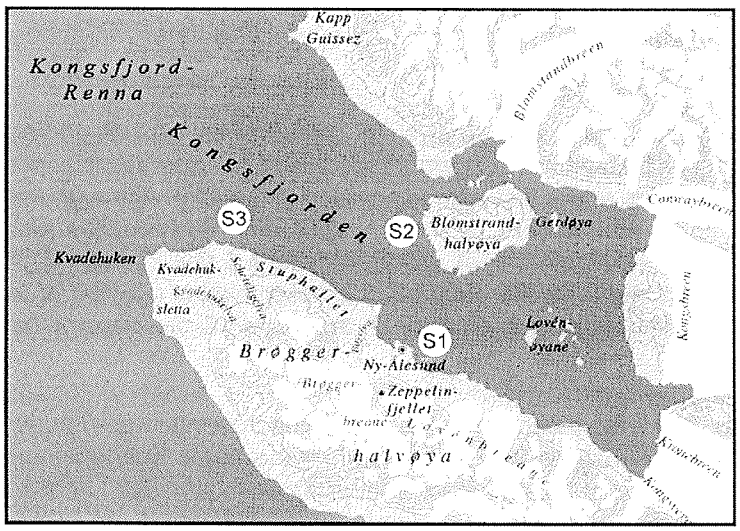

Fig. 1: Maps of the study area, sampling stations S1 (mainly soft bottom), S2 (mixed substrates) and S3 (bedrock) are indicated.

Results

A total of 47 species or morphospecies of 18 higher taxa (order to class) were identified. Table 1 list the species and their occurrences at the three stations.

Table 1: List of taxa identified, • indicates the presence of taxa at station S1 (mainly soft bottom), S2 (mixed substrates) and S3 (bedrock).

	Taxa	S1	S2	S3
Phaeophyta	*Laminaria* sp.	*		*
	Desmarestia aculeata	•		*
	Desmarestia viridis	•	•	*
	Acrosiphonia aff. *penicilliformis*	*		*
Rhodophyta	*Polysiphonia arctica*	*		*
	Phyllophora sp.	*		*
	Unidentified sp. 1	*		
Porifera	*Haliclona* sp.	•	•	
	Unidentified sp. 1		*	
	Unidentified sp. 2		*	
Anthozoa	*Urticina equees*	*	*	*
	Hormathia nodosa	*	*	
	Unidentified sp. 1		*	
Hydrozoa	*Eudendrium* sp.		*	
Polychaeta	*Thelepus* cf cincinnatus		*	
	Serpulidae		*	
	Sabellidae		*	
	Unidentified sp. 1		*	
	Unidentified sp. 2	*		
Gastropoda	*Buccinum* sp.	*		
	Neptunea sp.	*		
Bivalvia	*Mya truncata*	*	*	*
	Chlamys islandica	•	*	
	Hiatella artica	*	•	•
	Unidentified sp. 1	*		*

	Taxa	S1	S2	S3
Polyplacophora	*Tonicella* sp.	*	*	
Cirripedia	*Balanus balanus*	*	*	*
Decapoda	*Lebbeus polaris*	*	*	
	Eupagurus sp.	*	*	*
	Unidentified sp. 1		*	
	Unidentified sp. 2	*	*	
Pycnogonida	Unidentified sp. 1			*
Bryozoa	*Reteporella beaniana*[1]		*	
	Crisia denticulata[1]	*	*	*
	Porella cf *compressa*		*	
	Unidentified sp. 1			*
	Unidentified sp. 2		*	
Asteroidea	*Crossaster papposus*		*	*
	Henricia sp.[1]		*	
	Pteraster sp.[1]		*	
Echinoidea	*Strongylocentrotus* sp.	*	*	*
Ophiuroidea	*Ophiopholis aculeata*	*	*	*
Ascidiacea	*Boltenia echinata*		*	
	Styela rustica		*	*
	Halocynthia pyriformis		*	*
	Unidentified sp. 1		*	*
Pisces	Unidentified sp. 1			*
Total	47	25	34	22

K-dominance curves indicated that diversity was lower at the station mainly composed of soft bottom (S1) than at stations dominated by hard substrate elements (S2 and S3) (Fig. 2). Diversity increased with depths at all three stations. This pattern was more evident at station S1, which showed a constant increase. At station S2 and S3 the differences were clear between 15m and 30m, but species composition was more similar at 20m and 25m (Fig. 2).

Multivariate analysis of classification and ordination (MDS) clearly separated the samples in first term by location, substrate type and later by depth (Figs. 3 and 4). Three communities were separated according to the sampling stations. The two sites with significant hard bottom occurrences (S2 and S3) were further grouped and separated from S1. Not one single sample was included among the samples of another station. Additionally, there was a distinction following the bathymetric gradient at each station.

All stations showed a change from macroalgal dominance in shallow waters to faunal communities in deeper waters. This pattern was especially clear at stations S1 and S2, while at S3 macroalgae were less abundant (even at 15m).

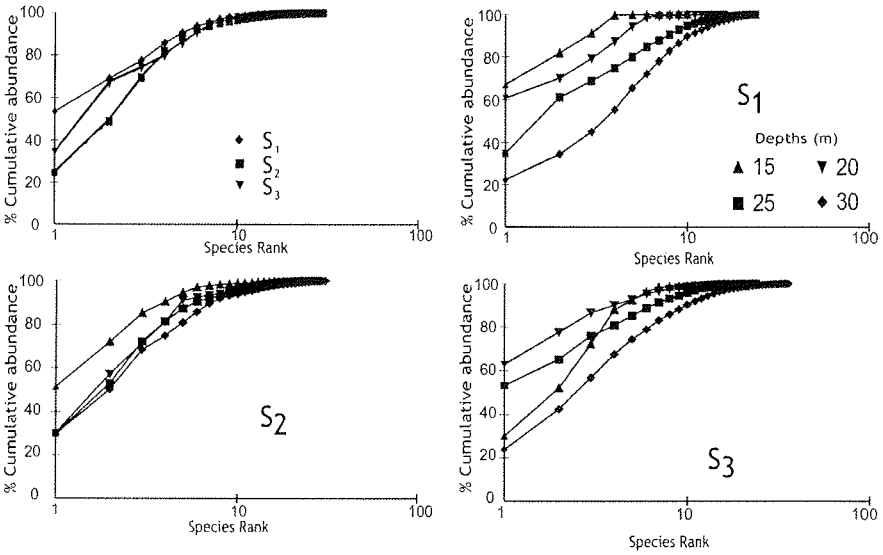

Fig. 2: K-dominance curves showing cumulative ranked abundances plotted against log species rank (following Lambshead *et al.* 1983). a: The most elevated curve (mainly soft bottom S1) shows a lower diversity than the two lower ones (S2 and S3). b-d: At all three stations cline of increasing diversity with depth was observed.

Discussion

The epibenthic community structures analysed in Kongsfjorden are coincident with previous studies of the area (Jørgensen & Gulliksen 2001, Lippert *et al.* 2001, Hop *et al.* 2002). However, communities showed marked differences in relation to substrate type and depth. Higher diversities of organisms were found in habitats mainly composed of hard bottoms (S2 and S3), while epifauna and –flora of habitats dominated by soft bottoms were less diverse. This observation reflects that most of the organisms inhabiting hard substrates are epifaunic, while the epifauna of soft bottoms in Kongsfjorden is a rather small proportion of the total soft bottom inhabitants, which are mainly infaunal species. Therefore, the macro-epibenthic communities of soft habitats may be less diverse than the communities of hard substrates, as only a special group is using the surface of the sediment. Correspondingly, Jørgensen and Gulliksen (2001) state that the infauna was underestimated by their photographic method. However, this non-destructive sampling provides reliable information about differences of larger epibenthic taxa between locations and along the bathymetric gradient.

The comparison of the present results with those achieved from a soft-bottom study of macrobenthos, which was located at Brandal, approximately 5km down-fjord (north-west) of our study location (Laudien *et al.*, this issue), revealed significant differences in species composition. The present species list includes rare, large species like the anthozoan *Hormathia nodosa*, the gastropods

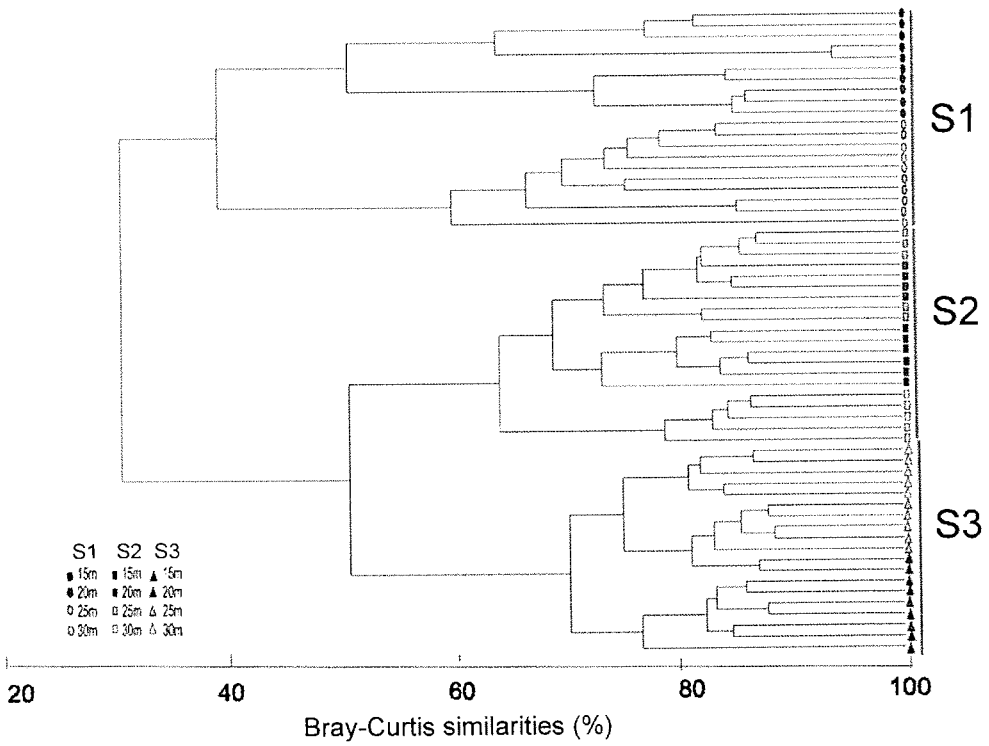

Fig. 3: Dendrogram resulting from classification analysis (Bray-Curtis similarities) of macroepibenthos from Kongsfjorden based on the UPGMA method. All samples are separated according to the stations (substrate type); the two communities inhabiting habitats dominated by hard bottom (S2 and S3) are more closely related and separated from the community inhabiting soft-bottom dominated habitats (S1).

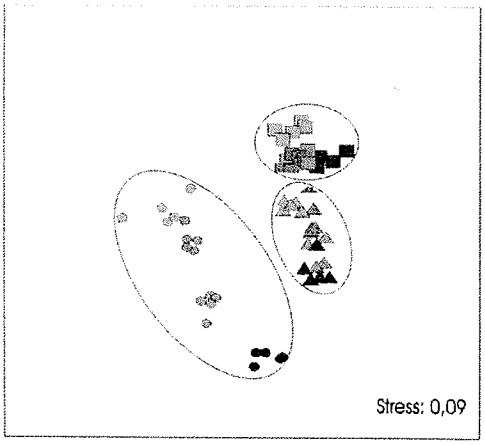

Fig. 4: MDS plot, circles are the superimposed results of the cluster analysis, ●: station with mainly soft bottom (S1), ■: station with mixed substrate (S2) and ▲: station with bedrock (S3). The shading of the sample symbols indicates depth, following the legend given in Figure 3.

Buccinum sp. and *Neptunea* sp., as well as highly mobile species like the decapods *Lebbeus polaris* and *Eupagurus* sp., which were not reported in the Brandal study. The latter sampled five replicates of a 20 × 20cm corer (surface: $0.2m^2$) by means of a suction method, while the present study covered a much wider area of 59 photo quadrates of 50 × 50cm (surface: $14.75m^2$). However, according to species-accumulation curves, the previous study already detected the majority of the total soft bottom fauna. Therefore both studies are contributing to a detailed description of the macrofauna of Kongsfjorden. Moreover, four species found in this study had been reported for Svalbard, but not for Kongsfjorden before (Gulliksen *et al.* 1999, Jørgensen & Gulliksen 2001) (Table 1).

Differences in species diversity might not only be substrate related, but also be correlated with environmental parameters (e.g., salinity, turbidity, temperature) following a gradient along the mayor axis of the fjord (for review: Svendsen *et al.* 2002). This explanation agrees well with the general trend that deposit feeding infauna (not quantitatively detectable with the applied photographic method) increases in dominance and filter feeders decrease in abundance – due to unfavourable conditions – with a decreasing distance from the glacier front (Farrow *et al.* 1983, Syvitsky *et al.* 1989, Holte *et al.* 1996, Wlodarska *et al.* 1996). Low species richness near the glacier fronts has also been related to the scarcity of food available to substrate detritivorous species as a consequence of low levels of primary production and the dilution of organic matter in the substrate by high sedimentation (Görlich *et al.* 1987). The same trend can be seen by comparing the two stations dominated by hard bottom substrates, where the station located down-fjords (S3, pure bedrock) showed a higher diversity than the intermittent station (S2). Under constant environmental conditions the opposite pattern would have been expected as the habitat heterogeneity of station S2 should result in a higher diversity (Gray 2001). Therefore, the environmental parameters could also have a strong effect in determining these communities. Both, this hypothesis and the explanation of substrates playing a major role in determining the benthic community structure will be analyzed in future studies.

Besides substrate and/or the location, depth appears important in structuring epibenthic communities. We detected increasing diversity along the bathymetric gradient at each of the three stations. This trend is most obvious in the station mainly composed of soft bottom showing a constant increase of diversity from 15m to 30m. The communities were characterized by a dominance of macroalgae at 15m and a shift to faunal dominance at 20m, diversity was highest at 30m. As boulders colonised by a typical rocky community (barnacles, actinians, ascidians and sponges) were more frequent at 30m, again the substrate could explain increasing diversity with depths. The previous study conducted at Brandal (Laudien *et al.*, this issue) revealed that species richness was lowest at 5m but highest at an intermittent depth of 10-15m; thereafter species richness steadily decreased (20, 25, 30m). This observation was explained according to the 'intermediate disturbance hypothesis' (Connell 1978): locations with minimal

disturbance show reduced species richness because of competitive exclusion between species. With increasing intensity or frequency of iceberg scouring (more icebergs ground in shallower areas) competition is relaxed, reflected in increasing species richness; while at higher or more frequent levels of disturbance species start to be eliminated by stress. These findings appear to be true for homogeneous substrates as sampled in the Brandal study. However, the results of the present study indicate that such a trend may be masked by substrate heterogeneity, especially when the density of hard bottom elements increase with depths.

The up-fjord bedrock station (S3) showed considerably lower abundances of macroalgae than the other stations. This observation could be explained with high densities of the green sea urchin *Strongylocentrotus droebachiensis* foraging on macroalgae (H. Wessels, Univ. Bremen, unpubl. data). In the year previous to this study abundances of 80 ind. m^{-2} were observed at the same location (F. Beuchel and B. Gulliksen, Univ. Tromsø pers. comm. in Hop *et al.* 2002). Grazed areas are commonly populated by sessile organisms such as actinians, barnacles and bryozoans. At deeper waters from 20m to 30m the increase in diversity is less marked than at the other stations and the community is dominated by suspension feeders (barnacles, actinians, bryozoans and ascidians). These organisms are favoured by rich Atlantic waters entering into the fjord. However, in the central and inner part of Kongsfjorden filter feeders might be under permanent stress, as the high inorganic load (800 g m^{-2} d^{-1} at the glacier front, Svendsen *et al.* 2002) is likely to clog their filter organs (e.g., Moore 1977).

The comparison with Potter Cove (King George Island, Antarctica), although still preliminary, appears to be coincident with the general trend of Antarctic communities being more diverse than the Arctic ones. There are striking differences: In the Potter Cove the highest diversities were found on soft bottoms (Sahade 1999) with pennatulaceans, bivalves and ascidians as dominant organisms, while in Kongsfjorden hard bottom habitats were more diverse. Besides that, soft bottom communities at Potter Cove were dominated by suspension feeders, especially ascidians and sponges, which are characteristic for many Antarctic faunal communities, but rarely seen in other areas. Diversity patterns along the major axis of both fjords are also different. While diversity is lower at the outer station of Potter Cove (hard bottom) compared with inner stations, the opposite is the case for Kongsfjorden. Future work will focus on further comparisons of both polar systems.

Conclusions

Photo-transects (15, 20, 25 and 30m) of macro-epibenthic invertebrate communities and algae from Arctic glacial Kongsfjorden (Spitsbergen) revealed a species list comprising 47 taxa. Mean species richness (17-20 species) was similar at all three stations. K-dominance curves showed higher diversity in stations

dominated by hard substrates than in habitats mainly composed of soft sediment. A bathymetric pattern was evident showing increasing diversity with depth, which is apparently related to increasing densities in hard bottom elements. The comparison with studies conducted in the Antarctic fjordic system Potter Cove, revealed major differences between the two polar systems.

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3. ECOPHYSIOLOGY OF KEY ORGANISMS IN THE ECOSYSTEM

A comparative analysis of photosynthetic pigments and tocopherol of some arctic-alpine plants from the Kongsfjorden area, Spitsbergen, Norway

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Introduction

From a human point of view both polar regions and the alpine and nival zones of the high mountains have an extreme climate and thus endanger all living matter with multiple stresses. Plants growing in high mountains or in high arctic ecosystems are comparable in the sense that they are exposed to temperature extremes and increased ratio of UV irradiation to PAR. Normally, they have developed a number of resistance mechanisms during evolution, which, observed on a tissue or cellular level, appear as a general system of adaptation to enable plant development and propagation.

Anthropogenic influences have triggered stratospheric ozone depletion, which enhances UV-B radiation. It has been observed first in the Antarctic, and is now of public concern also in Europe because of the increase in UV-B in the northern hemisphere. This means, that the general stress loads may now and in the future overrun the well balanced defence capacities of many plants resulting in changed species composition in the aforementioned ecosystems (Gehrke et al. 1996, Björn et al. 1999). UV-radiation in alpine environments is higher due to an increase in altitude and mostly at lower latitudes (Caldwell and Robberecht 1980, Blumthaler et al. 1997) and less influenced by ozone hole effects.

Higher plants strongly depend on photosynthetic effectiveness for carbon fixation. Photosynthesis adapts to field conditions in numerous ways, one adaptation, easy to measure, is the change in pool sizes and relative contents of chloroplast pigments. Some carotenoids, and the membrane bound lipophilic tocopherols are effective quenchers (Palozza and Krinsky 1992) to dissipate an overflow in energy, normally avoiding destruction of the photosynthetic apparatus (Havaux et al. 2000, 2003, Lütz 1996).

The main interest of our work is to understand whether the range of adaptation of plants from alpine and high arctic ecosystems is comparable or differs under natural influences (mainly climate stress), but also how the evolutionary selected stress resistance will be influenced by anthropogenic impacts. This communication compares adaptations in chloroplast pigments from plants grow-

ing in NW-Svalbard with an example of high alpine occurrence. The selected plants are characteristic members of both ecosystems (Ronning 1996, Körner 1999)

Materials and Methods

Plant material and growth sites: leaves of the following plants were harvested for these studies: *Dryas octopetala, Polygonum viviparum, Oxyria digyna.* Plants were collected about 2 – 5 km from the Ny-Alesund research station growing on the bare ground along Kongsfjorden near the Knudsenheia – area and close to the Lovenbreen glacier. At the selected sampling sites, the plants were abundantly growing.

Sampling procedure: for each plant, leaf material of 120 – 250 mg were cut from the plants, leaf fresh weight determined at an accuracy of 2 mg via a battery powered balance at the growth site, and finally the leaf material was transferred into vials containing 5 ml Dimethylformamide (DMF) per sample. On average, 5 samples per species per sampling date were prepared. DMF extracts and stabilises pigments for several weeks (Bergweiler and Lütz 1986). Sampling dates were July 14th, 22nd, 29th, 2002, mostly between 12 and 14 hours (noon) and in case of *Oxyria* and *Polygonum* additionally at July 30th, after midnight (24-1 hour).

Climate conditions:

Own observations at plant growth sites for sampling dates (12.7.02-30.7.02):

July 14th: overcast, air temperature 1 cm above soil: 10°C – 13 °C.

July 22nd: occasionally sunny, air temperature 1 cm above soil: 8°C – 10 °C.

July 29th: sunny, few clouds, air temperature 1 cm above soil: 10°C – 13 °C.

Meteorological climate:

	t : air	t: - 5 cm	UV-A+B	PAR -global	PAR direct
13.07.02	10,7	15,2	23	543	892
14.07.02	**6,5**	**12,4**	**8,6**	**143**	**0,6**
21.07.02	3,5	4	8,5	137	0,6
22.07.02	**6,2**	**9,4**	**16,3**	**307**	**11,9**
28.07.02	7,4	11,1	10,4	181,4	4,7
29.07.02	**8**	**9**	**17,5**	**360**	**407**

Tab. 1: Climate conditions in the Ny-Alesund area one day before, and at the sampling dates (bold). Data from noon, each day, kindly provided by the AWI climate recording group. Temperatures: in °C, air temp. in 2 m height, soil temp. in – 5 cm depth; radiation measurements: expressed in W/m^2. PAR direct: radiation on a horizontal surface.

Precipitation was negligible around 13/14.7.02; during July 21, precipitation varied from 0,4 mm at 6h to 1,8 mm at 18 h, while during 22nd of July precipitation was 0,1 mm at 6h and ceased for the rest of the day (mostly sunny).
Pigment assays: The pigments samples were stored until transport back to the university institute at –20°C; later until processing at –53°C. During storage most pigments were extracted into the solvent, therefore two small volume of DMF for re-extraction of the leaves removed remaining pigments completely. Separation and quantification of pigments and α-tocopherol was done on an Agilent – HPLC system with diode array detection according to Wildi and Lütz (1996).

Results and Discussion

The composition of photosynthetic pigments assembled in a functional thylakoid membrane determines light acclimation and thus efficiency of photosynthesis (Siefermann-Harms, 1985, Thayer and Björkman, 1990). Further, a number of adaptive or stress responses can be followed by detailed pigment observations. One possible influence comes from temperature and light climate at the growth site, accompanied by dry/wet soil conditions.
The light climate in Kongsfjorden depends very much from additional input by sea – or ice and snow - scattering, affecting also the free soil containing a tundra - like plant cover. During summer, PAR is reported to reach an average of 1300 µMol photons m^{-2} s^{-1}, and UV-A radiation of 19 W m^{-2} and UV-B radiation of 1.1 W m^{-2} (Bischof et al 1998). Table 1 shows meteorological data raised at July, 2002, which may be compared with own observation at the sampling plots. For better comparisons, always the noon records are presented, but occasionally PAR did reach nearly 900 W/m^2, and UV A+B radiation 26 W/m^2.
The main plastid pigments of plants from Svalbard are listed in tab. 2. An inter - species as well as sampling date - comparison shows that total chlorophylls and the carotenoids lutein and neoxanthin are similar in *Dryas* and *Polygonum*, only slightly changed by the prevailing weather and light conditions of the three harvests, but still in the range of normal differences between individual samples.
The pigments measured in *Oxyria* amount in average only to half of the values measured in the other plants, and also chl a/b shows the lowest values. This difference can be explained by the higher, often visible, accumulation of anthocyanins in *Oxyria* (Lütz, in preparation), which shield against harmful radiation especially in younger plants (Manetas et al. 2002), and this shading results in accumulation of chl b. The ratio chl/car does not change specific for species or date.

Species	Date	Neox	Violax	Zeax	Lutein	Chla+b	Chla/b	Car	X- Pig	Chl/Car
Dryas	**14.07.02**	**36**	**62**	**14**	**98**	**1234**	**4,5**	**284**	**90**	**4,3**
		2	3	9	6	43	0,1	11	8	0,2
Dryas	**22.07.02**	**47**	**45**	**18**	**106**	**1393**	**4,5**	**307**	**84**	**4,5**
		5	10	3	10	131	0,1	34	13	0,1
Dryas	**29.07.02**	**41**	**43**	**12**	**103**	**1182**	**4,5**	**273**	**70**	**4,3**
		2	8	2	4	45	0,1	16	7	0,1
Oxyria	**14.07.02**	**16**	**31**	**6**	**52**	**573**	**4,1**	**146**	**42**	**3,9**
		1	4	1	2	58	0,5	9	5	0,2
Oxyria	**22.07.02**	**20**	**34**	**6**	**63**	**713**	**4,0**	**176**	**59**	**4,1**
		1	5	1	2	55	0,2	24	22	0,6
Oxyria	**29.07.02**	**16**	**30**	**4**	**48**	**592**	**4,0**	**134**	**39**	**4,4**
		1	2	1	1	21	0,4	5	4	0,2
Oxyria N	***30.07.02***	***17***	***34***	***3***	***49***	***578***	***3,8***	***136***	***45***	***4,3***
		1	5	1	3	37	0,1	11	4	0,2
Polygonum	**14.07.02**	**34**	**66**	**7**	**116**	**1414**	**5,1**	**308**	**81**	**4,6**
		1	4	1	10	81	0,1	19	4	0,2
Polygonum	**22.07.02**	**42**	**62**	**13**	**137**	**1579**	**4,5**	**350**	**90**	**4,5**
		2	10	2	7	74	0,0	22	10	0,1
Polygonum	**29.07.02**	**32**	**41**	**16**	**105**	**1360**	**4,8**	**268**	**69**	**5,1**
		2	8	5	9	156	0,9	21	7	0,8
Polygonum N	***30.07.02***	***40***	***70***	***3***	***126***	***1481***	***4,0***	***317***	***79***	***4,7***
		1	2	1	9	41	0,1	14	1	0,1

Tab. 2. Contents (µg/g FW) of individual carotenoids and of chlorophyll separated for sampling date and plant species. *Dry: Dryas; Oxy: Oxyria; Pol: Polygonum.* In case of *Oxyria* and of *Polygonum* also midnight samples were added (N). Mean values of 5 independent samples with standard deviation (small numbers). Car:sum of all carotenoids and carotene; X-Pig: sum of xanthophylls cycle pigments including antheraxanthin.

As the xanthophylls are building components of the light harvesting complexes of both photosystems (Morosinotto et al. 2003), one can assume that photosystem composition is quite similar in the investigated plants and not drastically changed by this summer climate variations as described in methods (cf tab. 1).
In case of *Oxyria* and *Polygonum* it was possible to assay pigment contents around midnight when light intensity (PAR) decreased only slightly because of the high latitude (79°N). Again these data meet the data measured at noon; variations are in the range of normal sample variations. The xanthophyll cycle pigments were expected to respond because their relative proportions depend more on light level than other pigments. The data in tab. 2 show that there is a tendency to reduced zeaxanthin contents in favour of violaxanthin in *Oxyria*, even more clearly visible in *Polygonum*: the energy dissipative power of the xanthophyll pigments (Havaux et al. 2000) continues at the polar night. This is of course in contrast to high alpine plants where night triggers strong diurnal variations in carotenoids (Wildi and Lütz 1996). Plant harvest sites were located in the relatively small open area between the glaciers and the sea, interrupted by snowbanks. These conditions result in additional light reflections which can drastically increase the amount of irradiance absorbed by the leaves. Xanthophyll content of the plants obviously provided enough protection to sustain good photosynthetic activity (Germino and Smith 2000, Lütz and Holzinger, in preparation), with leaf temperatures reported between 8 to 13°C in the afternoon (Kongsforden, Crawford and Smith 1997)
A measure for the intensity of the light environment in a leaf is the ratio total chlorophylls to total carotenoids (chl/car in tab. 2 and 4). The data in tab 2 show values mainly ranging between 4 and 5, independent of species. Data calculated from tab. 4 for high alpine *Dryas* are 4.1, 3.3, 3.2; which meets the mean found in alpine trees because of carotenoid accumulation (about 3.6) (Lehner and Lütz 2003). These values show that alpine plants are exposed to higher irradiation (PAR), despite the missing midnight sun, and photosynthesis is protected by adapting xanthophyll content.
The most effective antioxidants in the thylakoid membrane, ß-carotene and α-tocopherol (Fryer 1992) are compared in tab. 3 and 4. ß-carotene shows a species specific accumulation, but no clear dependence from sampling date (weather condition before and during harvest). Similarly as is shown for other pigments, the data comparison for *Dryas* from Svalbard vs. alpine origin supports a strong similarity in formation of both antioxidants.
α-Tocopherol increased with sampling time in all samples. This is an age effect to protect the tissue (Molina-Torres and Martinez 1991). The high value for *Oxy-*

ria at night is unexpected, if compared with the noon/midnight change in *Polygonum*. For a species comparison, both antioxidants have the lowest values in *Oxyria*: probably a results of the shielding function of epidermal anthocyanine accumulation (see above).

Species	Date	ß-Car	a-Toc
Dryas	**14.07.02**	**61**	**158**
		2	15
Dryas	**22.07.02**	**70**	**209**
		7	25
Dryas	**29.07.02**	**59**	**238**
		2	20
Oxyria	**14.07.02**	**36**	**40**
		1	13
Oxyria	**22.07.02**	**35**	**66**
		9	21
Oxyria	**29.07.02**	**31**	**68**
		2	18
Oxyria N	***30.07.02***	***25***	***92***
		4	16
Polygonum	**14.07.02**	**78**	**88**
		5	8
Polygonum	**22.07.02**	**81**	**111**
		3	20
Polygonum	**29.07.02**	**62**	**123**
		7	15
Polygonum N	***30.07.02***	***72***	***116***
		3	25

Tab. 3: Contents (µg/g FW) of ß-carotene and α-tocopherol separated for sampling date and plant species, *Dry: Dryas; Oxy: Oxyria; Pol: Polygonum*. In case of *Oxyria* and of *Polygonum* also midnight samples were added (N). Mean values of 5 independent samples with standard deviation (small numbers).

Dryas octopetala is widely distributed in the alpine zone of the northern limestone Alps. Some pigment data from a 4-year research project are given in tab. 4, as mean values from summer harvests over three years. It is surprising that total chlorophylls, carotene and x-cycle pigments show amounts in the same range as observed in the high arctic *Dryas* samples. Only total carotenoid content is reduced from about 330 µg/g FW (alpine) to about 288 µg/g FW (polar) as a consequence of lower irradiation (see above).

This comparison supports a very conservative, but also flexible construction of the photosynthetic apparatus, which may enable establishment of plants via seeds from the Arctic to the Alps and vice versa.

Date	Chla+b	Car	X- Pig	ß-Car	a-Toc
01.07.00	1308	312	85	59	208
18.07.01	1123	337	93	79	126
19.06.02	1160	361	110	65	194

Tab. 4. Contents (µg/g FW) of total chlorophylls and carotenoids, of xanthophylls cycle pigments, of ß-carotene and tocopherol extracted from *Dryas octopetala* growing in the Northern Limestone Alps (Germany, Karwendel, 2200m a.s.l.) in three consecutive years.

In summary, the plants selected for this study represent members of different plant associations (Möller 2000) structured by microclimate conditions and often separated only by a few meters distance. In the arctic, species survival is more affected by unpredictable remaining snow banks than in an alpine environment, dominated by vertical relief. *Polygonum* and *Oxyria* adapted to survive under frequent snow cover, and *Dryas* developed different life forms according to snow patch (large leaves) or ridge growth sites (small leaves) (Crawford 1997). The comparison of the pigment and α-tocopherol equipment in plants from Svalbard has shown that the normal fluctuation in weather, especially in irradiation, during the arctic summer did not result in changes higher than normal biological variation. The adaptive range is broad enough to cope with minor climate changes. This holds also for the temperature and light conditions at midnight.

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Enzymatic activities related to nutrient assimilation in common seaweeds of the Arctic

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Introduction

The Arctic coastal environment is characterised by relatively constant and near-freezing water temperatures (typically -2 to +4 °C) and strong seasonal variations in light and nutrient availability (Hanelt et al. 2001, this issue; Hop et al. 2002). Long periods of low light, temperature and nutrient availability are attributes that indicate the high vulnerability to additional stress and environmental changes. Two of the most relevant global phenomena that threaten the ecosystems are the increase in atmospheric CO_2 concentration and the increased load of nitrogen in both the terrestrial and aquatic environments. Changing the availability of these nutrients will force a change in the acclimation strategies of the organisms. Nitrogen and phosphorus have been reported to limit macroalgal productivity (Lapointe and O'Conell 1989, Hernández et al. 1993). Nutrient uptake and assimilation characteristics of macroalgae from temperate waters have been shown to vary among populations and species in ways that optimise survival and growth under local nutrient supply conditions (e.g. Wheeler and Weidner 1983, Hernández et al. 1993, Gordillo et al. 2001a). Arctic species are also reported to show nutritional strategies that allow them to cope with the long periods of darkness in winter and nutrient depletion in summer (Korb and Gerard 2000a,b). Among the metabolic paths that are suspected to be most influenced by changing the nutritional conditions of the environment are the main nutrient assimilatory enzymes for CO_2, nitrate and phosphate. Key enzymes of the respective assimilation pathways are external carbonic anhydrase (CA_{ext}), nitrate reductase (NR) and alkaline phosphatase (AP).

Carboxylation in macroalgae is carried out almost solely by rubisco. Many species have evolved different carbon concentration mechanisms (CCMs) that allow high concentration of CO_2 around rubisco. Among them, CA_{ext} seems to play a key role in helping the uptake of inorganic carbon to the interior of the cell by conversion of HCO_3^- to CO_2 which enters the cell by diffusion or active transport (Haglund et al. 1992), or by supplying HCO_3^- for direct uptake over the plasma membrane through hydration of CO_2 (Badger 1987). However, there is no clear relation between the external carbonic anhydrase activity and the ability of macroalgae to use HCO_3^- (Giordano and Maberly 1989, Mercado et al. 1998).

The maintenance of CCMs is known to be highly costly for the cell. Under specific nutrient conditions, where other energy-costly processes are induced (e.g. nitrate assimilation), the operation of CA_{ext} could be compromised. Nitrogen assimilation is linked to carbon metabolism in some 50% (Turpin 1991). Nitrate reductase is usually regarded as the limiting step in nitrate assimilation, and is regulated by the external levels of nitrate (among other factors), so that studies on how changes in external nitrate availability affect nitrogen assimilation are commonly studied by measuring NRA (Gordillo et al. 1998, Gordillo et al. 2001b).

Cell surface alkaline phosphatase enables the P supply by using phosphate released from P-monoesters which are part of the dissolved organic P pool. In macroalgae, the alkaline phosphatase activity (APA) is inversely related to the external phosphate concentration (Hernández et al. 2002), and high APA values are considered to be indicative of P limitation.

The Spitsbergen current brings relatively warm and nutrient-rich waters from the south to the island of Spitsbergen (Svalbard, Arctic Norway), allowing the presence of a relatively rich macroalgal community of around 50 species (Hop et al. 2002, Wiencke et al. this issue). In this study we determine the extension of distribution of CA_{ext} among the most representative macroalgal species, covering green, red and brown algae, as well as the changes in the acitivities of nitrate reductase and alkaline phosphatase in a simulated nutrient-enriched situation.

Materials and methods

Experimental setup. Twenty two species of macroalgae were collected from the Kongsfjorden at Spitsbergen, Norwegian Arctic (78° 55' N, 11° 56' E) during July 2002 at depths of 4 to 6 m (except otherwise indicated); 4 Chlorophyta, 8 Rhodophyta, and 10 Phaeophyta (Table 1). Healthy thalli free from macroscopic epibiota were selected and incubated in 2 L aquaria for 72 h with or without daily additions of 10 µM NO_3^- and 1µM PO_4^{3-} at 5° C in continuous white fluorescent light (PFD 30 µmol m^{-2} s^{-1}). The aquaria were aerated at 0.5 l min^{-1}. All the enzymatic methods described below were applied on fresh material taken directly from the incubation aquaria.

External carbonic anhydrase assay. The external carbonic anhydrase (CA_{ext} EC 4.2.1.1) activity was measured potentiometrically, according to Haglund et al. (1992). The assay was carried out at 0-2 °C determining the time taken for a linear drop in pH in the range 8.5 to 7.5 in a 3 ml volume cuvette containing a buffer (50 mM TRIS, 25 mM ascorbic acid, and 5 mM EDTA). Small pieces of algae weighing a total of 150-250 mg FW were washed with distilled water and placed in the cuvette. The reaction was started by adding 1 ml of ice-cold CO_2-saturated distilled water. One unit of relative enzymatic activity was defined as (t_0/t_c)-1, where t_0 and t_c are the time taken for the pH change in the absence and the presence of the alga, respectively.

Nitrate reductase assay. Nitrate reductase (NR, EC 1.6.6.1) activity of fresh material was measured following the *in situ* method according to Corzo and Niell (1991). The in situ method has become a suitable method when the extraction procedure for in vitro determination of NRA is difficult or even impossible without the inactivation of the enzyme (Gordillo et al. 1998, Mercado et al. 2000, Gordillo et al. 2001). In the *in situ* procedure the enzyme is assayed in its original cellular location. The assay medium contained a buffer (0.2 M $H_2PO_4^-$ and 1 mM EDTA; pH = 8), a compound able to permeabilise the membrane (0.1% propanol v/v), nitrate in excess (50 mM $NaNO_3$), and a source of reducing power (10 µM glucose). The reaction was carried out by placing 30 to 70 mg FW of alga in a test tube containing 2 ml of the assay medium. The incubation time was 30 min at a temperature of 5° C. The assay medium was

bubbled with N_2 gas for 2 min before and 2 min after placing the algal sample in order to remove the dissolved O_2, that competes with NO_3^- in its reduction to NO_2^-. The incubation was performed in darkness to prevent further reduction of NO_2^- to NH_4^+. The activity is measured as the rate of NO_2^- produced. The NO_2^- in the assay medium was quatified spectrophotometrically according to Snell and Snell (1949). The observed activity is a potential measure of the NRA of the cell under the conditions prior to the assay.

Alkaline phosphatase assay. The alkaline phosphatase (AP, EC 3.1.3.1) activity (APA) was assayed in 4-6 replicate samples of 50-100 mg FW using the artificial substrate p-nitrophenyl phosphate (pNPP). This compound is cleaved by enzymatic hydrolysis rendering Pi and the coloured compound p-nytrophenol (pNP) determined by absorbance at 410 nm. The procedure followed the method adapted for marine macrophytes by Hernández and Whitton (1996). The assay medium consisted of 2 mL of 500 µM pNPP (which enables maximum velocity of the enzymatic reaction) and 500 mM HEPES-NaOH buffer, pH 8.3, dissolved in P-free artificial seawater of 33 psu. The samples were incubated for 30 min with constant gentle shaking under standard conditions of temperature for potential activity (20° C). Enzymatic activity was expressed as µmol pNP released g^{-1} fresh weight h^{-1}.

Results and discussion

Nutrient concentration in the Kongsfjorden is closely related to the presence of ice cover. The concentrations of nitrate and phosphate during winter and early spring are high, around 9 µM and 0.7 µM, respectively, decreasing drastically during the spring bloom, right after the ice cover melts (<0.5 µM NO_3^- and <0.2 PO_4^{3-}; Aguilera et al. 2002). We measured 0.4 µM of nitrate and 0.5 µM of phosphate at the time of our experiments (July 2002). CO_2 concentration in the system were calculated using the CO_2sys program (Lewis and Wallace 1998). The calculated values were 110 µM CO_3^{2-}, 2067 µM HCO_3^-, and 20 µM CO_2. Under these chemical conditions, all the 22 species examined showed significant CA_{ext} activity (Table 1).

Table 1. External carbonic anhydrase activity (REA g^{-1} FW) of species from the Kongsfjorden, incubated for 72 h with (enriched) or without (control) the addition of 10 µM NO_3^- and 1 µM PO_4^{3-}. The percentage of increase in activity of enriched plants with respect to the control is also shown. Data are mean of 4 to 6 replicates (± standard deviation)

Phylum	Species	Control	Enriched	% change
Chlorophyta	*Acrosiphonia sp.*	17.2 ± 1.4	12.3 ± 0.2	-28.2
	Chaetomorpha melagonium	9.7 ± 2.3	15.7 ± 6.0	+62.4
	Monostroma arcticum	33.4 ± 0.7	32.2 ± 2.3	-3.4
	Prasiola crispa	59.0 ± 0.8	46.6 ± 3.3	-21.1
Rhodophyta	*Ceramium strictum*	15.4 ± 0.8	16.3 ± 1.2	+6.2
	Devaleraea ramentacea	3.6 ± 1.3	4.0 ± 0.4	+11.8
	Odonthalia dentata	11.9 ± 3.2	7.4 ± 2.3	-37.4
	Palmaria palmata	3.93 ± 1.5	3.11 ± 1.4	-20.9
	Phycodrys rubens	24.9 ± 3.6	13.8 ± 0.1	-44.7
	Polysiphonia arctica	11.5 ± 0.0	7.3 ± 0.1	-37.1
	Ptilota gunneri	14.0 ± 0.7	10.8 ± 2.2	-22.8
	Rhodomela lycopodioides	17.2 ± 1.6	6.4 ± 1.4	-62.9
Phaeophyta	*Alaria esculenta*	26.8 ± 1.3	14.5 ± 1.1	-45.9
	Chorda tomentosa	32.0 ± 5.2	27.6 ± 3.9	-13.7
	Chordaria flagelliformis	14.7 ± 0.9	13.7 ± 1.2	-6.7
	Desmarestia aculeata	18.8 ± 2.1	7.8 ± 1.3	-58.6
	Fucus distichus	8.4 ± 3.0	8.8 ± 1.2	+4.7
	Laminaria saccharina	23.6 ± 0.8	9.5 ± 1.4	-59.6
	Laminaria solidungula	21.8 ± 0. 6	8.0 ± 0.9	-63.3
	Saccorhiza dermatodea	11.3 ± 0.1	9.9 ± 0.3	12.6
	Scytosiphon lomentaria	14.4 ± 2.4	15.3 ± 3.9	+6.5
	Sphacelaria plumosa	25.1 ± 0.6	24.7 ± 0.1	-1.6

On average, green algae showed the highest activity, followed by the brown algae, and the red, respectively. The lowest value were obtained in the red algal species *Pa. palmata* and *D. ramentacea* (3.1 and 3.6 REA g^{-1} FW, respectively) while the maximum was found in the green alga *Pr. crispa* (59

REA g^{-1} FW). High affinity for inorganic carbon under both emersed and submersed conditions has been previously reported for *Pr. stipitata* (Giordano and Maberly 1989, Raven and Johnston 1991). Theoretically, the impact of low temperature on photosynthesis of marine macrophytes is predicted to favour diffusive CO_2 entry rather than a CO_2-concentrating mechanism (Raven et al. 2002); however, average value of CA_{ext} (19 REA g^{-1} FW) was much higher for Arctic species than those reported for macroalgae from more temperate waters, like those from Australia (6.4 REA g^{-1} FW , Graham and Smillie 1976), Scotland (5.5 REA g^{-1} FW, Giordano and Maberly 1989), and Southern Spain (3.6 REA g^{-1} FW, Mercado et al. 1998). This can be considered what can be called the 'Arctic paradox' of the C_i uptake system. In principle, Arctic seawater has higher concentration of dissolved CO_2 than temperate waters, because of lower water temperature and lower salinity (we calculated 20 µM CO_2, see above, while typical concentration in temperate areas is 14 µM). Even though it is a well-known phenomenon that higher concentration of CO_2 represses CA activity, the explanation for this paradox could be that high levels of CA are present in Arctic species as part of a general strategy that allows to cope with low temperatures. The strategy would consist in a general increase of the cellular level of enzymes involved in photosynthesis, according to Davison (1987), and invoked to operate in Arctic macroalgae (Korb and Gerard 2000b). An alternative explanation might be related to discrepancies motivated by the methodology. CA activity is measured at 0-2 °C, which seems a more favourable temperature for Arctic species than for temperate ones. Seventeen out of the 22 species studied decreased their CA_{ext} activity when incubated in nutrient-enriched seawater. The exception was *Ch. melagonium* that increased its CA_{ext} activity by 62%, and *Ce. strictum*, *D. ramentacea*, *F. distichus*, *Sc. lomentaria* with more moderate increase (lower than 12%). The general decrease in energy-costly inorganic carbon uptake revealed by decreased CA_{ext} activity in nutrient-enriched situation can be ascribed to enhanced energetic demand by competitive mechanisms such as nitrate assimilation and presumably, macromolecule biosynthesis.

Nitrate reductase activitiy ranged from 0.7 to 12 µmol NO_2^- g^{-1} FW h^{-1} (Table 2).

Table 2. Nitrate reductase activity (μmol NO_2^- g^{-1} FW h^{-1}) of species from the Kongsfjorden, incubated for 72 h with (enriched) or without (control) the addition of 10 μM NO_3^- and 1 μM PO_4^{3-}. The percentage of increase in activity of enriched plants with respect to the control is also shown. Data are mean of 4 to 6 replicates (± standard deviation)

Phylum	Species	Control	Enriched	% Change
Chlorophyta	*Acrosiphonia sp.*	2.67 ± 0.48	3.81 ± 0.91	+42.7
	Monostroma arcticum	5.79 ± 0.00	3.97 ± 0.55	-31.4
	Prasiola crispa	5.16 ± 0.96	11.95 ± 1.43	+131.5
	Ceramium strictum	8.30 ± 0.47	8.94 ± 0.83	+7.7
Rhodophyta	*Devaleraea ramentacea*	3.54 ± 0.39	4.95 ± 0.66	+39.9
	Odonthalia dentata	1.16 ± 0.64	2.92 ± 0.50	+152.6
	Palmaria palmata	8.48 ± 0.51	10.14 ± 0.31	+19.6
	Phycodrys rubens	1.48 ± 0.31	2.70 ± 1.11	+82.1
	Polysiphonia arctica	2.19 ± 0.49	1.57 ± 0.48	-28.2
	Ptilota gunneri	2.42 ± 0.61	5.37 ± 1.60	+122.0
	Monostroma arcticum	4.18 ± 0.54	3.92 ± 0.35	-6.3
	Rhodomela lycopodioides	2.19 ± 0.53	3.14 ± 0.59	+43.2
Phaeophyta	*Alaria esculenta*	2.34 ± 0.49	11.01 ± 2.35	+370.8
	Chorda tomentosa	1.53 ± 0.04	0.74 ± 0.02	-51.5
	Chordaria flagelliformis	1.03 ± 0.39	3.35 ± 1.06	+225.5
	Desmarestia aculeata	2.27 ± 0.28	1.97 ± 0.44	-13.0
	Fucus distichus	1.22 ± 0.50	4.53 ± 0.35	+271.6
	Laminaria saccharina	2.20 ± 0.38	2.35 ± 0.32	+6.7
	Laminaria solidungula	3.44 ± 1.70	11.94 ± 1.17	+247.2
	Saccorhiza dermatodea	0.70 ± 0.18	1.20 ± 0.10	+71.4
	Scytosiphon lomentaria	1.10 ± 0.32	1.66 ± 0.47	+50.0
	Sphacelaria plumosa	2.45 ± 0.06	3.53 ± 0.35	+43.9

Five species showed comparatively higher NRA than the rest, the green alga *M. arcticum* and *Pr. crispa*, the red alga *O. dentata* and the brown alga *Al. esculenta* and *L. solidungula* with values around or above 10 μmol NO_2^- g^{-1} FW h^{-1}. These highest values were found after the incubation in nutrient enriched

conditions. Nutrient enrichment promoted the increase in NRA in 17 out of the 22 species examined. Among those species, the highest induction was found in *M. arcticum*, *D. ramentacea*, *Po. arctica*, *Al. esculenta*, *Ch. flagelliformis*, *F. distichus*, and *L. solidungula* with NRA values more than double the values registered in non-enriched conditions. The rest of species showed lower NRA in enriched conditions, *Ch. melagonium*, *Ph. rubens*, *Pt. plumosa*, *Ch. tomentosa* and *D. aculeata*, being the extent of decrease up to 50%.

Alkaline phosphatase activity was detected in all the species examined. Acitvity ranged from 0.13 to 2.79 µmol pNPP g^{-1} FW h^{-1} in non-enriched conditions, and 0.08 to 3.20 µmol pNPP g^{-1} FW h^{-1} in enriched medium (Table 3). On average, red algae presented APA 5 times higher than the green species and 3 times higher than the brown ones under control (non-enriched) conditions. In enriched medium, differences were lower, mainly due to a general decrease (about 25%) of the APA in the red species, except *Ce. strictum*. In general, 9 out of 22 species showed increased APA in enriched medium, the most drastic increase corresponding to the green *Acrosiphonia sp.*

The C:N:P ratios shown in Table 4 were directly derived from the ratio between the enzymatic activities shown above. Thus, they cannot be indicative of the overall C:N:P ratio for biomass, because other activities (e.g. rubisco) should be considered. However, they are indicative of nutritional preferences, and of changes in those preferences promoted by nutrient enrichment. The ratios indicated that green and brown algae had a similar pattern, while red algae were clearly different in general. C:P ratio was on average 67 and 75 for green and brown algae, respectively, under non-enriched conditions, decreasing to 54 and 38, respectively after nutrient enrichment. Red algae however averaged 6 and 5 for non-eriched and enriched conditions, respectively. Similarly, N:P ratio where in general higher in green and brown algae, with average values of 14 and 7 in non-enriched conditions, and 16 and 21 in enriched conditions, respectively. Differences were not so drastic in C:N ratios that averaged from 2.7 for enriched red algae to 11.8 for non-enriched brown algae, respectively.

Table 3. Alkaline phosphatase activity (µmol pNPP g-1 FW h^{-1}) of species from the Kongsfjorden, incubated for 72 h with (enriched) or without (control) the addition of 10 µM NO_3^- and 1 µM PO_4^{3-}. The percentage of increase in activity of enriched plants with respect to the control is also shown. Data are mean of 4 to 6 replicates (± standard deviation)

Phylum	Species	Control	Enriched	% change
Chlorophyta	*Acrosiphonia sp.*	0.39 ± 0.02	2.57 ± 0.32	+555.6
	Chaetomorpha melagonium	0.29 ± 0.03	0.29 ± 0.03	-0.2
	Monostroma arcticum	0.47 ± 0.25	0.32 ± 0.06	-32.6
	Prasiola crispa	0.49 ± 0.06	0.89 ± 0.08	+80.6
Rhodophyta	*Ceramium strictum*	1.45 ± 0.05	1.88 ± 0.05	+29.9
	Devaleraea ramentacea	1.14 ± 0.16	0.79 ± 0.08	-31.2
	Odonthalia dentata	2.76 ± 0.40	2.06 ± 0.46	-25.4
	Palmaria palmata	0.75 ± 0.13	0.59 ± 0.05	-21.4
	Phycodrys rubens	2.79 ± 0.83	2.33 ± 0.92	-16.4
	Polysiphonia arctica	2.69 ± 0.22	1.69 ± 0.22	-37.3
	Ptilota gunneri	2.34 ± 0.35	1.59 ± 0.21	-32.2
	Rhodomela lycopodioides	2.67 ± 0.49	2.58 ± 0.32	-3.4
Phaeophyta	*Alaria esculenta*	1.30 ± 0.73	0.57 ± 0.23	-56.5
	Chorda tomentosa	0.22 ± 0.02	0.43 ± 0.02	+97.7
	Chordaria flagelliformis	0.31 ± 0.06	0.34 ± 0.02	+9.5
	Desmarestia aculeata	0.22 ± 0.02	0.43 ± 0.02	+97.6
	Fucus distichus	1.35 ± 0.27	0.74 ± 0.03	-44.9
	Laminaria saccharina	0.15 ± 0.03	0.21 ± 0.05	+41.7
	Laminaria solidungula	0.14 ± 0.01	0.08 ± 0.01	-43.6
	Saccorhiza dermatodea	0.13 ± 0.04	0.26 ± 0.02	+103.0
	Scytosiphon lomentaria	0.53 ± 0.05	0.46 ± 0.07	-12.5
	Sphacelaria plumosa	2.45 ± 0.32	3.20 ± 0.20	+30.6

Nutrient enrichment changed the ratios significantly, specially in brown algae where C:P decreased around 50% and N:P increased threefold (on average). The decrease in C:N ratio was similar for the three groups (around 30 to 45% on average).

Table 4. Apparent C:N:P molar ratio derived from CA_{ext}, NR and AP activities of species from the Kongsfjorden, incubated for 72 h with (enriched) or without (control) the addition of 10 µM NO_3^- and 1 µM PO_4^{3-}

Phylum	Species	Control C:N:P	Enriched C:N:P
Chlorophyta	*Acrosiphonia sp.*	44:7:1	5:1:1
	Chaetomorpha melagonium	34:20:1	55:14:1
	Monostroma arcticum	71:11:1	102:38:1
	Prasiola crispa	120:17:1	53:10:1
Rhodophyta	*Ceramium strictum*	11:2:1	9:3:1
	Devaleraea ramentacea	3:1:1	5:4:1
	Odonthalia dentata	4:3:1	4:5:1
	Palmaria palmata	5:2:1	5:5:1
	Phycodrys rubens	9:1:1	6:1:1
	Polysiphonia arctica	4:1:1	4:3:1
	Ptilota gunneri	6:2:1	7:2:1
	Rhodomela lycopodioides	6:1:1	2:1:1
Phaeophyta	*Alaria esculenta*	20:2:1	26:19:1
	Chorda tomentosa	146:7:1	64:2:1
	Chordaria flagelliformis	48:3:1	41:10:1
	Desmarestia aculeata	86:10:1	18:5:1
	Fucus distichus	6:1:1	12:6:1
	Laminaria saccharina	162:15:1	46:11:1
	Laminaria solidungula	152:24:1	99:148:1
	Saccorhiza dermatodea	86:6:1	38:5:1
	Scytosiphon lomentaria	27:2:1	33:4:1
	Sphacelaria plumosa	10:1:1	8:1:1

Consequences of environmental nutrient enrichment would be quite drastic in the nutritional balance of the algal community of the Arctic. According to our results, a theoretical community with its biomass equally distributed among the species examined would have on average 25% less CA_{ext} activity,

and would assimilate 60% more N through NRA, while APA would show almost no change at the community level (3% increase).

Regarding the predicted increase in atmospheric CO_2 concentration, it is generally assumed that elevated CO_2 will enhance growth in species were N assimilation is also favoured (e.g. Gordillo et al. 2001b). The concomitant enrichment of inorganic N and P in a predicted future scenario would provoke enhanced growth of the macroalgal community of the Arctic. To our knowledge, there is still a lack of evidence of progressive eutrophication in the West coast of Svalbard. However, the fact that the Spitsbergen current brings rich Atlantic waters from the South makes the eutrophication a likely phenomenon. These changes in the fragile Arctic benthic ecosystem would have drastic consequences, as already warned by Hop et al. (2002). Biodiversity could be compromised since fast-growing species (e.g. the green *M. arcticum* and *Acrosiphonia* sp.) are more likely to show enhanced growth than resilient slow-growing species (e.g. the red *Ph. rubens*, and *Pt. plumosa*).

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EFFECT OF TEMPERATURE AND PHOTON FLUENCE RATE ON GROWTH RATES OF TWO EPIPHYTIC DIATOM SPECIES FROM KONGSFJORDEN

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INTRODUCTION

Although the structure of the pelagic and benthic food webs in Kongsfjorden are well described, even basic information on the primary production of the different phototrophic organisms are still for most groups lacking, particularly for macro- and microalgae (Hop et al. 2002). This is surprising since microphytobenthic communities in estuarine to marine shallow water environments from many temperate to tropical coasts are well known to be ecologically important due to their generally high primary production rates and consequently their function as a major food source for benthic suspension- or deposit-feeders (Cahoon 1999). In addition, many benthic microalgae such as diatoms secrete extracellular polymeric substances that stabilize sediments thereby reducing the erodibility under flow conditions. Although most benthic species grow on various geological hard and soft substrata, there are also taxa living epiphytically on macroalgae where they often form complex assemblages and high abundances. The effects of epiphytic microalgae on their host may be either beneficial (e.g. protection against herbivory) or detrimental (e.g. competition for light and nutrients) (Fong et al. 2000). If epiphytes are present in high cell numbers they may account for up to 50% of the primary production of a macrophyte community (Pollard and Kogure 1993). In contrast to temperate to tropical regions the ecological significance of microphytobenthic communities for polar waters is not well understood, although a recent pilot study on epilithic diatoms of a high Arctic fjord in Greenland demonstrated for the first time that these algae accounted for about 40% of the total benthic primary production (Glud et al. 2002).

Arctic microphytobenthos experiences strong seasonal amplitudes in solar radiation. During the long polar night that covers 116 days in the Kongsfjorden area primary production of all phototrophs is completely suppressed. Later in

the season during periods of persisting ice layers in spring, and of melting snow and glaciers in summer resulting in a high discharge of particles into the fjord light penetration into the water column may be extremely low and hence negatively affect photosynthesis and production of all phototrophs (Hanelt et al. 2001). While growth and photosynthesis as function of the radiation conditions has been investigated for many macroalgal species from Kongsfjorden (see various contributions of this volume), similar ecophysiological studies on benthic and epiphytic microalgae are completely missing.

Therefore, the aim of the present study was for the first time to isolate abundant epiphytic diatom taxa from Kongsfjorden macroalgae, to establish unialgal cultures and to determine their growth rate under different radiation and temperature conditions. From these data optimum, minimum and maximum photon fluence rates and temperatures for growth can be assessed and possible adaptation to the environmental conditions in the Arctic habitat better understood.

MATERIAL AND METHODS

Species isolation

Epiphytic diatom assemblages were sampled from the brown macroalga *Chordaria flagelliformis* growing at 1 m water depth at the station Hansneset in July 2002. Diatoms were transferred into sterile North Sea water (32 PSU) enriched by 20 ml Guillards f/2 and 30 mg l^{-1} sodium metasilicate after strongly shaking the host plant followed by incubation at <5°C and about 10 µmol photons m^{-2} s^{-1} for some months to obtain dense cultures. Subsamples were dispensed onto silicate-enriched seawater agar plates (1.5%) and incubated for several weeks under the same conditions. Individual colonies were transferred into 5 ml medium and allowed to grow dense cultures again. These were checked microscopically if they were unialgal. However, most of the cultures had to be further cleaned from rare, contaminating taxa by picking single cells or cell chains with a capillary and checking the droplet microscopically for unialgality. The unialgal suspensions were grown for biomass production.

Experimental set up

Log phase cell suspensions were derived from batch cultures grown at 5°C, 10-15 µmol photons m^{-2} s^{-1} in sterile enriched seawater medium. These were diluted prior to the experiment 1000-fold and portioned into 5 cm diameter polyethylene dishes at a volume of 15 ml each. Fifteen dishes per species were incubated at 0, 5, 10, 15, 20°C, respectively. Osram Daylight Lumilux Deluxe lamps were used as light sources. Different irradiation intensities were obtained by shading the dishes with up to 4 layers of black gauze resulting in 2, 5, 10, 15 and 20 µmol photons m^{-2} s^{-1}. Thus, the number of replicates was 3.

Growth rate determination

Growth rates were determined fluorometrically (Karsten et al. 1996). Each dish was placed into the fluorometer always in the same orientation to minimise noise originating from the dishes material and measured 3 times for chlorophyll fluorescence of the sedimented or attached cells. Medium blanks were measured daily and were subtracted from sample values. Each sample was monitored for up to 14 days every 12 hours or for faster growth for up to 7 days every 12 hours.

Curve fitting

The growth rate for each individual sample was calculated from the logarithmic growth equation by following procedure: According to

(1) $$N_t = N_0 \cdot e^{\mu \cdot t}$$

N_0	fluorescence at day 0
N_t	fluorescence at a given day
μ	growth rate in logarithmic phase (d^{-1})
t	time (d)

N_t was calculated for each measurement from an estimated µ. The squared differences between measured and calculated N_t were summed. With the Solver function of the Microsoft Excel calculation program µ was iterative optimised for a minimum deviation between measured and calculated N_t. Mean µ were calculated from 5 replicate samples for each temperature and irradiation. Temperature and irradiation optimum curves were fitted after Blanchard et al.

(1996) from this equation:

$$(2) \qquad \mu(F) = \mu_{max} \cdot \left(\frac{F_{max} - F}{F_{max} - F_{opt}} \right)^{\beta} \cdot \exp\left[-\beta \cdot \left(\frac{F_{max} - F}{F_{max} - F_{opt}} - 1 \right) \right]$$

μ_{max}	maximum growth rate (d^{-1})
F	factor, i.e. temperature or irradiation
F_{opt}	factor value for optimal growth
F_{max}	maximal value at which growth occurs
ß	sensitivity parameter describing the curve steepness

Symbol für diesen Parameter erscheint bei mir nicht!

The values for μ_{max}, F_{opt}, F_{max} and ß were optimised in the way as the μ for equation (1) with the Excel Solver function after calculating μ for given environmental factors.

RESULTS

During a pilot study about the occurrence of epiphytic diatoms on macroalgae from Kongsfjorden we observed high abundances particularly on shallow-water host plants (0.5-5 m). Thalli area of macroalgal species such as the chlorophyte *Acrosiphonia* aff. *penicilliformis* or the phaeophyte *Ectocarpus siliculosus* can be 70-90% covered by diatoms (Karsten et al., unpublished results). In addition, the assimilation hairs of the brown alga *Chordaria flagelliformis* exhibited also an almost complete coverage with approximately 20-25 epiphytic diatom taxa, of which 2 were isolated and successfully established as unialgal cultures at the University of Rostock.

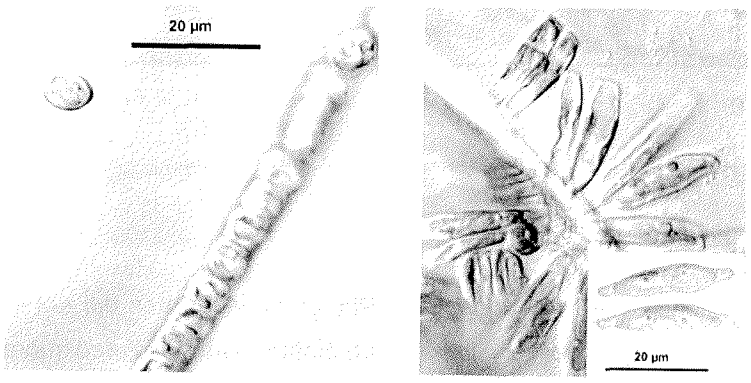

Figure 1 Isolates 99 (a) and 125 (b) (Differential Interference Contrast, Olympus IX 70, ColorView12)

Isolate ROS D99 is a 6-12 µm long, 6-7 µm wide and 4-6 µm high pennate diatom of the genus *Fragilaria* (Fig. 1a). It forms chains of about 50-100 cells. Isolate ROS D125 is also a *Fragilaria* species and about 20-45 µm long, 6-7 µm wide and 5-7 µm and has a slightly asymmetric shape in top view (Fig. 1b). This species attaches to surfaces with one end and forms only short chains of up to 10 cells if unattached.

At temperatures >5°C both species grew with only a short lag phase of <1d logarithmically as long as the chlorophyll *a* fluorescence could be monitored. Standard deviation of the pigment fluorescence in replicate dishes was <18% on average at 15°C for isolate 125 (Fig. 2).

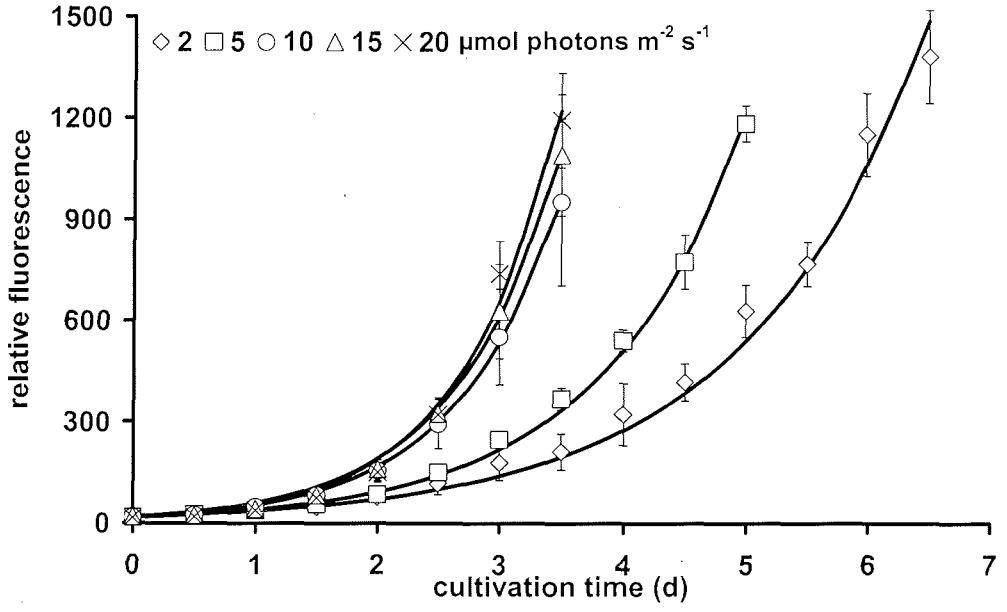

Figure 2 Increase in chlorophyll fluorescence over time during logarithmic growth of isolate 125 at 15°C under different photon fluence rates (µmol m^{-2} s^{-1}).

At 0°C both species grew exponentially. Photon fluence rates did not influence growth rates or only to a very low extent. Both isolates differed more in the duration of lag phases, which was about 4 days for isolate 125 and 1-2 days for isolate 99 (Fig. 3).

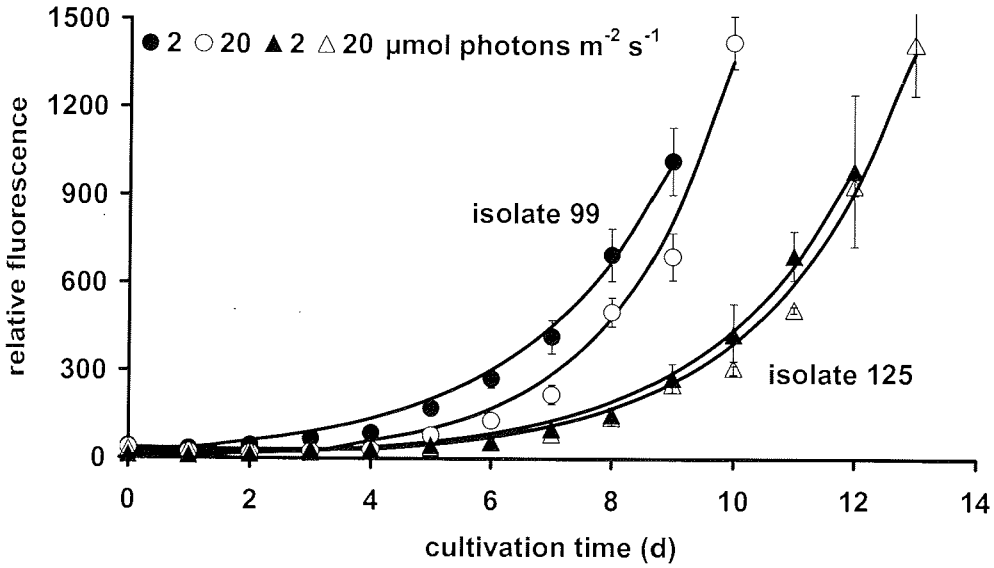

Figure 3 Increase in chlorophyll fluorescence over time during logarithmic growth of isolate 99 (circles) and 125 (triangles) at 0°C, 2 and 20 µmol photons m^{-2} s^{-1}.

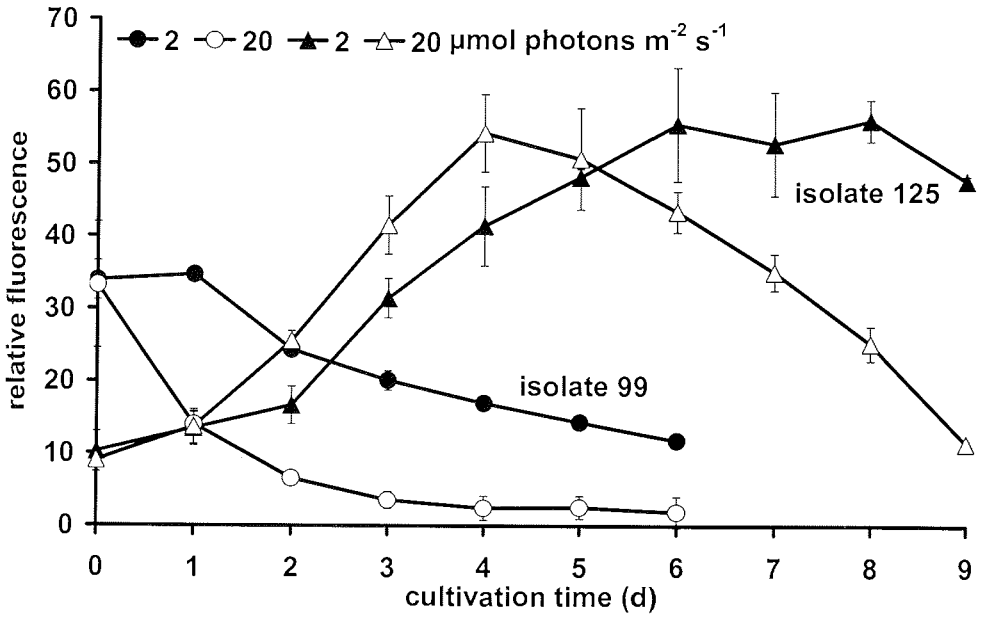

Figure 4 Changes in chlorophyll fluorescence over time of isolate 99 (circles) and 125 (triangles) at 20°C, 2 and 20 µmol photons m^{-2} s^{-1}.

Isolate 99 died immediately after transfer to 20°C as seen by cells starting to bleach which was reflected by the decreasing fluorescence signal. In contrast, isolate 125 could survive this temperature for at least 4 days, particularly at lower photon fluence rates (Fig. 4).

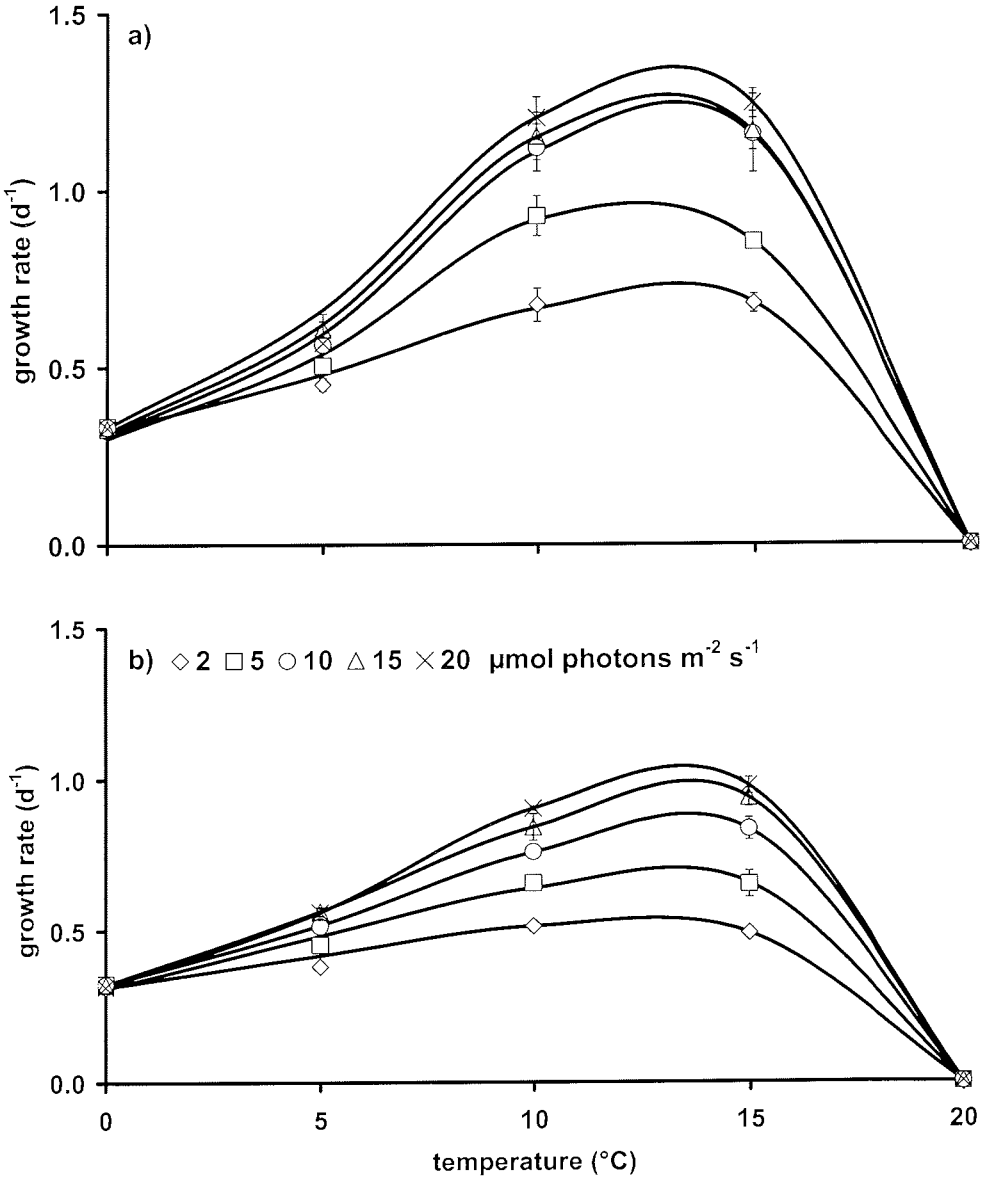

Figure 5 Growth rates (d^{-1}) of isolate 125 (a) and 99 (b) at different temperatures (°C) and photon fluence rates (µmol photons m^{-2} s^{-1}).

If growth rates were fitted against temperature according to Blanchard et al. (1996, Fig. 5) optimum temperatures of 12-14°C were calculated for both isolates. At 0°C both isolates could still sustain one third of optimal growth. Photon fluence rates above 10 µmol photons m^{-2} s^{-1} did not increase growth rates further for isolate 125 and only slightly for isolate 99. At the very low irradiation of 2 µmol photons m^{-2} s^{-1} growth measured at 15°C (near the calculated optimum rate) was still half as fast compared to the highest measured rate at 20 µmol photons m^{-2} s^{-1}.

DISCUSSION

For the first time 2 epiphytic diatom taxa from macroalgae of the Arctic Kongsfjord were isolated, established as unialgal cultures and ecophysiologically characterised in terms of growth under different temperatures and photon fluence rates. Both species of the genus *Fragilaria* exhibited optimum growth rates at 12-14°C, grew still well but with a reduced rate at 0°C and did not survive 20°C. Therefore, both taxa from Kongsfjorden can be characterised as eurythermal organisms. This is in contrast to Antarctic benthic diatoms which showed maximum growth at 0°C and full inhibition of cell division already at 7-9°C (Longhi et al. 2003). Consequently, the studied Antarctic microphytobenthic taxa are characterised as polar stenothermal organisms. These obvious differences in the temperature requirements for growth in Arctic and Antarctic benthic microalgae can be related to the geological cold water history of both regions. While macroalgae from Antarctica are exposed to low temperatures for almost 14 million years (Wiencke et al. 1994), and diatoms even longer (Barron 1993), species from the Arctic have experienced these environmental conditions for a much shorter period of only 3.5 million years, which explains the high and low degree of endemism in phototrophic organisms from Antarctic and Arctic waters, respectively. Particularly endemic algae have evolved various physiological and biochemical adaptations to low temperatures (Wiencke et al. 1994). While the Antarctic benthic diatom taxa investigated by Longhi et al. (2003) are indeed characterised as endemic species, which well explains the low temperature demands for growth, the respective information on both *Fragilaria* strains from the Arctic is still missing. However, due to the eurythermal physiological

features it may be speculated that both strains occur besides the Arctic also in more temperate regions.

Both *Fragilaria* species grew optimally already at very low photon fluence rates of 10-20 µmol photons m^{-2} s^{-1}, indicating shade adaptation. Even at the lowest photon fluence rate tested (2 µmol photons m^{-2} s^{-1}) half of the maximum growth rate could be measured. Similar results were reported for Antarctic benthic diatoms. *Gyrosigma subsalinum* exhibited saturated growth at 11.4 µmol photons m^{-2} s^{-1} (Longhi et al. 2003), and *Amphora antarctica* and *Trachyneis aspera* grew also best under very similar conditions at 10-15 µmol photons m^{-2} s^{-1} (Rivkin and Putt 1987). However, while these authors documented maximum cell divisions for the Antarctic benthic diatoms in the range of 0.25-0.4 d^{-1} under optimum photon fluence rates, both Arctic *Fragilaria* species grew much faster under similar conditions with cell divisions of 0.9-1.2 d^{-1}.

The extremely low light requirements for growth of the epiphytic diatoms studied guarantee biomass production under the fluctuating radiation conditions in Kongsfjorden. Hanelt et al. (2001) monitored over several years solar radiation in Kongsfjorden, and described the underwater light climate as seldom stable. Due to rapidly changing weather conditions not only on a seasonal scale, but also diurnally extremely variable radiation can be recorded. In addition, during summer the underwater light climate of Kongsfjorden is further affected by calving glaciers and strong melt water influx resulting in increasing turbidity due to suspended particles and hence in a strong decrease of the water column transmittance. Under these conditions Kongsfjorden can be optically characterised as coastal water type 9 according to Jerlov (1976) indicating very low light conditions already below 1-2 m depth. The shade adaptation of the epiphytic diatoms can be well explained by the prevailling photon fluence rates in the water column. In addition, in some years after a long cold winter sea-ice plus a top layer of snow may persist until late spring resulting in a very strong attenuation of the impinging solar radiation (Hanelt et al. 2001). Under such circumstances PAR of only 6.5 µmol photons m^{-2} s^{-1} can be measured under the ice, which is very low but still sufficient to support growth of both *Fragilaria* species studied.

In conclusion, the epiphytic diatoms studied seem to be well adapted to the temperature and radiation conditions in the Arctic Kongsfjord. Measurement of

growth rates under different environmental conditions are ecologically very important because they reflect an integrating process for all physiological responses in the cell.

ACKNOWLEDGEMENTS

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4. EFFECTS OF UV RADIATION ON BIOTA FROM KONGSFJORDEN

Photosynthetic and bacterial activity in Kongsfjorden, Svalbard: Dependence of ambient PAR and UV-B radiation.

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1 INTRODUCTION

Kongsfjorden is a high Arctic bay located 79°N on the west-coast of Spitsbergen. The physical and biological conditions have been investigated in some detail, stimulated by the location of Ny-Ålesund research station in the bay. Most of those results are summarised in the reviews by Hop *et al* (2002) and Svendsen *et al* (2002). These reviews indicate that we have a good knowledge about the physical environment (metrology, sun radiation) and higher trophic levels (birds, mammals). Substantially less, however, is known about the pelagic microorganisms. The present knowledge regarding pelagic microorganisms is based on sporadic measurements of phytoplankton and bacteria distribution and production reviewed in Hop *et al* (2002).

Kongsfjorden is surrounded by a series of glaciers and is highly affected by runoff that normally starts in late May/early June. During this period a thin layer of surface water with a high content of silt and clay strongly reduces the penetration of radiation (Hanelt *et al* 2001). In the inner part of the fjord, the transmission of radiation is low and is almost constantly reduced to below 1 meter in summer periods. In the large central part of the fjord the penetration of radiation is very variable. Current and tides move the turbid surface water in and out of the fjord and the spatial variation in time and place are very large, thus making it of special interest to describe how primary and bacterial production varies in a short-term timeframe and how it is dependent on the light conditions.

To increase our knowledge about the pelagic microorganisms in Kongsfjorden, photosynthetic and bacterial activity is studied in a summer situation where runoff substantially affects penetration of radiation.

2 METHODS

2.1 Water sampling

Water was sampled with a non-metal water sampler (Ruthner) 200-300 m NW of Brandalspynten in the central part of Kongsfjorden (Figure 1).

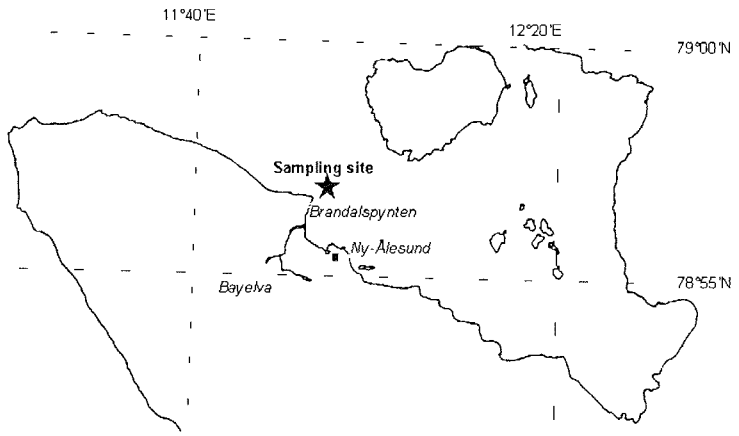

Figure 1: Map of inner part of Kongsfjorden and location of sampling and incubation site.

This location was chosen to avoid influence from local freshwater run-off, e.g., from Bayelva which transports large amounts of silt and clay into the bay at Ny-Ålesund. The whole bay is, however, strongly influenced by runoff from rivers and glaciers, which strongly increases the turbulence of the water (see below). Water was sampled at 2 and 4 m depths and mixed in equal proportion (to give representative samples of the well-mixed surface water). Prior to mixing the water was filtered through a 200 µm net to exclude larger grazers. 10 ml water was directly distributed into polyethylene bags (Whirl-Pac™) for incubations (see below). All handling of the water was done in shaded light to avoid light shocks. For determination of species, composition and salinity, water was filled into glass flasks. The water for algal counting was preserved with acid Lugol solution.

2.2 Incubation

Incubations were done *in situ* at the same location as the sampling. Normally incubations were done at five depths (0.5, 1, 2, 4 and 8 m). Each treatment and depth was run in triplicates. Incubations started around noon on July 6, 8, 11,12 and 15. In addition, a series of incubations was done on July 14-15 that together covered all of the 24 h of a day-night cycle.

For measurement of the primary production (PP) ^{14}C-labelled carbonate (10 µCi) was added at the start of the incubation. After incubation (normally 4 h) the carbon incorporation was terminated by adding 0.5 ml formaldehyde (25%). Formaldehyde was added To two samples before the incubation as blanks for non-biological carbon fixation. In the laboratory 9 ml of water from each bag was transferred to a scintillation vial. The water was acidified with hydrochloric acid and aerated to eliminate non- incorporated ^{14}C-carbonate. Scintillation cocktail (Beckman Ready-Gel) was added and the radioactivity was measured by liquid scintillation. Resulting DPM values were converted to carbon uptake assuming a carbonate$_{(total)}$ concentration of 2.1 mM.

The incubation for bacterial production (BP) was done in parallel with the incubations for PP. After *in situ* incubation BP was measured as incorporation of ^{3}H-labeled thymidine during 30 minutes at the same temperature but in darkness. The ^{3}H-thymidine incubation started within 10 minutes after the *in-situ* incubation.The incubation was terminated by addition of trichloracetic acid, TCA (5% final concentration). Samples with TCA added before the thymidine addition were used as blanks. In the laboratory the water was filtered down on cellulose nitrate filters (0.45 μm) and was carefully washed with ice-cold TCA (5%). Scintillation cocktail (Packard Ultima Gold) was added and the radioactivity was measured by liquid scintillation. The thymidine uptake (DPM) values were converted to cell production rate using values in Ducklow and Carlson (1992).

2.3 Environmental conditions

Data for surface level PAR were supported from Koldeway station in Ny-Ålesund. The PAR values are calculated by subtracting the infrared radiation and the ultraviolet radiation from the global measurement (see Hanelt et al. 2001).

At each sampling and at the end of the *in situ* incubation period, the secchi depth (Z_{SD}) was measured and in addition, on July 15, the wavelength specific transmittance of the water in the range 300-800 nm, was measured with an underwater spectroradiometer Li-1800 (LiCor Bioscience) at 1, 2, 4 and 8 m depth. Data on wind speed and direction were obtained from weather station in Ny-Ålesund, run by the Norwegian Polar Institute.

2.4 Calculations

The PAR levels at the different depths were estimated from the measured secchi depths (the mean of the value at sampling and termination of the incubation was used), and the ambient PAR levels. K_d-values were calculated from the secchi depth as $K_d=1.44*Z_{SD}^{-1}$ (Kirk 1994). The validity of these calculations were confirmed by comparing the K_d values calculated from the spectroradiometer measurement on July 15 with the ones calculated from the secchi disc measurements on the same day. The K_d calculated from the Z_{SD} values was 0.41 and the mean K_d value calculated from the spectroradiometer data between 1 and 2 m was 0.45, between 2 and 4 m the mean K_d was 0.44 and between 4 and 8 m it was 0.42.

Statistical analysis of UVBR effects on production was done using an ANOVA test. Checks of homogeneous variances were done with Cochran C. P-values <0.05 are regarded as significant.

3 RESULTS

3.1 Surface light conditions and wind speed.

The light conditions during the early and late part of the campaign were sunny while the conditions in the middle part of the campaign were overcast (Figure 2a). During July 6 to 9, the wind speed was generally low, while it was strong on July 10. From July 11 to 15, the wind speed increased slowly (Figure 2b).

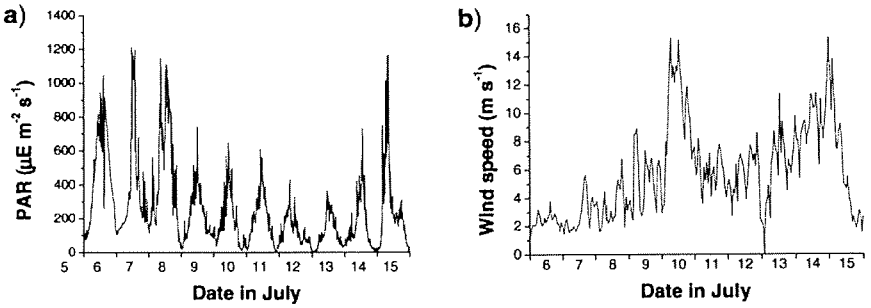

Figure 2: Light and wind conditions. a) PAR radiation; b) Wind speed

3.2 Algal species composition

The microalgal flora was dominated by dinoflagellates and chrysophytes of which several are mixotrophic or heterotrophic. Some diatoms were found but in very low numbers (Table 1). There were large changes in species composition, especially the species composition on the first two days which was very different from the others.

Table 1: **Algal species composition (cell/l)**

Date of July	6	8	11	12	14	14	15	15	15
Time of Day (hh.mm)	12.30	12.00	11.30	12.30	15.00	19.25	07.00	11.00	01.00
DIATOMS	40	560	0	160	700	0	240	0	200
DINOFLAGELLATES									
Amylax triacantha	0	0	80	0	200	240	240	80	160
Dinophysis spp	0	240	80	0	160	240	160	80	240
Gymnodinium wulffii (x 10^{-3})	43.2	8.64	8.64	33.5	72.4	18.0	16.2	3.98	17.3
Gyrodinium spp(x 10^{-3})	60.5	4.88	30.2	12.0	11.9	2.24	3.36	2.48	1.60
Helgolandinium subglobosum	0	0	0	240	0	0	0	0	0
Peridiniella danica (x 10^{-3})	38.9	0.720		0.48	0.440	0.800	0.080	0	0.04
Protoperidinium spp	120	480	640	880	1286	480	1440	400	160
Scrippsiela trochoidea	0	0	0	0	0	0	0	0	120
CHRYSOPHYTES									
Dinobryon balticum (x 10^{-3})	1749	1233	17.2	52.9	0.620	48.2	11.6	0.080	58.3
Apedinella cf. *spinifera* (x 10^{-3})	155	46.3	1468	978	0	0.160	0.320	0.240	0

3.3 Production rates

The primary production rates varied substantially during the campaign (Figure 3a). On July 6 and 8 the production did not decrease by depth. The production integrated over the first 8 m differed from 0.6 mg C m^{-2} h^{-1} July 15 to 8.5 mg C m^{-2} h^{-1} on July 11 (Table 2, Figure 3).

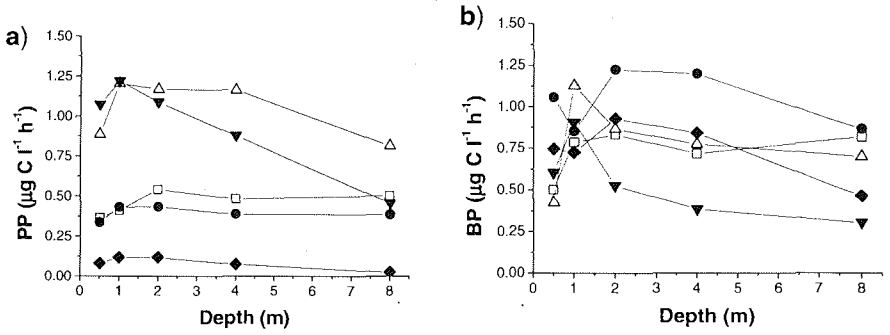

Figure 3: **Primary and bacterial production** incubated at different depths. □ July 6; ● July 8; △ July 11; ▼ July 12; ◆ July 15 a): Primary production; b): Bacterial production

The primary production, at all depths was higher on July 11 and 12 than both before and after these dates. The higher efficiency on the aforementioned days than on July 6 and 8 can mostly explained by the lower light intensity as they fit well together in a PP/PAR vs PAR plot (Figure 4). The lower production rates on July 14-15 cannot be explained by low light intensities.

The bacterial production (BP) varied much less between days and depth (Figure 3b). Integrated over the first 8 m the highest production was 8.6 (July 8) and the lowest 3.7 (July 12) mg C m^{-2} h^{-1}(Table 2).

Table 2: **Radiation data, estimated attenuation coefficient (K_d), primary production (PP) and bacterial production (BP) during incubations**

date	incubation time	PAR (surface) (W m^{-2})	Estimated K_d start	Estimated K_d end	PP 0-8 m mg C m^{-2} h^{-1}	BP 0-8 m mg C m^{-2} h^{-1}	PP/BP
July 6	12.40-16.45	155	0.26		3.8	6.0	0.6
July 8	12.10-16.10	198	0.24	0.32	3.2	8.6	0.4
July 11	11.33-15.40	81	0.32	0.32	8.4	6.2	1.4
July 12	12.28-16.35	21	0.41	0.26	6.9	3.7	1.9
July 14	15.35-19-30	27	0.41	0.24			
	19.30-01.30	4	0.24	0.21			
July 15	01.30-7.40	85	0.21	0.32			
	7.40-11.50	116	0.32	0.41			
	12.00-16.20	42	0.41		0.6	5.9	0.1

The ratio between primary and bacterial production (mean for 0-8 m) was <1 on July 6 and 8 but increased to >1 on July 11 and 12 whilst it was very low (0.1) on July 15 (Table 2).

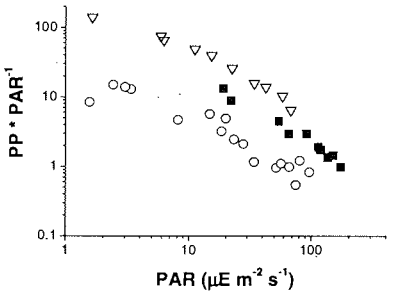

Figure 4: **Ratio between primary production and PAR relative PAR.**: ■ Phase 1 (July 6-8); ▽ Phase 2 (July 11-12); ○ Phase 3 (July 14-15)

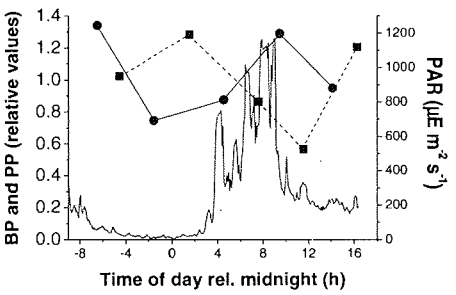

Figure 5: **Primary and bacterial production (0-2 m) and PAR during the diurnal incubation.** Primary and bacterial production is expressed relative to the mean production during 24 hours. The time value used for primary production is the mean of the *in situ* incubation, for bacterial production the start of the incubation. ● Primary production; ■ Bacterial production

As expected, the light dependence in bacterial production was less than for primary production, however, a tendency to enhanced BP at 1 m depth was seen on July 11 and 12. In the diurnal measurement of July 14-15, both PP and BP showed a weak diurnal variation, partly out of phase (Figure 5).

4 DISCUSSION

These data show a substantial variation in primary production within a limited time-frame at one location in Kongsfjorden. Three phases can be identified: July 6-8; July 11-12; and July 15. The first change between phases, but not the second, is closely correlated to changes in phytoplankton species composition. A reasonable scenario is that the stable weather condition that prevailed until July 9 changed with increased wind that augmented the turnover in the water sample and thus increased the nutrient condition and introduced new species to the surface layer.

The changes in bacterial production were much smaller and it is not possible to classify changes in bacterial production into phases. The ratio between primary and bacterial production was variable and between 0.1-1.9 (Table 2). BP higher than PP show that there is a significant supply of organic matter from benthic and terrestrial ecosystems. Our calculations, however, do not conflict with the estimates of daily loss of phytoplankton due to

sedimentation in Kongsfjorden range between 2 to 5% (Keck 1999, Wiktor 1999).

The primary production in the upper water levels, is only slightly dependent on the time of the day. The production rates in the incubation around midnight were around 60% of the highest PP the day after (incubation between 07.40 and 11.50). This is well in accordance with Eilertsen *et al.* (1989) who found that the production rate between 00.00-06.00 was 50% of that between 06.00 and 12:00 in Smeerenburgfjorden. The PP vs PAR values at the different time of the day/night cycle showed a correlation with PAR (Figure 4, phase 3) indicating that the main reason for the reduction in PP during the night is the reduced PAR levels, not a biological cycle in the algae.

In summary: by measuring the primary and bacterial production during a 14-day period we showed a strong variation in primary production, a change that is correlated with weather changes. During midnight sun conditions the amplitude in diurnal variation in PP is limited but a minimum is shown around midnight. The BP also shows a fluctuation but it was out of phase with the PP, with highest production being shown at midnight. Estimates indicate that the major part of the PP is consumed in the pelagic microbial system.

This is the first series of combined BP and PP measurements in Kongsfjorden. The data show the risk in using a single or a few production measurements as typical for longer periods and that apart from the annual variations in production, a strong short-term fluctuation also occurs.

ACKNOWLEDGEMENT

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Impacts of solar ultraviolet-B radiation on marine macroalgae from Kongsfjorden: Inhibition and acclimation of photosynthetic activity

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Introduction

Since the first records of stratospheric ozone depletion above the Northern Hemisphere, serious concerns have arisen on the impact of enhanced UVB radiation on the Arctic environment (Hessen 2001). Since 1995, major research efforts were directed to elucidate the impact of solar UVB radiation on the marine environment of Kongsfjorden, with major emphasis on the macroalgal flora. The particularly dense macroalgal vegetation along the shores of Kongsfjorden (Wiencke et al.: this issue) is a major source of marine primary production in that area. Seventy macroalgal species with a total wet biomass of up to 21 kg m^{-2} have been recorded there (Hop et al. 2002). Depending on the strong seasonal changes in the availability of major abiotic resources such as nutrients, light etc., algal productivity exhibits marked variations in the course of the year. In particular, light availability is a crucial requisite for maintaining algal populations as it drives photosynthesis. The underwater radiation regime the benthic macroalgal flora is exposed to, is subject to strong seasonal variation due to the change in the seasons (polar night, polar day) and also to sea ice conditions (Svendsen et al. 2002). Consequently also the degree of UVB exposure is highly determined by season, sea ice as well as actual weather conditions and the turbidity of the water column (Hanelt et al. 2001). Macroalgal species inhabiting the high latitudes of both hemispheres are generally regarded as being low light adapted (Kirst and Wiencke 1995). Therefore, sudden and strong increases in radiation conditions may have marked effects on macroalgal physiology. Particularly, UVB radiation may confer serious adverse effects on *in situ* photosynthesis. The impact of UVB radiation on photosynthesis of macroalgae of Kongsfjorden has been readily studied, focussing on the inhibition by, acclimation to and protection against UV-exposure (Bischof et al. 1999; Hanelt et al. 1997a; Karsten et al. 1999). In short, the adverse effects of UVB exposure to photosynthesis are a result of absorption of radiation of high energy content by biomolecules such as proteins and nucleic acids. As major target sites of UV-exposure, the D1 protein in the core complex of photosystem II and the carbon dioxide fixing enzyme RubisCO have been identified (Bornman 1989; Vass 1997; Bischof et al. 2000). In addition to the direct disruptive effects, that UV-absorption causes in proteins, it may also favour the generation of reactive oxygen species within the primary reactions of photosynthesis (Bischof et al. 2003), thus contributing to photooxidation of components of the photosynthetic machinery (e.g. pigments such as chlorophylls, etc.). Here, we summarise the research hitherto performed on UV-impacts on macroalgal photosynthesis with special reference to studies conducted in the Kongsfjorden area.

Mechanisms of UVB induced inhibition of photosynthesis

The process most intensively studied in relation to UV-effects on plant life is photosynthesis, as the light absorbing/converting apparatus will be clearly affected. There is evidence that UV-radiation (UVR) has a great impact on the photosynthetic apparatus and that the mechanisms of its impairment are manifold (see Sisson 1986; Bornman 1989; Vass 1997). The mechanisms how adverse UVB effects on photosynthesis are mediated are predominantly studied in higher, terrestrial plants and as well in macroalgae, but from areas usually exposed to higher irradiance than in the Arctic. Recent field studies on the green macroalga *Ulva rotundata*, conducted in warm-temperate regions, provided evidence for a UVB-induced inhibition of the xanthophyll cycle, which is regarded as a regulatory mechanism to harmlessly dissipate excessively absorbed energy as heat (Osmond 1994). The consequence of an impairment of this important regulatory and protective mechanism is an impaired ability of the alga for dynamic photoinhibition/photoprotection to adequately respond to excessive irradiances of photosynthetically active radiation (PAR) (Bischof et al. 2002b,c). This may result in an elevated production of reactive oxygen species (ROS), which are destructive to any cellular component (Foyer and Mullineaux 1994; see Fig. 1).

These results were obtained on an alga from warm-temperate regions, but it is highly probable that the proposed mechanism is a common feature of inhibition of photosynthesis under field conditions, and may thus be also applied to Arctic macroalgae.

In situ experiments in Kongsfjorden on the abundant intertidal species *Fucus distichus*, were conducted by covering specimens with different cut-off filters, thus absorbing radiation from the shorter wavelengths range. Here it is confirmed that beside UVR, high levels of PAR have strong negative effects on photosynthetic activity (Bischof et al. 2001). Specimens exposed to the natural PAR+UVR do not show a stronger inhibition than samples exposed to solar PAR only, but have a significantly slower recovery rate. Samples receiving only UVR deprived of PAR are not much affected. This shows the synergistic effects of PAR and UVR in the field. Photosynthesis in eulittoral algae is predominantly inhibited by high PAR while UVR is more likely to cause a delay in recovery. These results are confirmed by mesocosm studies conducted on various macroalgal species from Kongsfjorden (Hanelt et al. 1997a; Aguilera et al. 1999; Karsten et al. 2001) and indicate different mechanisms and target sites for PAR and UVR induced inhibition of photosynthesis. In addition, laboratory studies on the effects of UV-exposure of Arctic/cold-temperate macroalgae from Kongsfjorden revealed further mechanisms of inhibition of photosynthesis. Amongst others, the activity and concentration of RubisCO was significantly impaired, with the degree of inhibition and decomposition depending on the respective species (Bischof et al. 2000).

Acclimation and adaptation

In the context of potentially increasing irradiances of UVB on the earth´s surface, and consequently also in the water column, the aspect of acclimation becomes a central point of concern. Various studies on macroalgal acclimation patterns to UVB exposure have been performed at Kongsfjorden at different temporal scales.

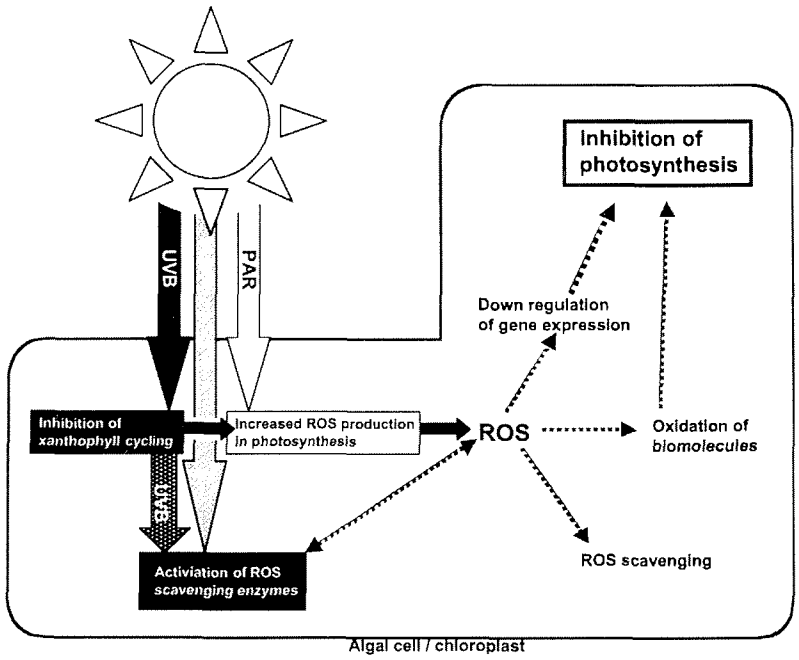

Figure 1: Conceptual model illustrating the interactive effects of different spectral ranges contributing to the inhibition of photosynthesis under field conditions (based on Bischof et al. 2002b,c, 2003): The UVB-range inhibits regulatory mechanisms such as the xanthophyll cycle, thus the alga is no longer able to respond to high irradiances of PAR by dynamic photoinhibition. Consequently, the production of reactive oxygen species in photosynthesis is elevated, resulting in photooxidation and/or down regulation of gene expression. UVA may contribute to inhibition of photosynthesis, but also activate enzymatic repair and protective mechanisms.

Adaptation: Adaptation implies the species-specific genetical prerequisites to cope with the respective environmental conditions. In a certain habitat it is best reflected by the vertical zonation pattern of macroalgal species at the shore. In all studies on marine macroalgae, there is common opinion that the sensitivity of photosynthesis to UVR is a function of vertical zonation of the species under examination (Larkum and Wood 1993; Dring et al. 1996a,b; Bischof et al. 1998a). Moreover, Maegawa et al. (1993) regard solar UVR as a major factor controlling the upper zonation limit of red macroalgae on the shore. Mesocosm experiments conducted at the shore of Kongsfjorden support these findings. Various macroalgal species were collected from growth sites at depths where the respective species exhibited highest coverage within Kongsfjorden and transferred to mesocosms where each specimen was exposed to the natural course of solar radiation at about 10 cm water depth. Exposure under different cut-off filters allowed discrimination of the inhibitory effects of both PAR and UVR. As algal species largely differed in terms of UV-susceptibility, they were exposed under different time duration. To account for the different doses received during the respective exposure periods (due to different times and weather conditions) an inhibition factor was defined by the ratio of the degree of inhibition and received dose during exposure. After exposure, all samples were transferred for 48 h of recovery in dim white light. In this experiment (see Fig. 2) species from shallow waters exhibited only minimal degrees of inhibition of photosynthesis, with hardly any additional contribution of the UV-range to PAR-induced inhibition. With

increasing growth depth, the degree of inhibition in the mesocosm experiment increased and, in contrast to the shallow water species, species from deeper waters show a pronounced impairment of photosynthesis due to UVR.

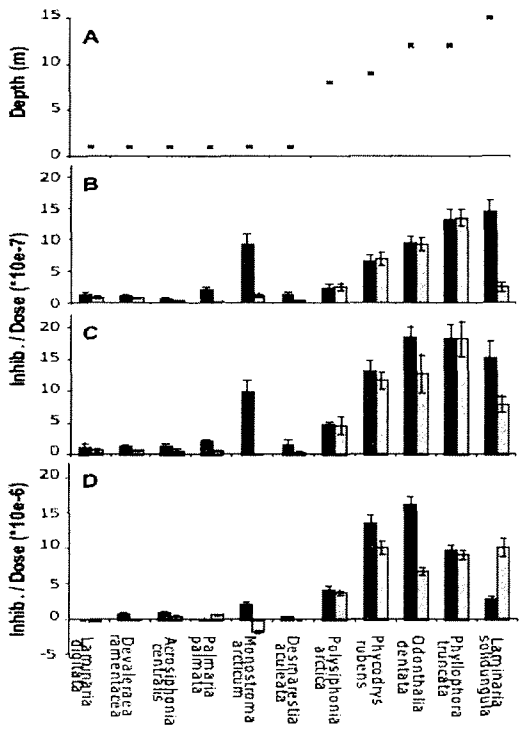

Figure 2: Collecting depth and degree of inhibition of optimal quantum yield of photosynthesis in algae exposed in mesocosm experiments. A: collecting depth; B: Inhibition induced by PAR; C: Inhibition induced by PAR and UVR; D: Inhibition induced by UVR. Black bars: Degree of inhibition at the end of experimental exposures; white bars: after 48h of recovery in dim white light. Algal samples were collected from typical growth sites within Kongsfjorden at their main distribution depth and then transplanted into mesocosms at 10 cm water depth and exposed to the natural course of solar radiation. To account for the different fluences received during exposure, an inhibition factor was defined by the ratio of the degree of inhibition and the respective dose received (Bischof et al. hitherto unpublished data).

The close relation between vertical zonation patterns of species and differences in UV-susceptibility also becomes visible when measuring daily cycles of photosynthetic performance in algae from different shore levels transplanted into mesocosm experiments (Hanelt et al. 1997a; Fig. 3). The degree of inhibition of photosynthesis induced by solar UVR and the rate of recovery from inhibition differed in the exposed specimens with respect to the shore depths that specimens usually occupy (Fig. 3). Algae with a generally lower position on the shore are more sensitive to the natural UVR, for instance, the red alga *Palmaria palmata* from the upper sublittoral zone (Hanelt et al. 1997a). By successively cutting off the shorter wavelength ranges, the degree of photoinhibition decreases and recovery commences earlier during the course of the day. In contrast to intertidal algae, UVR alone can cause photoinhibition to a similar degree as PAR, in species from the deep sublittoral zone, such as *Laminaria solidungula, Phycodrys rubens* and *Ptilota plumosa* (Hanelt unpublished). Similar results obtained from laboratory experiments on cultivated algae raised from stock cultures provide evidence that the differences in response are partly based on the genetically-fixed ability to cope with UV and high light-stress (Hanelt et al. 1997b;

Bischof et al. 1998a). During cultivation, specimens were exposed always to identical conditions so that short-term responses upon exposure can solely be based on genetically fixed adaptive mechanisms.

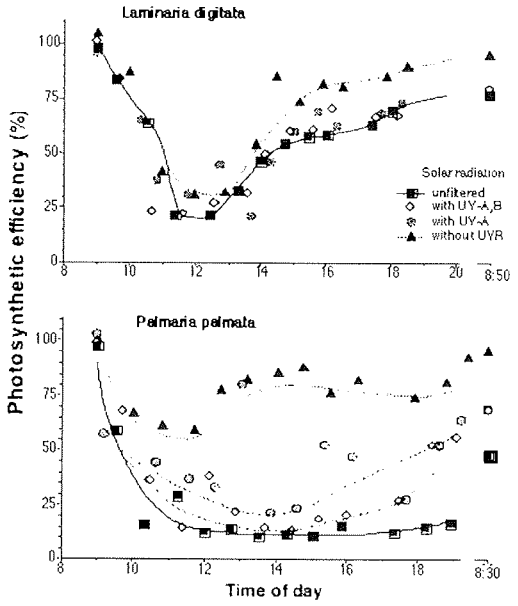

Figure 3: Daily cycles of photosynthetic quantum yield measured as variable chlorophyll fluorescence in two species from different shore levels exposed in mesocoms experiments. The blades of *Laminaria digitata* floating near the water surface show a minor impact of UVR on the daily pattern of photoinhibition of photosynthesis and a fast recovery in the afternoon. Most of photoinhibition was caused by high PAR levels. In contrast, the sublittoral red alga *Palmaria palmata* is strongly inhibited by the solar UV-radiation and recovery from photoinhibition occurs only very slowly in the afternoon. PAR (solar radiation without UVR) causes a minor depression of photosynthetic efficiency around noon (modified after Hanelt et al. 1997a).

Adaptation also sets the range for potential acclimation mechanisms, as can be easily demonstrated by the differential reactions of specimens collected along depth gradients (Bischof et al. 1998b). Species-dependent acclimation to radiation conditions was studied in three dominant brown algal species (*Alaria esculenta, Saccorhiza dermatodea, Laminaria digitata*) from Kongsfjorden. At their natural growth sites, specimens were collected along the depth gradient. Subsequent exposure to artificial UV-radiation revealed different growth-site specific acclimation potential, and thus acclimation to the respective *in situ* light climate. Photosynthetic acclimation was found for both ambient PAR and UVR. Exposure to artificial UVR reduces photosynthetic rates in deep-water plants significantly while photosynthesis in specimens collected from shallow waters is unaffected. Exposure to defined doses of artificial UV-radiation of specimens collected from different water depths and monitoring of inhibition of photosynthesis were applied as an easy test-system to demonstrate growth site-specific acclimation to UV-exposure (Bischof et al. 1998b). In Figure 4, the contribution of the different wavelength ranges of UVR to the inhibition of photosynthetic capacity (ETRmax) in specimens of *Laminaria saccharina* collected in different water depths is displayed in pairwise comparisons. Positive values represent the respective contribution to the overall inhibition of photosynthesis, while negative values indicate promoting effects. Together, UVA and UVB caused substantial inhibition of ETRmax values in accordance with the growth depth of specimens. In shallow water specimens, experimental UV

exposure resulted in elevated photosynthetic capacity, while algae collected from 14 m depth were mostly negatively affected. Severe inhibition due to UVA was only present in the specimens collected from 11 to 14 m depth to a similar degree. In samples from 6 to 11 m depth, additional UVB effects were small, especially after the recovery period.

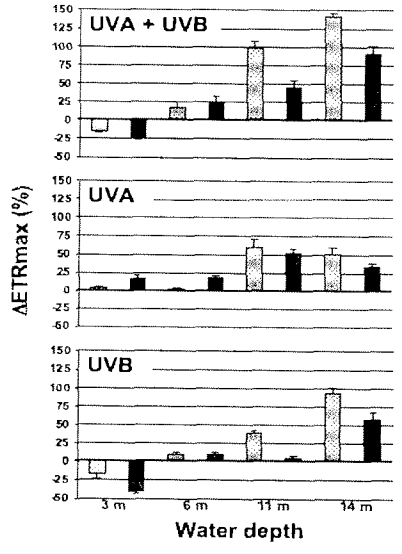

Figure 4: Contribution of the different wavelength ranges to the overall inhibition of photosynthetic capacity in *Laminaria saccharina* sampled along the depth gradient of Kongsfjorden and subsequently exposed to artificial UV-radiation for four hours (7 Wm^{-2} UVA and 0.7 Wm^{-2} UVB) followed by 24 h for recovery in dim white light. Values are expressed as the differences in maximal relative electron transport rates as measured by PAM-fluorescence and calculated as % of control values. Top: contribution of UVA + UVB (= difference in ETRmax between PAR and PAR + UVA + UVB); middle: contribution of UVA (= PAR - [PAR + UVA]); bottom: contribution of UVB (= [PAR + UVA] - [PAR + UVA + UVB]). Grey bars: after 4 h of exposure; black bars: after 24 h of recovery (modified after Bischof et al. 1998b).

Under conditions of high water transparency soon after the break-up of sea ice, the maximal 1%-depth of UVB inside Kongsfjorden does not exceed 13 m (Hanelt et al. 2001). Thus, specimens of *L. saccharina* are hardly exposed to harmful radiation. Algae growing at 11 m depth experience only slightly higher irradiances of UVB, but are significantly less sensitive to artificial UVB-exposure than algae from 14 m. The explanation for this striking difference in UV-sensitivity is still lacking. It seems likely that acclimation and protection mechanisms require a certain threshold irradiance to become activated. However, the molecular mechanisms behind them are still unknown and require intense research efforts.

Seasonal changes: Evidently various factors contribute to the individual sensitivity of algae towards solar radiation, e.g. growth depth (Sagert et al. 1997; Dring et al. 1996b; Bischof et al. 1998b), position within and below the canopy (Stengel and Dring 1998; Hanelt et al. 2003), life history stage, as well as the different parts and sizes of the thalli (Hanelt et al. 1997c; Karsten and Wiencke 1999). Seasonal aspects at an intermediate timescale contribute also to the sensitivity. These are acclimation of macroalgal physiology to seasonal changes in the *in situ* light climate, such as changes in pigment composition, the concentration of UV-absorbing compounds and the activity of reactive oxygen scavenging enzymes (Aguilera et al. 2002), which might result in a stepwise reduction of susceptibility of algal photosynthesis to UV-exposure (Bischof et al. 2002a). In the course of the season, the brown alga *Desmarestia aculeata* from Kongsfjorden typically exhibits a continuous decline in UV-sensitivity of photosynthesis, including both maximal quantum yield as well as maximal photosynthetic rates (Fig. 5). In this species, changes in UV-susceptibility might also be related to morphological changes of the alga: in spring to early summer,

D. aculeata exhibits tufts of delicate assimilatory filaments but its phenology changed later in the season to rather spine-like corticated branchlets (Fletcher 1987).

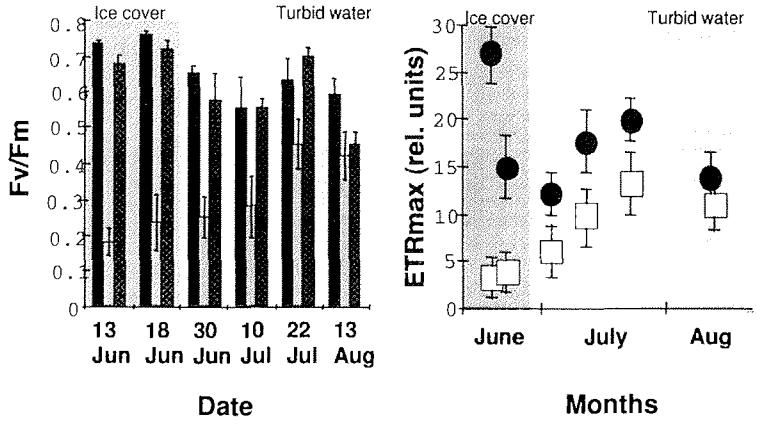

Figure 5: Seasonal response of *Desmarestia aculeata* to UV-radiation. Left: Maximal quantum yield of photosynthesis (Fv/Fm) as measured immediately after collection (black bars), after 2 h of artificial UV-exposure (open bars) and after 18 h of recovery under UV-exclusion (shaded bars); right: the respective maximal photosynthetic rate (ETRmax) before (filled circles) and after (open squares) UV-exposure (modified after Bischof et al. 2002a).

Similar findings were also derived from studies on two abundant red macroalgal species collected from Kongsfjorden in the course of the spring/summer season of 1998. In that year, the fjord was covered by sea ice for an unusually long time, specimens could be collected from under the ice, without them being exposed to neither high PAR nor to UVR prior to the experiment. In the following weeks, the influence of the different hydrographic conditions affecting radiation climate inside the water column could be studied, including sea ice and snow cover, sea ice break-up, open and clear water conditions, and inflow of turbid melt water. For the red algae *Devaleraea ramentacea* and *Palmaria palmata*, the results clearly show the increasing concentration of UV absorbing mycosporine-like amino acids (MAAs) and the high levels of superoxide scavenging superoxide dismutase (SOD) after the break-up of sea ice (Aguilera et al. 2002). These changes in the biochemical characteristics resulted in a reduced susceptibility of photosynthesis to UV-induced inhibition (Bischof et al. 2002c).

Short-term acclimation: After all, life in a highly variable environment, such as the coastal areas of polar regions, requires flexible responses. In the brown alga *Alaria esculenta* from Kongsfjorden, short-term acclimation of photosynthetic activity was studied by subjecting experimental specimens to repeated UV-exposures (Bischof et al. 1999; Fig. 6). The algae used in this experiment were collected under a persisting sea ice cover, thus specimens were not exposed to high irradiances of either PAR or UVR prior to the experimental treatments. Acclimation to changing radiation conditions occurred within a few days. This is of substantial ecological importance as these algae, shielded for six months during the Arctic winter and under sea ice and snow in spring, become suddenly exposed to high radiation as soon as sea ice breaks up. *A. esculenta* showed two different responses involved in the acclimation of photosynthesis. Firstly, after a few days of exposure to artificial UVR, the recovery from UV-induced inhibition of

photosynthesis proceeds faster. Later, the degree of inhibition decreases. The initial response might be due to changes in the activation state of repairing and protecting enzymes, e.g. those involved in photorepair (Karentz 1994) or in scavenging of reactive oxygen species (Lesser 1996). The subsequent reduction in UV-sensitivity in the inhibitory phase is likely to be related with the establishment of a physical barrier, shielding the photosynthetic apparatus from damaging radiation (Karentz 1994). In brown algae, the accumulation of phenolic compounds has been described as a potential mechanism of shielding against UVR (Pavia et al. 1997; Schoenwaelder 2002). For red algae from Kongsfjorden, a similar function has been attributed to the group of mycosporine-like amino acids (Karsten et al. 1999; Karsten and Wiencke 1999; Karsten and Hoyer: this issue). Apparently, there are different molecular mechanisms involved in acclimation of macroalgal photosynthesis, which still need to be revealed at the molecular level.

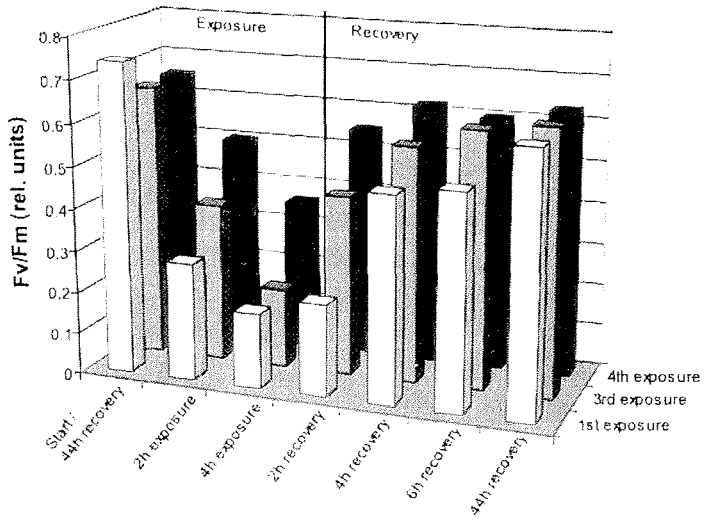

Figure 6: Changes in maximal quantum yield of photosynthesis (Fv/Fm) in the Arctic/cold-temperate brown alga *Alaria esculenta*, collected under the sea-ice and exposed to repeated exposure cycles of 60 µmol m^{-2} s^{-1} PAR, 8 W m^{-2} UVA and 0.8 W m^{-2} UVB, for 4 h and subsequent recovery in dim light for 44 h, for the 1st (white), 3rd (grey) and 4th (black) exposure cycle. Figure modified after Bischof et al. (1999).

Conclusions

The hitherto available information on UV-effects on photosynthesis of polar macroalgae is predominantly derived from studies conducted in Kongsfjorden area. Based on these data, a general concept of adaptive responses of macroalgal species towards UV-exposure can be outlined. Photosynthesis of

macroalgae from the intertidal zone is rather resistant to natural UVB radiation; inhibition of photosynthesis is predominantly evoked by high irradiances of solar PAR (see Table 1). Algae from the upper sublittoral are able to acclimate rapidly to fast changes of solar irradiance, consequently reducing the adverse effects of UVR exposure. Deep water algae lack the adaptive ability to acclimate to UVR and thus are highly sensitive to exposure (Bischof et al. 2000). However, these specimens are restricted to water depths, where harmful irradiances of UVR hardly penetrate the water column. Based on the data sets available on UV-effects on macroalgal photosynthesis, and extrapolating results obtained from Kongsfjorden area to other Arctic sites, we may conclude that ozone depletion will hardly affect the function and composition of Arctic ecosystems via a *direct* inhibition of macroalgal photosynthesis due to the high ability for acclimation. However, other studies conducted in Kongsfjorden revealed that parameters other than photosynthesis respond more sensitively upon UV exposure, such as zoospore viability (Wiencke et al. 2000; Clayton and Wiencke: this issue). Enhanced UVR may still exert adverse effects on the Arctic marine environment and the macroalgal community via other mechanisms apart from photosynthesis. In addition, the energetic costs of acclimation mechanisms are still unknown, and may indirectly contribute to an inhibition of primary productivity. More detailed research is needed on the level of communities and ecosystems, and also at the molecular level in order to describe the mechanisms behind acclimation, such as signal transduction pathways. Thus, UV research on Arctic macroalgae still represents a challenge in marine biology.

Table 1: Summary of results from transplantation experiments, indicating the relative contribution of the PAR and UV range to the overall inhibition of photosynthesis due to exposure to the whole solar spectrum (PAR + UVA + UVB). The respective difference in Fv/Fm values measured in initial controls and in samples exposed to the total wavelength range was set to 100%; see legend to Fig. 2 for experimental details.

Species	Inhibition by PAR (% of total inhibition by PAR + UVR)	Inhibition by UVR (% of total inhibition by PAR + UVR)	Sampling depth
Laminaria digitata	100	0	1 m
Devaleraea ramentacea	67	33	1 m
Acrosiphonia centralis	55	45	1 m
Palmaria palmata	96	4	1 m
Monostroma arcticum	86	14	1 m
Desmarestia aculeata	84	16	1 m
Polysiphonia arctica	46	54	7 m
Phycodrys rubens	48	52	9 m
Odonthalia dentata	48	52	12 m
Coccotylus truncatus	68	32	12 m

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UVB-induced DNA damage and its repair in marine macroalgae from Kongsfjorden (Svalbard)

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Introduction

Stratospheric ozone depletion has been detected over the Arctic when light returns to this region in early spring (WMO 1998, Dahlback 2002). Although the spatial and temporal scale of this phenomenon is not comparable to the Antarctic ozone hole, prospects of increasing ultraviolet-B radiation (UVBR, 280-315 nm) have raised concern over the potential impact on the productive Arctic marine ecosystem. Macroalgal vegetation that grows close to the water surface can experience high UVBR (Franklin and Forster 1997). UVBR is considered to be harmful because it causes direct damage to organic molecules such as DNA and indirectly increases the production of reactive oxygen species (Setlow et al. 1963, Rijstenbil et al. 2000). Approximately 70-90% of UVB induced DNA damage consists of cyclobutane pyrimidine dimers (Mitchell and Nairn 1989, Dany et al. 2001). These lesions interfere with processes that are crucial for cell function by obstructing DNA expression and replication (Sauerbier and Hercules 1978, Draper and Hays 2000). UV-induced DNA damage has also been linked to reduced growth and survival in marine algae (Poll et al. 2001, Buma et al. 2003). It has been found that ambient UVBR at the water surface can depress the growth of Arctic macroalgae, when compared to PAR exposed plants (Makarov 1999, Aguilera et al. 1999). Although algae have evolved various UV-tolerance mechanisms that allow them to survive harmful UVB effects, variable responses to UVB exposure have been observed among algal species (Karentz et al. 1991a, b, Poll et al. 2001). This variation is presumably caused by differences in the occurrence and effectiveness of their UVB-tolerance mechanisms. Some macroalgae produce polyphenolic compounds and mycosporine like amino acids, which absorb UV radiation and therefore provide protection for sensitive components (Cockell and Knowland 1999). In addition, algae deploy an array of enzymatic repair pathways that remove reactive oxygen species (Aguilera et al. 2002). To remove UVB-induced DNA damage, macroalgae appear to possess the same repair pathways as higher plants. Apart from versatile DNA repair pathways such as nucleotide excision repair, macroalgae probably use light dependent photolyase enzymes that specifically remove UVB-induced DNA damage (Pakker et al. 2000a, b). Although the production of UV-absorbing compounds has been studied in considerable detail, relatively little is known on the active repair of UVB-induced DNA damage in Arctic macroalgae. Low CPD repair activity could make these algae vulnerable to increases in UVBR. In this contribution, we describe experiments that were designed to determine macroalgal sensitivity to UVB-induced DNA damage. To this end, CPD accumulation and repair were tested in field collected Arctic macroalgae, whereas DNA dosimeters were used to assess macroalgal exposure to summertime UVBR.

Method

The experiments were performed in Ny-Ålesund, Svalbard (78° 55.5' N, 11° 56.0' E) in the summer of 2001. Experimental details were published elsewhere (Poll et al. 2002). In short, algae were obtained from Kongsfjorden between 6 and 13 m depth by SCUBA diving and kept overnight in running seawater under low irradiance. Algal fragments of *Laminaria saccharina* (L.) Lamour, *Phycodrys rubens* (L.) Batters, *Palmaria palmata* (L.) Kuntze, *Devaleraea ramentacea* (L.) Guiry, *Coccotylus truncatus* (P.) Wynne and Heine, *Odonthalia dentata* (L.) Lyndberg and *Monostroma arcticum* (Wittrock) were briefly exposed to high artificial UVBR for CPD induction (45 min, 2814 J m^{-2} weighted with Setlow's DNA damage action spectrum). Afterwards, CPD removal was monitored over time in the presence of PAR (14 W m^{-2}) and UVA (9 W m^{-2}). In addition, we tested if CPD accumulation was detectable under natural irradiance conditions. Small fragments of *P. rubens* and *P. palmata* were exposed to unfiltered sunlight just below the water surface for 4 h around noon, whereas PAR exposed fragments served as control. All samples were preserved on silica gel and analyzed for CPDs as described in Poll et al. (2001). Furthermore, DNA dosimeter (Boelen et al. 1999) exposures and spectro-radiometer derived UVB doses were correlated to estimate the maximal CPD concentration that would be induced after 24 h exposure of bare DNA in Svalbard. In addition, DNA dosimeters were repeatedly exposed for two days at several depths in Kongsfjorden to determine attenuation of the wavelengths that cause DNA damage.

Results and discussion

Most algae showed fast repair of accumulated CPDs when challenged for 45 minutes with a UVB dose that would require 8 days of sunshine at highest solar angle in Svalbard (Fig. 1). Around 20 to 75 minutes after CPD accumulation, repair was observed in *P. palmata*, *D. ramentacea*, *L. saccharina* and *P. rubens*, with ~10% of the CPDs remaining after 5 h.

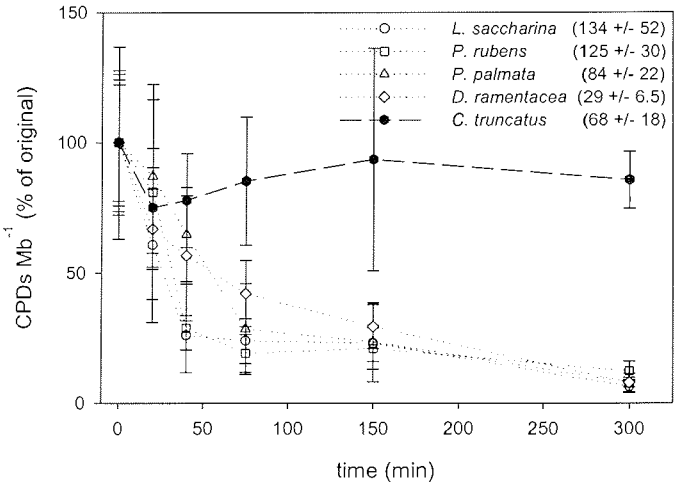

Figure 1. CPD concentrations (CPDs per Mb, 10^6 nucleotides) in *L. saccharina*, *P. rubens*, *P. palmata*, *D. ramentacea* and *C. truncatus* after 45 min of high UVBR, and 20, 40, 75, 150 and 300 min of recovery under PAR and UVAR. CPDs are expressed as a percentage of the initial concentration after 45 min UVBR. Initial CPD concentrations (+/- sd) are indicated in bracelets. Six replicates were analyzed for each species.

Despite the differences in accumulated CPD concentrations after the UVB treatment (ranging from 29 CPDs Mb^{-1} for *D. ramentacea* to 134 CPDs Mb^{-1} in *L. saccharina*, Fig. 1), no significant differences in repair rates could be observed between these species. The differences in CPD accumulation may originate from morphological features and the presence of UV-absorbing compounds, which influence the exposure of the algal DNA. Morphology may be the most important determinant in these experiments because the algae were collected from depths where they contain low concentrations of UV-absorbing compounds (Karsten et al. 1999, Karsten and Wiencke 1999). Experiments with *P. palmata* indicated that repair was light dependent, since no repair was observed in darkness (not shown).

In contrast, no efficient repair was observed after 5 h of recovery in *C. truncatus* (Fig. 1) when challenged by high CPD accumulation, whereas low repair rates were also found in *M. arcticum* and *O. dentata* (not shown). This may point to down regulation of CPD repair under the low (PAR) irradiance present at the collection depth, or to differences in the condition of the tested algae. In terrestrial plants, CPD repair in the form of photolyase activity is regulated by irradiance quality and quantity (Hada et al. 1999, 2001, Ries et al. 2000). Furthermore, photolyase levels differ between tissue types and appear to be adjusted to the concentration of UV-absorbing compounds (Waterworth et al. 2002, Hada et al. 2003). However, it is unknown to what extent Arctic macroalgae regulate CPD repair responses. Apparently, CPD repair was not linked to the distribution pattern of the tested algae as it was observed in deep water and upper subtidal species alike. Probably, the ability to repair UV-induced DNA damage is a feature that is shared by all macroalgae.

Despite the low summertime UVB irradiances, exposure to 4 h of sunlight at noon produced significant CPD accumulation in *P. rubens* (Fig. 2).

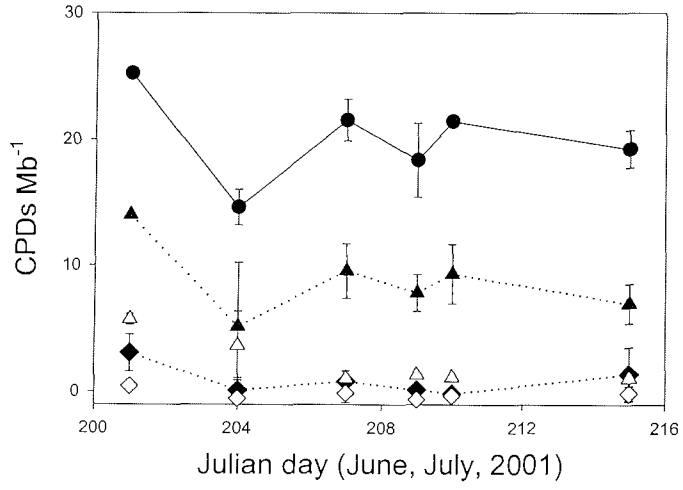

Figure 2. CPDs in DNA dosimeters (circles), *P. rubens* (triangles) and *P. palmata* (diamonds) after exposure to 4 h of sunlight around noon just below the water surface on six days in the summer of 2001. Open symbols show the CPDs in PAR exposed samples. Mean and standard deviation are shown for two dosimeters and six algal fragments.

For *P. rubens*, accumulated CPDs correlated positively with CPDs measured in simultaneously exposed DNA dosimeters (R^2 = 0.86). The rapid bleaching of red pigments under UV exposure and also UV exclusion treatments indicated that the high irradiance levels were extremely stressful for this alga. Consequently, stress caused by high PAR and UVAR may have interfered with the CPD repair response. Less CPD accumulation was observed in *P. palmata*, which can inhabit shallower habitats than *P. rubens*. Although exposure close to the water surface was unnatural for both algae, these experiments show that CPD accumulation is possible in the upper part of the water column in Svalbard.

By correlating dosimeter and spectro-radiometer data it was estimated that maximally ~ 53 CPDs Mb^{-1}can accumulate in bare DNA when exposed for 24 hours just below the water surface in Svalbard (406 J m^{-2}, weighted daily dose (Setlow), Björn and Murphy 1985, data not shown). This value is 20 times lower when compared to those of tropical regions, where up to 1000 CPDs Mb^{-1} can accumulate over the day (Regan et al 1992, Jeffrey et al. 1996, Boelen et al. 1999). The DNA damaging irradiances that reach high latitudinal regions like Svalbard are reduced by reflection and attenuation in the water column before they reach the macroalgal vegetation. Therefore, actual exposure of upper subtidal algae will strongly depend on the attenuation of UVBR in the water column. The DNA dosimeter incubations revealed that the 1% depths for DNA damage ranged between 4 and 8 m for the examined period (Table 1, Fig. 3).

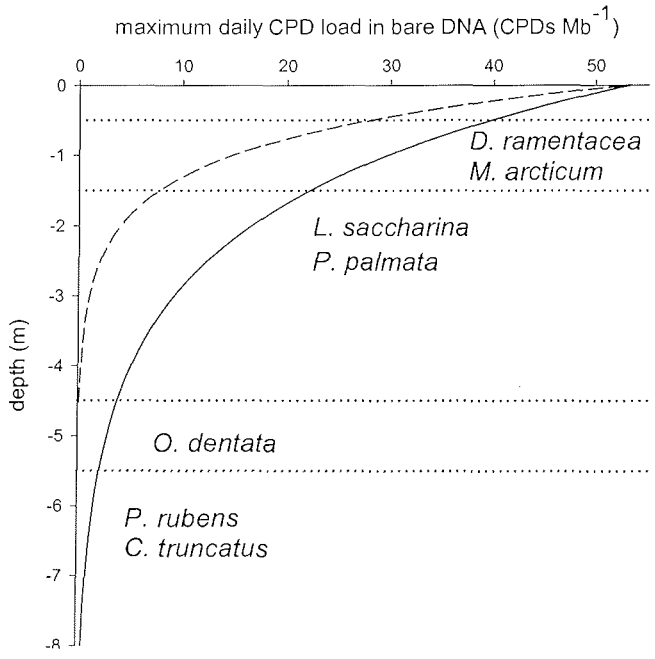

Figure 3. Attenuation of the maximum daily CPD load in DNA dosimeters in Kongsfjorden (Svalbard), calculated for the minimum (line) and maximum (dashed line) attenuation as derived from dosimeter incubations at several depths. The upper vertical distribution boundaries of several species that inhabit Kongsfjorden are indicated with horizontal dotted lines.

Table 1. Diffuse attenuation coefficients (K_d) and 1% depths for DNA damage in Kongsfjorden (Svalbard) calculated from dosimeter data for several time intervals in June and July (2001).

Date (2001)	K_d (m^{-1})	1% depth (m)
13-16 June	0.58	7.94
16-18 June	0.66	6.98
18-20 June	0.74	6.22
20-24 June	0.68	6.77
26-28 June	1.28	3.60
29 June-2 July	1.24	3.71
2-7 July	1.17	3.94

In Kongsfjorden, attenuation is strongly influenced by the influx of sediment rich melt water, as has been described previously (Hanelt et al. 2001). The attenuation of UVBR was much stronger than that found for the open ocean (Boelen et al. 1999). Nevertheless, the dosimeter data confirm that upper subtidal species such as *D. ramentacea* are potentially exposed to DNA damaging wavelengths in the upper part of their vertical distribution range in summer, whereas deep subtidal species like *P. rubens* never receive significant short wavelength radiation (Fig. 3). However, it is uncertain to what extent CPDs are actually formed in the DNA of upper subtidal species like *D. ramentacea*, *P. palmata* and *L. saccharina*, because they produce UV-absorbing compounds in response to the high irradiance present in shallow habitats that may protect their DNA (Karsten et al. 1999, Karsten and Wiencke 1999, Pavia et al. 1997). Consequently, upper subtidal algae have a much higher UV-screening capacity than their low light adapted equivalents that were used in these experiments. Thus, due to the presence of efficient repair and prevention mechanisms in the algae and the relatively low UVB irradiances in the water column, we consider UVB-induced DNA damage to be a minor stress factor for macroalgal vegetation in Kongsfjorden, Svalbard.

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UV-ABSORBING MYCOSPORINE-LIKE AMINO ACIDS IN MARINE MACROALGAE AND THEIR ROLE IN UV PROTECTION

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INTRODUCTION

Compared to the „ozone hole" over Antarctica which has been known since the 70's, the increase in ozone depletion over the Arctic represents a more recent phenomenon (Rex et al. 2000; Hanelt et al. 2001). As a consequence of ozone springtime reduction in the polar regions the UVB-radiation waveband (280-320 nm) markedly rises. Although the ecological consequences of changes towards higher doses of UV-radiation in marine ecosystems are not fully understood, many phototrophic organisms living in the intertidal as well as in the upper subtidal zone of the coasts are strongly affected (Franklin and Forster 1997).

UV-B wavelengths are predominantely absorbed by nucleic acids and proteins causing photodamage and conformational changes that can subsequently disturb vital metabolic functions such as transcription, DNA replication and translation (Buma et al. 1997). The cellular and physiological consequences of damage to these biomolecules can be manifold resulting in negative effects on cell division and reproduction, inhibition of photosynthesis and growth, and, finally, death (Franklin and Forster 1997; Wiencke et al. 2000). Of major interest is the identification of repair and/or protective mechanisms that allow phototrophic organisms living in high-radiation habitats to survive and reproduce.

An important physiochemical mechanism against biologically harmful UV-radiation involves the biosynthesis and accumulation of photoprotective sunscreens. Typically absorbing in the UVA (320-400 nm) and UVB, these compounds were invoked to function as passive shielding substances by dissipating the absorbed radiation energy in form of harmless heat without

generating photochemical reactions (Bandaranayake 1998). The most common photoprotective substances in marine organisms are the mycosporine-like amino acids (MAAs), a suite of chemically closely related, water-soluble compounds. MAAs have been identified in taxonomically diverse marine organisms including bacteria, cyanobacteria, micro- and macroalgae, invertebrates and fish (Dunlap and Shick 1998). Their function as intracellular passive screening agents has been inferred from UV-induced delays in the first division of sea urchin embryos having low concentrations of MAAs compared to embryos with high MAA contents (Adams and Shick 1996). In addition, marine phototrophic microorganisms that are capable to synthesise and accumulate MAAs under UV-exposure exhibit relatively UV-insensitive growth and photosynthesis compared to cells lacking MAAs (Garcia-Pichel et al. 1993, Neale et al. 1998).

Although MAAs are widely present in various types of marine organisms, few data exist of their type and quantity, as well as of their physiology and biochemistry in macroalgae (Nakamura et al. 1982, Karentz et al. 1991, Karsten et al. 1998b,c), in particular from polar waters. Isolates of the red macroalgal species *Devaleraea ramentacea* and *Palmaria palmata* from the Arctic Kongsfjorden are capable to acclimate to variable and enhanced radiation conditions by the synthesis and accumulation of MAAs (Karsten and Wiencke 1999, Karsten et al. 1999). However, in terms of sensitivity of photosynthesis under UV-stress, *D. ramentacea* is more resistant than *P. palmata* (Karsten et al. 2003), which seems to be related to the vertical zonation on the shore. At the study site in Kongsfjorden, *D. ramentacea* preferentially grows in depths from 0.2 m down to 7-8 m, while *P. palmata* inhabits the upper subtidal zone from 2 m down to 8-10 m. While for many Antarctic red algae increasing MAA contents with decreasing depths are well documented (Hoyer et al. 2001), much less is known for Arctic species.

Therefore, the aim of the present investigation was the evaluation of quantitative MAA inventories in macroalgae of Kongsfjorden collected from well defined depths. Although macroalgae are ecologically important in coastal primary production serving as a food source for herbivores and detritivores, as well as a habitat and nursery area for juvenile invertebrates and fish, many gaps

in knowledge still exist on how these plants are currently affected by UV radiation, and whether and how they can acclimate to enhanced ambient doses.

MATERIAL AND METHODS

Plant material

The locations and respective depths of collection of the macroalgal species studied are listed in Table 1. All plants were sampled directly from the shore as attached material by SCUBA diving. Afterwards macroalgae were oven-dried at 50°C, and then stored in sealed plastic bags under cool, dry and dark conditions until analysis.

MAA extraction and analysis

Samples of about 10-20 mg dry weight (DW) were extracted for 1.5-2 h in screw-capped centrifuge vials filled with 1 mL 25% aqueous methanol (v/v) and incubated in a waterbath at 45°C. After centrifugation at 5000 g for 5 min, 800 µL of the supernatants were evaporated to dryness under vacuum (Speed Vac Concentrator SVC 100H). Dried extracts were re-dissolved in 800 µL 100% methanol and vortexed for 30 s. After passing through a 0.2 µm membrane filter, samples were analysed with a Waters HPLC system. MAAs were separated on a stainless-steel Phenomenex Sphereclone RP-8 column (5 µm, 250 x 4 mm I.D.) protected with a RP-8 guard cartridge (20 x 4 mm I.D.). The mobile phase was 25% aqueous methanol (v/v) plus 0.1% acetic acid (v/v) in water, run isocratically at a flow rate of 0.7 ml min^{-1}. MAAs were detected at 330 nm and absorption spectra (290-400 nm) were recorded each second directly on the HPLC-separated peaks. Identification was done by spectra, retention time and by co-chromatography with standards extracted from the marine red macroalgae *Chondrus crispus* (Karsten et al. 1998a), *Mastocarpus stellatus* and *Porphyra umbilicalis*, which originated from the rocky island Helgoland, North Sea, Germany. Quantification was made using the molar extinction coefficients given in Karsten et al. (1998c). All amounts are given as concentration on a dry weight basis (n=4).

RESULTS

In this study 4 green, 12 brown and 11 red macroalgae which are typical members of the macrophytobenthos of Kongsfjorden were chemically profiled for their MAA inventories. MAAs were extracted from the dried macroalgal samples collected in Kongsfjorden at well defined depths, characterised by HPLC, and identified and quantified based on their retention times, absorption spectra, co-chromatography with standards and molar extinction coefficients (Karsten et al. 1998 b,c). Except *Prasiola crispa* traces or no MAAs were found in most Chlorophyta (Tab. 1).

Table 1 Collecting location, depth and presence (+) (>0.1 mg/g dry weight) or absence (-) of mycosporine-like amino acids (MAAs) in the investigated macroalgal species from the Arctic Kongsfjorden. t: trace (>0.01, <0.1 mg MAAs/g dry weight).

Species investigated	Collecting location	Depth (m)	MAAs
Chlorophyta			
Acrosiphonia arcta (Dillwyn) J.Agardh	Hansneset	0 - 2	t
Chaetomorpha melagonium (F.Weber *et* Mohr) Kütz.	Harbour	6 - 8	-
Monostroma arcticum Wittrock	Brandal	4 - 6	-
Prasiola crispa (Lightfoot) Kütz.	aeroterrestrial underneath seagull colony	+ 5 - + 2	+
Phaeophyta			
Alaria esculenta (L.) Grev.	Hansneset	3 - 5	-
Chorda filum (L.) Stackh.	Hansneset	1 - 3	-
Chorda tomentosa Lyngb.	Hansneset	1 - 3	+
Chordaria flagelliformis (O.F.Müll.) C.Agardh	Brandal	1 - 3	-
Desmarestia aculeata (L.) Lamour.	Brandal	4 - 6	-
Dictyosiphon foeniculaceus (Huds.) Grev.	Brandal	2 - 4	t
Fucus distichus L.	Hansneset	0 - 2	-
Laminaria digitata (Huds.) Lamour.	Hansneset	1 - 3	-
Laminaria saccharina (L.) Lamour.	Brandal	3 - 5	-
Laminaria solidungula J. Agardh	London	12 - 15	-
Pylaiella littoralis (L.) Kjellm.	Epiphytic on *L. digitata*	0 - 2	+
Saccorhiza dermatodea (de la Pylaie) J.Agardh	Brandal	9 - 12	-
Rhodophyta			
Coccotylus truncatus (Pall.) M.J.Wynne & J.N.Heine	London	5 - 7	-
Devaleraea ramentacea (L.) Guiry	Hansneset	1 - 2, 6 - 7	+
Odonthalia dentata (L.) Lyngb.	Hansneset	6 - 8	-
Palmaria palmata (L.) O.Kuntze	Brandal	2 - 3, 7 - 8	+
Phycodrys rubens (L.) Batters	Hansneset	10 - 12	-
Polysiphonia arctica J. Agardh	London	3 - 4, 7 - 8	+
Porphyra spec.	Hansneset	8 - 10	+
Ptilota gunneri P.C.Silva, C.A.Maggs & L.M.Irvine	Hansneset	10 - 12	t
Ptilota serrata Kütz.	Hansneset	10 - 12	-
Rhodomela confervoides (Huds.) P.C.Silva	Hansneset	6 - 8	+
crustaceous deep water species	Hansneset	15 - 18	-

Within the Phaeophyta only 3 species exhibited MAAs in trace or low concentration, while the remaining taxa lack these compounds. In contrast to the green and brown macroalgae, most Rhodophyta contained high MAA contents (Table 1). In total 10 different MAAs could be detected within all samples investigated, of which seven were identified as mycosporine-glycine, shinorine, porphyra-334, palythine, asterina-330, palythinol and palythene (data not shown).

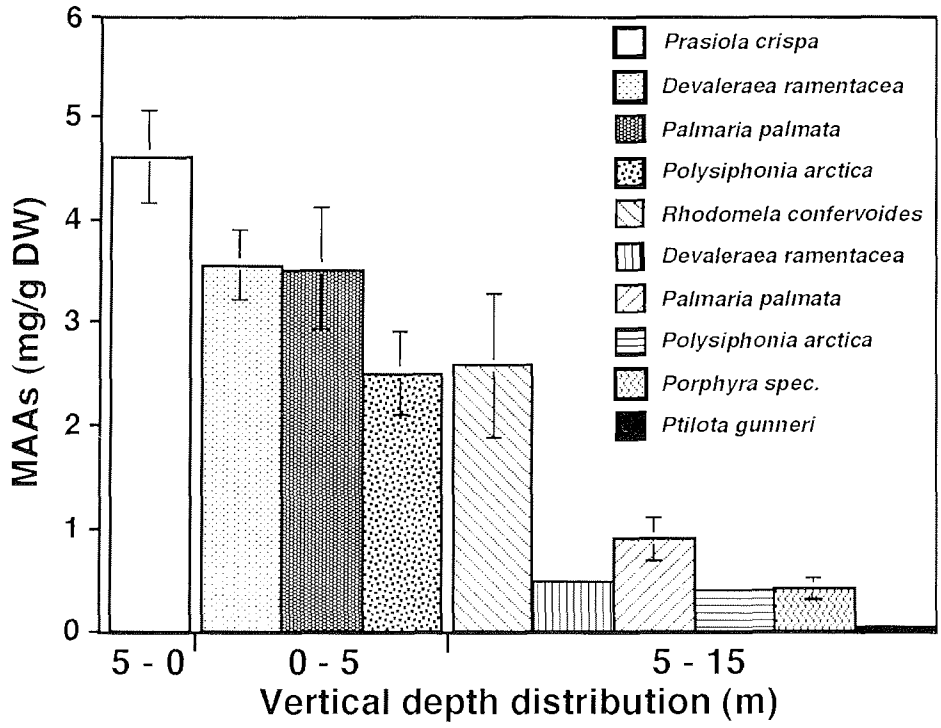

Figure 1 Total concentration of MAAs in macroalgae at different depths in Kongsfjorden. Given are the mean values ± SD (n=4).

Two unknown UV-absorbing compounds were found in some red algal species such as *Palmaria palmata* and *Polysiphonia arctica* exhibiting different retention times but identical absorption spectra peaking at 357 nm. It is possible that one of these compounds represents usujirene, the cis-isomer of palythene, which is characterised by an absorption maximum at 357 nm. Although Sekikawa et al. (1986) reported the occurrence of usujirene in *P. palmata*, without the availability of a usujirene standard the experimental verification in some red

algae from Kongsfjorden is not possible. In the green alga *P. crispa* a high concentration of a chemically not-identified UV-absorbing substance could be detected that exhibited identical features (retention time, absorption spectrum) as reported for *Prasiola crispa* ssp. *antarctica* from Antarctica (Hoyer et al. 2001) (data not shown).

The total MAA concentration in macroalgae from Kongsfjorden is strongly dependent on the vertical depth zonation (Figure 1). The highest MAA contents were detected in the aeroterrestrially living green alga *P. crispa* (>4.6 mg/g DW). While red algal species inhabiting the eulittoral and upper sublittoral zone between 0 and 5 m depth also exhibited high amounts of MAAs (2.5-3.5 mg/g DW), in Rhodophyta from the lower sublittoral zone between 5 and 15 m depth much smaller values were measured (0.1-2.5 mg/g DW) (Figure 1). This is particularly well documented for *Devaleraea ramentacea, P. palmata* and *P. arctica* in which isolates of the same species were collected from populations in shallow and deeper waters.

DISCUSSION

In contrast to brown and green macroalgae, UV-absorbing substances have been widely observed in many species of the Rhodophyta (Hoyer et al. 2001; Karsten et al. 1998 a,b,c). In the present study, the MAA concentrations measured in typical shallow water species such as *Devaleraea ramentacea* are approximately >7-fold higher compared to deeper sublittoral species such as *Porphyra* spec. However, it should be mentioned that the vertical distribution of the *Porphyra* species from Kongsfjorden is atypical for the genus, because most members of this group are living in the intertidal zone of all continents where they experience strong insolation, and consequently synthesise and accumulate very high MAA contents. In contrast, many species growing in the deep sublittoral such as *Phycodrys rubens* are physiologically not capable to synthesise MAAs, which well explains a strong degree of photoinhibition or even photobleaching after transplantation to shallow water followed by exposure to ambient solar radiation (Karsten et al. 2001). This is in good agreement with reports on Rhodophyta from Antarctica which indicate that species from deeper water also exhibit trace amounts only or even completely lack MAAs (Hoyer et al. 2001).

The presence of increasing MAA concentrations with decreasing depth in macroalgae has already been documented for a few species (Franklin et al. 1999, Hoyer et al. 2001, Karsten et al. 1999). These observations are also valid for the Rhodophyta of Kongsfjorden in which isolates of the same taxa were collected from different depths and the MAA amounts comparatively analysed. All data presented strongly support the hypothesis that MAAs are formed as sunscreen compounds in response to a more stressful situation in shallow waters where plants are exposed to increasing UV-B and higher PAR. Although UV-B penetration into the water column is strongly attenuated with depth, and is also highly variable depending on weather conditions, seasonality and inherent optical properties of the water body, the 1% depth for this waveband can reach about 10 m (Hanelt et al. 2001), and consequently affect macroalgae inhabiting shallow waters. In contrast, sublittoral macroalgae are generally never exposed to high irradiances including UV, at least not for long periods, and hence there is no physiological need to synthesise and accumulate metabolically expensive MAAs. This in turn would save energy to better support other essential pathways such as, for example, light-harvesting phycobilisomes to guarantee sufficient PAR absorption under rather dim-light conditions.

However, besides the stimulating effect of increasing solar radiation on the biosynthesis and accumulation of MAAs in macroalgae other environmental factors may also act as controlling variable. Particularly temperature (Hoyer et al., unpublished data) and nutrient availability (Korbee Peinado et al., personal communication) have been experimentally proven to influence the MAA concentration of macroalgae.

In recent studies, the photobiological function of MAAs as a cellular defense system against the harmful effects of UV-radiation on growth, photosynthesis and other processes has been reported for various marine phototrophic organisms (Garcia-Pichel et al. 1993, Dunlap and Shick 1998, Neale et al. 1998). Ishikura et al. (1997) measured maximum MAA concentrations in the outermost surface layer of the siphonal mantle of the giant clam *Tridacna crocea*. The occurrence of MAAs in the animal tissue prevented an inhibition of photosynthesis of its zooxanthellae *Symbiodinium* sp., which outside the protecting animal tissue exhibited strong photoinhibition under UV radiation. These authors calculated that the sunscreen capacity of the measured MAAs

were sufficient to absorb 87% of 310-nm radiation and 90% of 320-nm radiation before reaching 0.2 mm depth in the siphonal mantle.

Although Chlorophyta generally lack UV-absorbing substances and MAAs (Hoyer et al. 2001, Karsten et al. 1998b,c), there are some biochemical exceptions such as the warm-temperate *Dasycladus vermicularis* from the Mediterranean Sea which synthesises and excretes UV-absorbing coumarins in the surrounding medium (Pérez-Rodríguez et al. 1998), as well as *Prasiola crispa* ssp. *antarctica* containing high concentrations of an unknown UV-absorbing compound with an absorption maximum at 324 nm (Hoyer et al. 2001). *Prasiola crispa* from Kongsfjorden exhibited exactly the same 324 nm absorbing molecule which due to the chromatographic properties and the shape of the absorption spectrum most probably represents a MAA or MAA-like compound. This is in agreement with data on the closely related *Prasiola stipitata* from Helgoland (Gröninger and Häder 2002), which exhibited a wavelength-dependent induction of a 324 nm UV-absorbing compound. Although until now the chemical structure of this 324 nm substance is still not elucidated these authors also assume a putative MAA. Members of the genus *Prasiola* often grow as aeroterrestrial algae in the supralittoral zone or even several meters above normal seawater level underneath bird cliffs and in pinguin-rockeries where they take advantage from nitrogeneous excrements (Hoyer et al. 2001). Here they are exposed to strong gradients of all environmental factors including UV radiation. The accumulation of high concentrations of this unknown 324 nm substance can be interpreted as photoprotective strategy against UV stress.

Similar to the Chlorophyta, most Phaeophyta do not contain MAAs. Instead, these plants typically synthesise phlorotannins under UV exposure, which strongly absorb UV radiation between 280 and 320 nm, and hence are regarded as photoprotective substances (Pavia et al. 1997). The traces or low concentrations of MAAs found in some Chlorophyta and Phaeophyta can be related to epiphytic diatoms (stalked taxa such *Licmophora* sp., *Gomphonema* sp.) and filamentous cyanobacteria (*Phormidium* sp.) that often occur in high cell numbers on macroalgal species of Kongsfjorden (Karsten, unpublished results). Many diatom and cyanobacterial taxa are known to synthesise and accumulate MAAs (Dunlap and Shick 1998).

In conclusion, the Arctic macroalgae studied, particularly from shallow waters, seem to be well adapted to the prevailling UV conditions in Kongsfjorden through their biochemical capability to synthesise and accumulate MAAs.

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Effects of H_2O_2 on the green macroalga *Chaetomorpha linum* (Müller) Kützing from Spitsbergen

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Introduction

Oxygen is essential for the metabolism of aerobic organisms, however, its participation in cellular metabolism results in the appearance of toxic reactive oxygen species (ROS): superoxide anion radicals ($O_2^{\bullet-}$), hydroxyl radicals ($OH^{\bullet}$), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2). Production of ROS occurs especially under various stress conditions as, for example, during exposure to excessive light or UV radiation (McKersie and Lesham, 1994; Collen and Davison, 2001). If accumulation of ROS exceeds the capacity of the protective systems, lipids, proteins and nucleic acids are destroyed leading to damage of the photosynthetic apparatus and finally to cell death (Asada and Takahashi, 1987; Halliwell and Gutteridge, 1989).
ROS except hydrogen peroxide are characterized by a short lifetime and interact rapidly with water or cellular components (Asada, 1994). Hydrogen peroxide itself is not particularly reactive with most biologically important molecules, but is a precursor for more reactive oxidants in different cell components through its ability to pass quickly through membranes.
Therefore cellular protection mechanisms are essential (Asada and Takahashi, 1987). Enzymatic detoxifying systems and antioxidants of different chemical groups are known in higher plants to diminish oxidative stress by elimination and reduction of ROS to less toxic and less reactive products (Pedersen et al., 1996). Common powerful detoxifying systems are the enzymes superoxide dimutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR), as well as phenolic compounds such as flavonoids, coumarins and tocopherols, nitrogen containing compounds including alkaloids, chlorophyll derivates, amino acids and amines and other compounds such as carotenoids, ascorbic acid, glutathione and uric acid (Fujimoto et al., 1985; Larson, 1988; Potterat, 1997). In marine algae ascorbic acid, β-carotene and α-tocopherol are well described antioxidants (Aguilera et al., 2002b; Castillo et al., 1986; Collen and Davison, 1999b; 1985; Potterat, 1997). In addition, the presence of enzymatic defense systems was recently reported in marine macroalgae (Aguilera et al., 2002a, b; Aguilera this issue; Potterat, 1997) with particular emphasis on photooxidative stress and discussed in respect to the respective habitat. However, data on further properties of these antioxidants in Arctic species, particularly under direct H_2O_2 stress, are missing.
In aquatic environments, H_2O_2 predominantly derives from UV-driven photoactivation of dissolved organic material (DOM) (Cooper and Zika, 1983;

Zika et al., 1985). H_2O_2 concentrations in seawater normally range between 20 and 300nM (Pamatmat, 1990; Zika et al., 1985). However, during low tide in summer, H_2O_2 was found to accumulate to micromolar (up to 5 µmol L^{-1}) concentrations in shallow intertidal pools on the German Wadden Sea coast (Abele-Oeschger et al., 1997). But also in polar regions high H_2O_2 concentrations in surface or tidal pool water up to 2µmol L^{-1} were measured in Antarctica (Abele et al., 1998; 1999), deriving here from wet deposition in form of snow, wherein H_2O_2 levels amounted to 13µmol L^{-1} (Abele et al., 1999). The stratospheric ozone depletion and the resulting increase in UVB radiation lead to an enhanced photochemical H_2O_2 production. A 10 % ozone reduction leads to a doubling of UVB surface irradiance at 300 nm, which entails a 40% increase of the apparent intertidal H_2O_2 concentrations (Abele-Oeschger et al., 1997).
The aim of the present study was to investigate the antioxidative reaction patterns of the green macroalga *Chaetomorpha linum* from Kongsfjorden, a species characterised by a high ascorbic acid content. Over a period of one week *Chaetomorpha linum* was incubated in seawater enriched with a series of ascending H_2O_2 concentrations. Extremely high concentrations (up to 2 mM H_2O_2) were used to investigate the tolerance range of this species to high H_2O_2 stress, as natural H_2O_2 concentrations in seawater were tolerated without problems. As control for the physiological status of the alga under stress photosynthetic efficiency was measured.

Material and Methods

Algal material and study site

Algal material was collected by SCUBA diving in summer 1999 and 2000 at the study site in Kongsfjorden (Ny Ålesund, Spitsbergen, Norway 78°55,5'N; 11°56,0'E). After sampling, the algal material was kept under dim white fluorescent lamps (Philips) and a fluence rate of 25µmol m^{-2} s^{-1} at 3-5°C for at least 24 hours in running seawater pumped directly from the fjord.
Algal thalli with a fresh weight of ca. 4g were incubated at a temperature of 5°C in 1-2 L glass vessels containing pure seawater (control) or seawater enriched with 0.5 to 2 mM H_2O_2. Media were changed daily to keep H_2O_2 concentration constantly high. The algae were illuminated with white fluorescent lamps covered with neutral grey filter foil to reduce photosynthetically active radiation to 25 µmol m^{-2} s^{-1}. Samples were taken and deep frozen in liquid nitrogen, lyophilizied and subsequently stored at -30°C until analysis.

Photosynthesis

Photosynthetic efficiency was determined as ratio of variable to maximum chlorophyll-fluorescence (Fv/Fm) of photosystem II (PSII) after 5-10 min dark-adaptation with leaf clip using a portable pulse amplitude modulated fluorometer (Diving-PAM, Walz, Effeltrich, Germany). F_v/F_m values of all algae acclimated for 24 h to dim light conditions in the laboratory were characteristic for photosynthetically non-inhibited algae and consequently set to 100% (=control). All data recorded are expressed in relation to this value.

Activities of antioxidative enzymes

Samples (0.010-0.012 g DW) were ground in liquid nitrogen and extracted with 1-1.5 mL 50 mM potassium phosphate buffer (pH 7.0) containing Complete protease inhibitor cocktail (Boehringer, Mannheim, 2 tablets in 100 mL buffer). Extracts were centrifuged for 15 min at 15,000 r.p.m. at 4° C. Enzymes were analysed in the supernatant according to Aebi (1984) for CAT, Chen and Asada (1989) for APX, Goldberg and Spooner (1983) for GR and Mc Cord and Fridovich (1969) for SOD as described by Aguilera et al. (2002b). The method was modified for use of a microtiterplate spectrophotometer (Spectramax, Molecular Devices, Sunnyvale CA94089, USA) as described in Dummermuth et al. 2003. Ascorbic acid was measured according to Foyer et al. (1983) and adapted for use in the microtiterplate spectrophotometer as described by Dummermuth et al. (2003). Total soluble protein (TSP) content in crude extracts was determined using a commercial Protein Assay (BioRad, Germany).

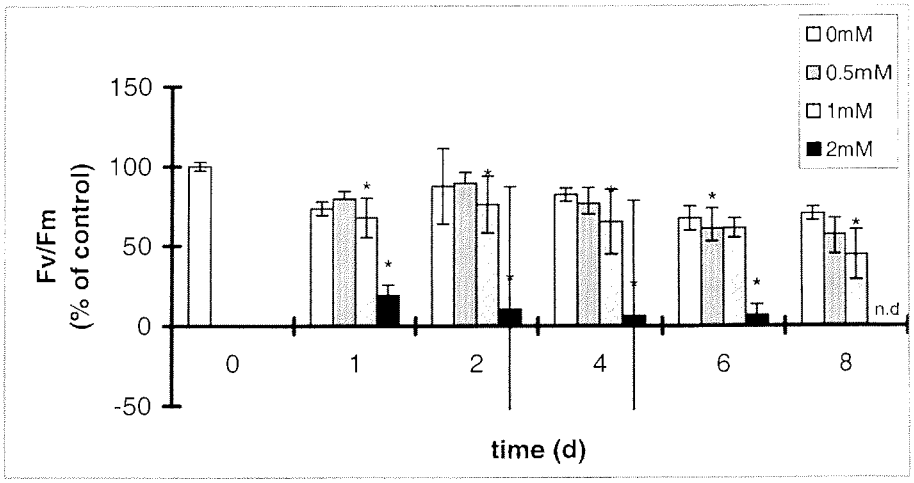

Fig. 1: Photosynthetic efficiency (Fv/Fm) mean values ± SD of *Chaetomorpha linum* exposed for up to 8 days to a series of ascending hydrogen peroxide concentrations, n=12. Mean values marked with * significantly differ to the preceeding lower H_2O_2 concentration. n.d. not detectabel Fv/Fm value after 8 days in 2 mM H_2O_2.

Results

The photosynthetic efficiency of *Chaetomorpha linum* decreased under increasing H_2O_2 concentrations. Exposure to 0.5 mM H_2O_2 was generally tolerated. Fv/Fm decreased slightly when exposed to 1mM H_2O_2 (Fig. 1). After 6 days of exposure Fv/Fm values of 67% for thalli exposed to pure seawater and 61% and 44% of the control for thalli exposed to 0.5 and 1 mM H_2O_2 were recorded. Fv/Fm was further reduced to 57% and 44% in thalli exposed to 0.5 and 1 mM H_2O_2 after 8 days of exposure (Fig. 1). However, a H_2O_2 concentration of 2 mM resulted in a drastic reduction of Fv/Fm to below 20 % of the control after 24 h of exposure and to below 10 % after 2 days (Fig. 1). After 4 days of exposure in 2 mM H_2O_2 the thalli of *C. linum* started to bleach, concomitantly the alga exhibited a much softer consistence. After 8 days under such extreme H_2O_2 concentrations no photosynthetic activity could be recorded (Fig.1).

Enzyme activities showed different reaction patterns. SOD activity decreased strongly in the first 24 h of exposure under all conditions. After two days of

exposure a certain recovery or acclimation could be observed up to concentrations of 1 mM, which was complete after 4 days. In contrast, exposure to 2 mM H_2O_2 was accompanied by a total loss of activity after two days (Fig. 2). APX activity rose after 6 days significantly from originally 0.54 U mg^{-1} TSP to 1.6 U mg^{-1} TSP and 2.2 U mg^{-1} TSP when exposed to 1 and 2 mM H_2O_2, respectively (Fig. 3). However, longer exposure times (Fig. 3) and higher H_2O_2 concentrations led to increasing bleaching and loss of activity (data not shown). CAT activity increased with rising H_2O_2 stress after 6 days from 0.77 to 1.52 U mg^{-1} TSP in 2 mM H_2O_2, showing a similar pattern as APX but with much lower activities (Fig. 4). GR activity showed an unclear pattern with very low values between 0.005 and 0.05 U mg TSP $^{-1}$.

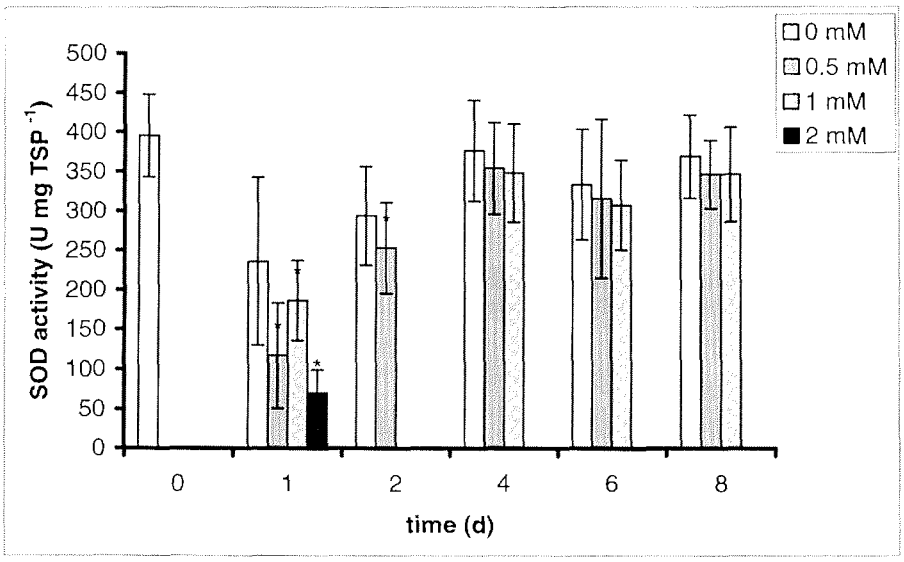

Fig. 2: Superoxide dismutase activity in *Chaetomorpha linum* (U mg TSP^{-1}) exposed for up to 8 days to a series of ascending H_2O_2-concentrations (0-2mM), mean values ± SD, n=5. * as in Fig. 1

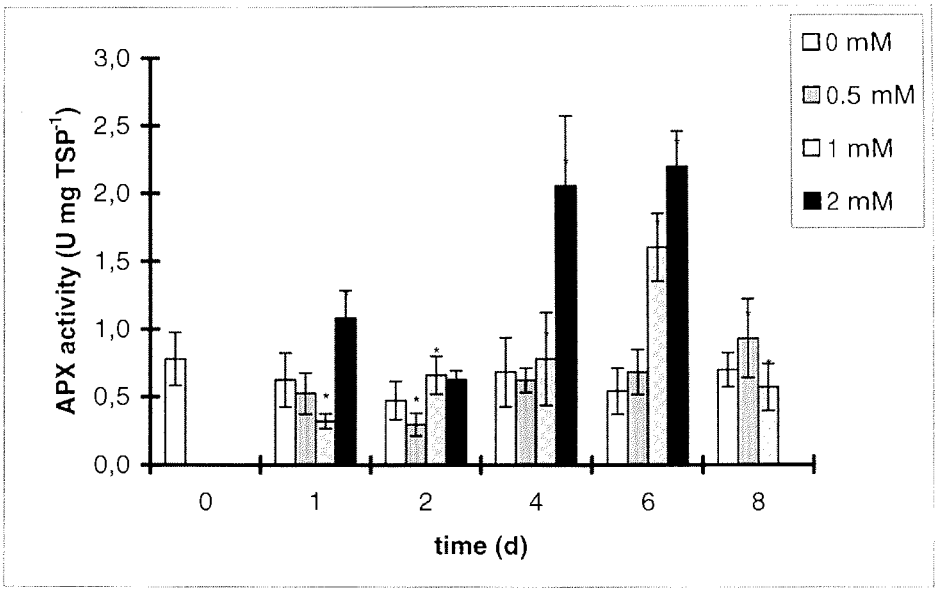

Fig. 3: Ascorbate peroxidase activity in *Chaetomorpha linum* (U mg TSP^{-1}) exposed for up to 8 days to a series of ascending H_2O_2-concentrations (0-2mM), mean values ± SD, n=5. * as in Fig. 1

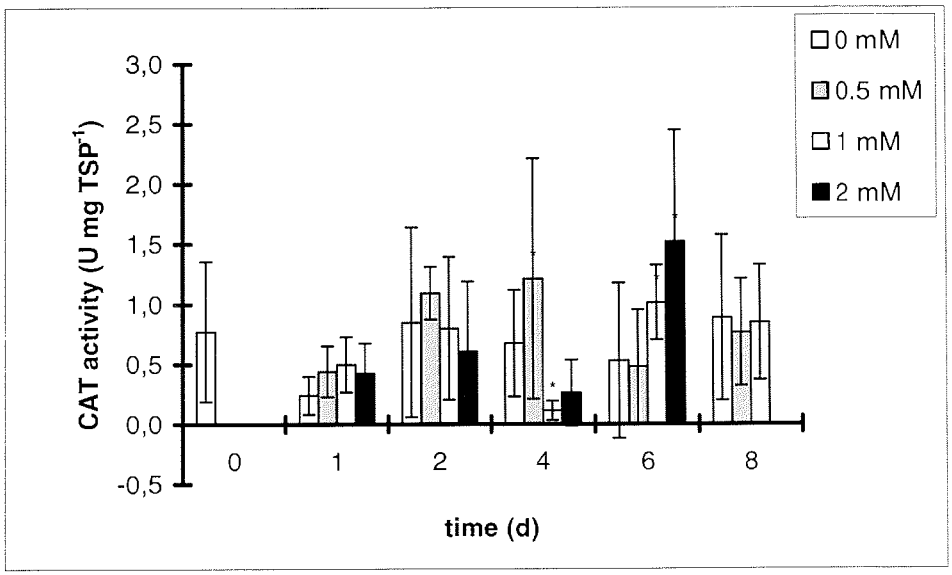

Fig. 4: Catalase activity in *Chaetomorpha linum* (U mg TSP^{-1}) exposed for up to 8 days to a series of ascending H_2O_2-concentrations (0-2mM), mean values ± SD, n=5. * as in Fig. 1

The content of the antioxidant ascorbic acid decreased in most cases in the first 24 h of exposure especially when exposed to 2 mM H_2O_2 (Fig. 5), showing the same pattern as photosynthetic efficiency (Fig. 1). After 4 days ascorbic acid contents was similar to the control in samples exposed to 0 – 1 mM H_2O_2 (Fig. 5), whereas at H_2O_2-concentrations ≥ 2 mM and longer exposure times ascorbic acid content was fully depleted (data not shown).

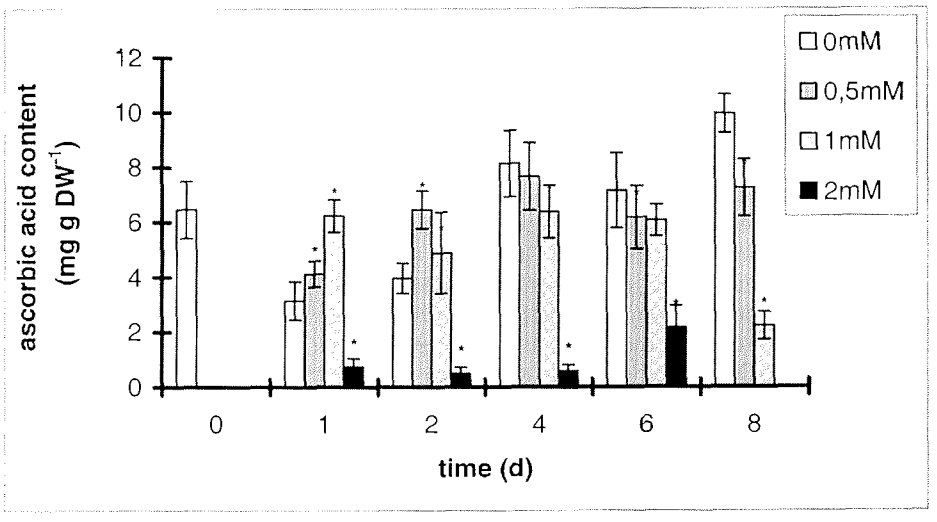

Fig. 5: Ascorbic acid content in *Chaetomorpha linum* exposed for up to 8 days to a series of ascending H_2O_2 concentrations. * as in Fig. 1

Discussion

The main result of this study is the high tolerance of *Chaetomorpha linum* against H_2O_2. H_2O_2 stress up to 1 mM was generally tolerated while concentrations higher than 1 mM reduced the photosynthetic efficiency significantly. *C. linum* showed extremely low activities in GR but high activities of the other detoxifying enzymes. Especially with increasing H_2O_2 concentration and exposure times enzyme activities increased. Ascorbic acid is an important scavenger up to H_2O_2 concentrations of 1 mM.

Assuming that H_2O_2 stress in the field in first order derives from photochemical H_2O_2 accumulation often driven by UV radiation as well as the fact that the H_2O_2 concentration in the surface layer is higher than in deeper waters, *C. linum* shows good adaptation to H_2O_2 stress under laboratory conditions. The effect of increasing H_2O_2 concentrations (0-20 mM) on the maximum quantum yield of macroalgae from Kongsfjorden was determined in 14 species and their tolerance against H_2O_2 indicates their antioxidative capacities (Dummermuth et al. 2003). Results for *C. linum* are comparable to the other green algae tested in this assay for the detection of the antioxidative potential.

Although measurements in H_2O_2 data from the Arctic Kongsfjorden are missing, the above hypothesis is well supported in Antarctic coastal waters (Abele et al., 1999) as well as in other coastal and estuarine areas (Johnson et al., 1989; Zika et al., 1985). Water column depth profiles typically show decreasing H_2O_2

concentrations, reflecting downward mixing below UV penetration depth (Cooper and Lean, 1989).

Enzyme activities measured here are in the same range as data given by Aguilera et al. (2002a; b; this issue) who carried out a general screening for antioxidative enzymes in Arctic marine macroalgae as well as a seasonal study with respect to changes in biochemical defense systems against radiation stress. Compared to macroalgae from temperate waters enzyme activities detected in *Chaetomorpha linum* are in the same range as in *Fucus* species (Phaeophyceae), the two intertidal red algae *Chondrus crispus* and *Mastocarpus stellatus* and the green alga *Ulva rigida* (Collen and Davison, 1999a; c; Collen and Pedersen, 1996). Ascorbic acid content in *C. linum* is similar to data about the same species from Aguilera et al. (2002b) and Collen and Davison (1999a, 2001).

The decrease in SOD activity after exposure to H_2O_2 concentrations >1 mM correlates to Fv/Fm data in *C. linum* and can be explained by a direct toxic effect as observed by Collen and Pedersen (1996) in similar experiments with the green alga *Ulva rigida*. Exposure to high levels of H_2O_2 (3 mM and higher) in this species caused intolerable oxidative stress accompanied by decease as in *C. linum* after 8 days of exposure to 2 mM H_2O_2. In *C. linum* H_2O_2 seemed to directly affect SOD activity and a decrease, especially in the first 24 h, was recorded for all treatments. These results are comparable to those observed in *Palmaria palmata* where SOD activity was directly affected after 1 day of exposure to UV radiation (Aguilera et al., 2002b) as well as in the green microalga *Chlorella vulgaris*, which showed a decrease of SOD activity after long-term exposure to UV radiation inducing oxidative stress (Malanga and Puntarulo, 1995). Inhibition of gene expression for this enzyme may be the reason for this negative effect as observed by Strid (1993) in *Pisum sativum*. *Zea mays* leaves, in contrast, showed increased APX and SOD activity after 12 h incubation in 1 mM H_2O_2 (Pastori and Trippi, 1993) which is in agreement to the increased APX activity in *C. linum*.

It has been shown that plants increase GR activity in response to stress. For example, in *Arabidopsis* GR activity was enhanced under UV radiation (Kubo et al., 1999) as well as in several Arctic macroalgae under artificial and natural UV stress (Aguilera et al., 2002b). This was, however, not evident in the species studied here.

The increase in APX activity in *C. linum* correlates to the decrease in ascorbic acid content indicating a switch in antioxidant strategy with increasing H_2O_2 concentration changing from the scavenger ascorbic acid to the enzymatic defense system of APX.

The data shown here agree well to other studies who have documented high antioxidant enzyme activities and ascorbic acid contents in green algae (Aguilera et al., 2002b). Green algae occupy the upper part of the rocky shore, where they are exposed to high amplitudes in environmental conditions, especially those related to rapid and drastic changes in UVR causing oxidative stress. So *C. linum* is adapted to high oxidative stress and has a high acclimation potential.

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Biochemical properties of antioxidative enzymes and the effect of radiation conditions in marine macroalgae from Kongsfjorden and other regions

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In contrast to the relatively stable low temperature conditions in the Arctic Ocean and in Kongsfjorden throughout the year there is a strong seasonal variation of the radiation conditions. Moreover, the penetration of solar radiation into the water column of Kongsfjorden also experiences drastic and rapid changes (Hanelt et al. 2001; this issue). In winter and early spring, a sea ice layer covers Kongsfjorden, resulting in darkness or very low underwater light conditions. On the other hand, following sea ice break-up the water column is highly transparent for photosynthetically active (PAR) and ultraviolet radiation (UVR). These rapid changes in underwater light conditions directly affect various processes, e. g. photosynthesis and growth as well as biological macromolecules, e. g. the DNA and proteins (Aguilera et al., Bischof & Hanelt, Clayton & Wiencke, Karsten & Hoyer, van de Poll et al., Wängberg & Gustafson, this issue).

Photosynthesis can be damaged due to high PAR or UVR as a result of an overreduction of the photosynthetic electron transport chain leading to the formation of superoxide radicals (O_2^-) and other reactive oxygen species such as singlet oxygen ($^1O_2$) and hydrogen-peroxide (H_2O_2). Reactive oxygen species can result in lipid peroxidation, damage proteins and have many other effects (Fridovich, 1986).

Cellular mechanisms of protection against such toxic oxygen species are essential for the maintenance of photosynthetic activity and other metabolic

functions (Halliwell 1982). The enzymes superoxide dismutase (SOD), catalase (CAT) or specific scavengers such as ascorbate and glutathione mediated by ascorbate peroxidase (APX), glutathione peroxidase and glutathione reductase (GR) are the main antioxidative mechanisms in plants. Studies on the properties of antioxidant enzymes and especially on the radiation-induced sensitivity of the protective mechanisms against oxidative damage are rare for macroalgae. The ability to resist excessive PAR and UVR may be one of the major factors controlling vertical macroalgal zonation patterns (Hanelt 1998; Bischof et al. 1998), and may at least in part be the result of an effective biochemical potential against oxidative stress.

Biochemical properties of antioxidative enzymes

In order to set a baseline for future studies the biochemical properties (Km, Vmax, pH- and temperature dependence) of GR and CAT from the green alga *Monostroma arcticum* from Kongsfjorden have been analysed and compared with species from the North Sea (*Enteromorpha intestinalis*, *Porphyra umbilicalis*) and Antarctica (*Lambia antarctica*; Table 1). Enzyme activities were analysed as described in Aguilera et al. (2002a).

GR catalyses the reaction: $GSSG + NADPH \longrightarrow 2GSH + NADP^+$,

where GSSG is oxidised glutathione and GSH is reduced glutathione. Assays for GR activity in algae were performed as follows: Approximately 50 μL of algal extract were added to 90 μL of a buffer containing 80 mM Tris buffer (pH 8), 1 mM EDTA, 0.1 mM NADPH, and 0.5 mM GSSG. Subsequently, the reaction was monitored by measuring the oxidation of NADPH at 340 nm. Assays for Km, Vmax, and optimum pH were performed at 20° C. The effect of temperature on GR activity was studied between 0 and 50º C. The pH dependence was determined in the range between 5-10. Km and Vmax for the substrate NADPH were calculated using the same assay protocol at NADPH concentrations between 5 and 300 mM. Km and Vmax for GSSG were determined at concentrations between 25 and 2000mM.

CAT catalyses the reaction: $2H_2O_2 \longrightarrow 2H_2O + O_2$

The biochemical properties of this enzyme was determined as follows: Approximately 50μL of algal extract was added to 850 μL potassium phosphate buffer (50 mM, pH 7) containing 15 mM of H_2O_2. Subsequently the reaction was followed by monitoring the decrease in absorbance at 240 nm at 20° C. Km, Vmax, and optimum pH were determined at 20° C, the temperature effect in enzyme activity was studied between 0-50 ºC, and pH dependence was determined between pH 5 and 10. Km and Vmax for the substrate H_2O_2 was calculated from assays with concentrations between 5 and 250 mM. Results for both CAT and GR are expressed as units (U) of enzyme activity [1 U= 1 μmol substratum (H_2O_2 and NADPH respectively) converted min^{-1} mg^{-1} of total soluble protein].

Km and Vmax values for the substrate GSSG in the GR reaction showed a great variation in the studied species. They were higher than in the sea ice alga *Entomoneis kufferathii* from Antarctica. In this species the Km and Vmax values for GSSG are 62.5 μM and 0.3 U mg SP^{-1} min^{-1}, respectively (Schriek 2000). A similar low Km value for GSSG of 55 µM has been measured in the microalga *Euglena gracilis* (Shigeoka et al. 1987), a value of 70 µM in the bacterium *Escherichia coli* (Scrutton et al. 1987) and a value of 14 µM in maize (Mahan and Burke 1987). Higher Km values for GSSG similar to the ones measured here were, however, reported for the cyanobacterium *Anabaena* (160 µM; Jiang and Manervick 1999) or *Spinacea oleracea* (196 µM; Halliwell and Foyer 1978). The Km and Vmax values for NADPH are lower in the studied species compared to *E. kufferathii*, which exhibits values of 20 μM and 0.6 U mg SP^{-1} min^{-1}, respectively. However, the values determined here are in the same range as reported for *Euglena*, *Anabaena*, *E. coli* , maize or spinach (same literature as above). Moreover, the optimal pH for the enzyme is similar in all studied species with the maximum of the activity ranging between pH 7-8. The only exception is the pH optimum of GR in *E. kufferathii* exhibiting a broad optimum between 7 and 9.5 (Schriek 2000).

Table 1. Biochemical parameters of Glutathione reductase (GR) [Km and Vmax for the substrates oxidised Glutathione (GSSG) and NADPH] and for catalase (CAT) [Km and Vmax for H_2O_2)] and optimal pH of reaction for both enzymes. Data are given as mean values ± SD (n=3) and expressed as units (U) of enzyme activity (1 U = 1 μmol substrate min^{-1}) per milligram of total soluble proteins (SP). Standard deviation was less than 20 % in all cases.

	GR					CAT		
Species	Km(μM) GSSG	Vmax GSSG $UmgSP^{-1}$	Km (μM) NADPH	Vmax NADPH $UmgSP^{-1}$	pH	Km (mM) H_2O_2	Vmax H_2O_2 $UmgSP^{-1}$	pH
Monostroma arcticum Spitsbergen, Arctic	288	2.96	10.46	2.51	8	42	221	7.5
Lambia antarctica King George I, Antarctica	176	1.12	6.1	0.91	8	106	44	7
Enteromorpha intestinalis (Bremerhaven, North Sea)	110	2.83	10.46	2.83	7.5	22	200	8
Porphyra umbilicalis (Helgoland, North Sea)	276	1.48	10.15	1.18	7.5	52	845	7.5

CAT has generally a very high Km constant (in the order of mM), as the reaction centre requires two substrate molecules. This has been shown also for other organisms. In comparison to *L. antarctica*, low Km values and high Vmax values were measured in *M. arcticum*, *E. intestinalis* and *P. umbilicalis*. The optimal pH varied between 7-8 for all species. A similar heterogeneity in catalase kinetic parameters has been observed also in different organisms with Km values ranging from 64 to 125 mM in *E. coli*, the yeast *Sacharomyces cerevisiae*, in Bovine liver and in human erythrocytes (Switala and Loewen 2002). Vmax values showed an even stronger variation between 70 and 587 in the same studies.

What is the role of temperature for the activity of antioxidative enzymes? One might assume that temperature optima in species from Polar Regions are lower than in species from the Temperate Zone. So we examined the activities of GR and CAT in these species from the Arctic, the Antarctic and from the North Sea (Fig. 1).

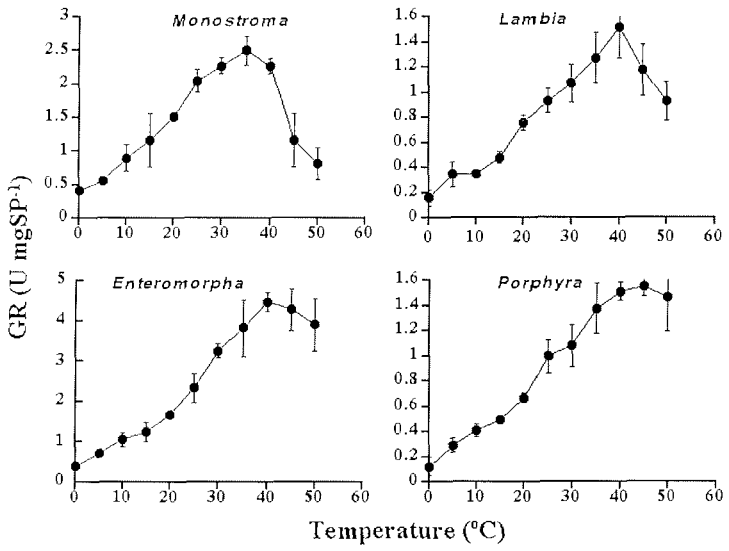

Fig. 1. Changes in GR activities of *Monostroma arcticum* from Spitsbergen, *Lambia antarctica* from King George Island (Antarctica), *Enteromorpha intestinalis* from Bremerhaven, North Sea and *Porphyra umbilicalis* from Helgoland, North Sea with respect to the temperature. All other parameters under optimum conditions. Data are given as mean values ± SD (n=3) and enzymatic activities are expressed as in Table 1.

Interestingly there were no big differences in the temperature optima of GR, which varied between 35 and 45 °C. In the two species from the North Sea, however, the optima were somewhat broader. On the other hand, CAT exhibited a broad optimum between 15 and 35 °C in *Monostroma arcticum*, whereas in *E. intestinalis* and *P. umbilicalis* CAT activity increased more slowly to 45 and 50 °C (Fig.2). The CAT activity of *P. umbilicalis* was about 5 times higher compared to *M. arcticum* and *E. intestinalis*. Overall it seems that a shift of the temperature optimum of these antioxidative enzymes in the polar species to the low seawater temperatures of the Polar Regions is not necessary for the success of the studied species. These species probably increase the amount of enzyme to maintain the activity at a sufficient level at low temperatures. Similar data are also available on other enzymes such as Rubisco. For example, temperature optima of Rubisco from two

temperate and two Antarctic diatoms were also in the same range at 40 and 50 °C (Descolas-Gros & de Billy 1987). The only example in which low temperatures stimulate the activity of an antioxidative enzyme has been described in the ice alga *Entomoneis kufferathii* (Schriek 2000). In this species CAT exhibits two optima, one at 0 °C and one at 60 °C, indicating the presence of isoenzymes. The other two studied enzymes, GR and glutathione peroxidase had temperature optima at 45 °C. Certainly more algal species from different biogeographical regions have to be studied to get a more complete overview.

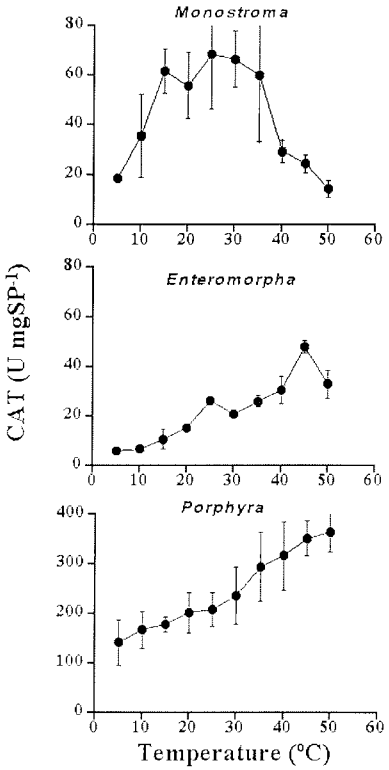

Fig. 2. Changes in CAT activities in *Monostroma arcticum* from Spitsbergen, *Enteromorpha intestinalis* from Bremerhaven, North Sea, and *Porphyra umbilicalis* from Helgoland, North Sea, with respect to the temperature. Data are given as mean values ± SD (n=3) and enzymatic activities are expressed as in Table 1.

Activities of antioxidant enzymes in green, brown and red algae from different water depth

In a second series of experiments we have characterised the oxidative stress tolerance in field material of 22 different green, red and brown macroalgal species from the Arctic by the analysis of a set of antioxidant enzyme activities as shown in Table 2. The experimental protocol is given in Aguilera et al (2002a). Clear differences were found between the three macroalgal groups. Green algae showed in general higher antioxidant enzyme activities than red algae whereas brown algae showed the lowest values in all enzymatic activities. Maximum SOD, GR, APX and CAT activities were found in the green algae *Monostroma sp.*, followed by *Acrosiphonia penicilliformis* and *Chaetomorpha linum*. Within the red algae, *Devaleraea ramentacea* showed highest values of APX and CAT activities being 6 times higher in APX and almost 2 times higher in CAT compared to the other investigated red algae. Within the group of brown algae, relatively low antioxidative enzyme activities were found especially in comparison to the studied green algae. However, we hypothesise that other protection mechanisms may be effective here such as the accumulation of UVB absorbing phenolic compounds (Clayton et al., this issue). When exposed to 2 mM H_2O_2 the activities of CAT and APX increased in *C. linum* within a few days (Dummermuth et al.; this issue). Similarly the activities of these enzymes increased more than 10 times compared to the control in *Polysiphonia arctica*, when exposed for 8 days to 2 mM H_2O_2 (Dummermuth et al. 2003), indicating very flexible responses to oxidative stress.

Table 2 Activities of superoxide dismutase (SOD), glutathione reductase (GR), catalase (CAT) and ascorbate peroxidase (APX) in different green, red and brown algae from Kongsfjorden (Spitsbergen). Data taken from Aguilera et al. (2002a). Data are given as mean values ± SD (n=3) and enzymatic activities are expressed as in Table 1. Standard deviation was less than 20 % in all cases.

Species	SOD $UmgSP^{-1}$	GR $Umg\ SP^{-1}$	APX $Umg\ SP^{-1}$	CAT $UmgSP^{-1}$
Chlorophyta				
Acrosiphonia penicilliformis	674	2.30	0.2	1.0
Monostroma sp.	1004	1.58	0.97	27.11
Chaetomorpha linum	395	0.10	0.778	0.77
Chaetomorpha melagonium	200	1.54	0.5	30.00
Prasiola crispa	153	0.10	0.12	3.86
Rhodophyta				
Coccotylus truncatus	165	0.08	0.05	9.91
Devaleraea ramentacea	245	0.32	0.60	15.5
Palmaria palmata	185	0.22	0.45	4.5
Phycodrys rubens	94	0.08	0.04	-
Odonthalia dentata	109	0.09	0.10	6.24
Polysiphonia arctica	87	0.06	0.02	7.58
Ptilota gunneri	122	0.11	0.01	-
Phaeophyta				
Alaria esculenta	102	0.07	0.05	-
Chorda tomentosa	128	0.11	0.07	-
Chordaria flagelliformis	112	0.10	0.03	-
Desmarestia viridis	143	0.10	0.08	-
Elachista fuciola	191	0.10	0.05	-
Fucus distichus	151	0.09	0.07	3.60
Laminaria saccharina	181	0.18	0.04	7.97
Laminaria solidungula	142	0.08	0.01	-
Laminaria digitata	68	0.10	0.05	-
Saccorhiza dermatodea	88	0.09	0.06	-

Apart from the differences of the antioxidative potential between the different taxonomic groups there is also a positive relation between the activities of the antioxidative enzymes and the depth distribution of the different species. Most green algae showed relatively high antioxidant activities and typically inhabit the upper sublittoral in Kongsfjorden. Similarly, the red algae *Devaleraea ramentacea* and *Palmaria palmata*, occurring frequently in the upper sublittoral, exhibited higher SOD activities compared to red algal species living in deeper waters, such as *Phycodrys rubens*. In the case of the brown alga –*Fucus distichus* low enzymatic activities were determined in comparison to green or red algae living in almost the same water depth. This species clearly has a different strategy to survive stressful conditions. Accumulation of UV absorbing phenolic compounds as discussed above as well as the morphology, i.e. a thicker thallus, obviously provide a good protection against high solar radiation similar as in *Laminaria*. In these leathery species the fluence rate of the harmful radiation decreases strongly towards the inner cell layers as already pointed out by Dring et al. (1996).

Seasonal changes in antioxidant enzymatic activities

As discussed above, the summer season in Spitsbergen is characterised by rapid and strong changes in water characteristics, especially with regard to the underwater light regime. To characterise the response of various algal species to the different radiation conditions, we monitored the seasonal changes of enzymatic activities of GR and SOD from late spring to autumn (Aguilera et al. 2002b).

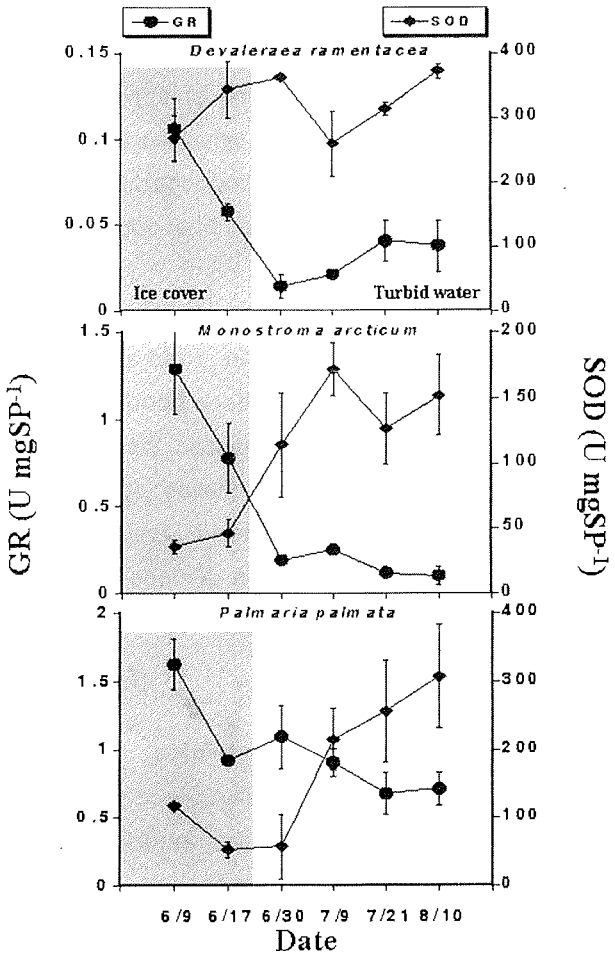

Fig. 3. GR and SOD activities of the red algae *Devaleraea ramentacea*, *Palmaria palmata* and the green alga *Monostroma arcticum* from field samples collected along the summer of 1998. Data under grey background indicates that algae were exposed to low solar underwater radiation due to winter ice-cover in spring and to turbid meltwater at the end of the summer. Data are given as mean values ± SD (n=3) and enzymatic activities are expressed as in Table 1. Data taken from Aguilera et al. 2002b.

High photosynthetic activities during summer (Bischof et al. 2002; Bischof & Hanelt, this issue) due to an increase in the underwater light irradiation may enhance oxidative stress. This is reflected in the pattern of SOD activities in the red alga *Palmaria*

palmata and the green alga *Monostroma arcticum*. An increase of the ambient irradiance as consequence of the rapid break up of the ice cover in the middle of June 1998 coincided with an increase of SOD activity and this was related also to an increase of the photosynthetic activity, especially for *P. palmata*. *M. arcticum* is very sensitive to the increase of underwater irradiance and exhibits a marked decrease in photosynthetic performance and also a bleaching of the thallus, which may be related to its seasonal development (cf. Kornmann and Sahling 1977). Nevertheless SOD activity increases strongly during the open water period. This period with high underwater radiation conditions in Kongsfjorden is typically relatively short. In late summer a high influx of turbid meltwater into the fjord from melting glaciers and snow results in a strong decrease of transmittance but under these conditions SOD activities stay at a high level in both species. In the red alga *Devaleraea ramentacea* high SOD activities were maintained over the summer, a feature directly related to high photosynthetic activities. Induction of SOD by high PAR and UV radiation was also demonstrated in different microalgae (Lesser 1996). The contrary has been observed for the enzyme GR exhibiting a significant decrease of its activity during the course of the summer. It might be possible that other enzymatic systems detoxifying H_2O_2, such as CAT are involved during this time of the year. Similarly, seasonal changes in antioxidative enzyme activities have been described for SOD and some H_2O_2 detoxifying enzymes of the ascorbate pathway like ascorbate peroxidase, glutathione reductase and dehydroascorbate reductase in the freshwater dinoflagellate *Peridinium gatunense* (Butow et al. 1997).

The above-described effects of high solar radiation on the activities of some antioxidant enzymes can be demonstrated also in the laboratory under controlled conditions. When exposed to different levels of PAR, *Monostroma arcticum* increases both the activities of CAT and total peroxidase (PX) under increasing photon fluence rates (Fig. 4).

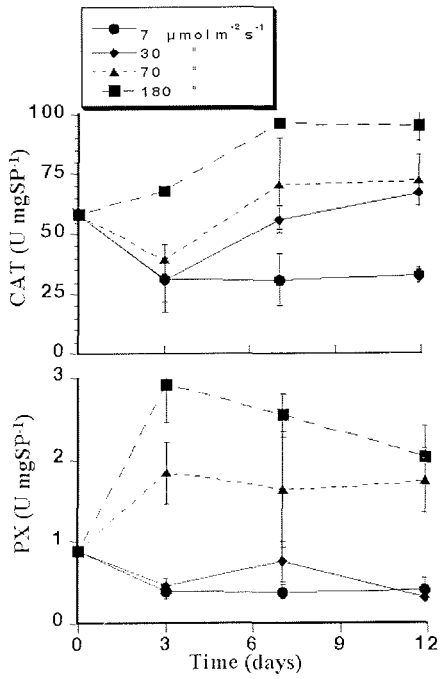

Fig. 4. Changes in Catalase (CAT) and peroxidase (PX) activities of *Monostroma arcticum* grown under different PAR irradiances. Data are given as mean values ± SD (n=3) and enzymatic activities are expressed as in Table 1.

In conclusion, the general biochemical characteristics of the studied enzymes are – as far as we can say now – comparable to published data on other organisms including land plants and mammals. There seems to be no temperature adaptation of the enzymes to the temperature regime in the environment of the species studied here. But more studies are urgently needed.

The antioxidant enzyme activities are generally higher in green algae in comparison to red and brown algae. Moreover they are higher in species that grow in shallow waters where they are exposed to drastic changes in the environmental conditions, especially those related to rapid and drastic changes in the visible and UV region of the solar spectrum.

The activity of the antioxidative enzymes varies in response to the seasonal changes of the radiation conditions as shown here for three species as examples. The responses of the species show certain differences, which are related to the life strategy of the species. Similar radiation effects can be demonstrated also in laboratory experiments. Many question as are, however, unresolved and many more studies are needed.

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UV effects on growth of macroalgae from Kongsfjorden (Svalbard)

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So far, there are only few published accounts on long-term growth of macroalgae of Arctic regions (Chapman and Lindley 1980, 1981; Dunton 1985, 1990; Dunton and Schell 1986; Aguilera et al. 1999a,b; Michler et al. 2002) despite their known importance in coastal marine ecosystems in terms of primary production (Mann 1982, Clendennen et al. 1996), biodiversity (Norton et al. 1996) and as a source of useful natural products (Lüning 1990; Guiry and Blunden 1991). Moreover, they have an important function as nursery and shelter for many juvenile animals.

Macroalgae, particularly those inhabiting higher littoral zones are mostly negatively influenced by an increase in UVB radiation (UVBR) due to stratospheric ozone depletion. Most of the information of UVB-effects on marine macroalgae is available from studies concerning photosynthetic activity, the synthesis of possible photoprotective pigments, pigmentation, enzyme reactions and nutrient uptake (Larkum and Wood 1993; Hanelt et al. 1997a,b; Bischof et al. 1998a,b; Post & Larkum 1993; Karsten et al. 1998a,b; Aguilera et al. 1999a,b; Bischof et al. 2000; Flores-Moya et al. 1998). However, these single physiological parameters are not sufficient to explain long-term effects induced by enhanced UVBR in an ecological context. Therefore, there is an urgent necessity for studies focusing on whole organisms and at the population level. To address this question, growth measurements are helpful in estimating possible changes of productivity due to enhanced UVR as growth is considered to be an important parameter that integrates stress effects at several levels.

In this study, we have investigated changes in the growth of Arctic macroalgae collected in the Kongsfjord Spitsbergen (Norway) and subsequently exposed to natural solar radiation or to treatments with controlled fluence rates of artificial UVR. Several experiments have been carried out in order to assess the UV effect on growth of different macroalgae. Algae were collected from the field and directly exposed to natural surface solar radiation depleted of UVR by use of cut-off filters and by outdoor incubation in basins with running seawater. Individuals of six macroalgal species were sampled from the same water depth in the course of the spring/summer season in 1998, before, during and after the break-up of sea ice, firstly under clear and later under turbid water conditions. Growth rate of different species was followed by incubations of three weeks duration, starting on the date shown in Fig. 1.

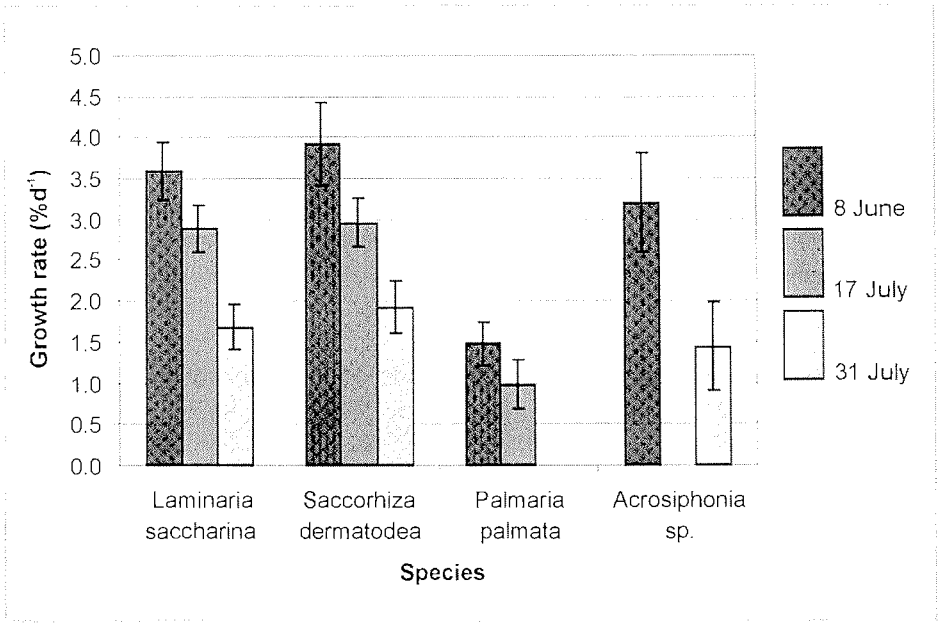

Fig. 1 Growth rate (% day^{-1}) of selected macroalgal species exposed to solar radiation depleted of UVR in basins at sea level during summer 1998. Experiments were initiated on each date given and subsequently run for three weeks. Plants were exposed to filtered UV radiation (PAR> 400 nm). Vertical bars indicate the standard deviation of 16 replicates per treatment.

The highest growth rates in all the species studied were always found in June followed by a gradual decrease in July and August. It is obvious that solar radiation is one of the key factors controlling algal growth. In spite of the positive correlation between the growth activity of the algae and solar radiation reaching the earth's surface, this decrease in the growth rate may reflect the life strategies of the studied species. For example, the seasonal course of daylength can also determine seasonality of growth and other physiological processes (Weykam & Wiencke 1996, Gómez et al. 1995, 1997, Gómez & Wiencke 1997). Species such as *L. saccharina* from the Alaska Arctic Coast grow with maximal growth rates between late April and late July (Dunton 1985). In contrast, *L. solidungula* from the same area completes most of its annual growth in darkness during late winter and spring (Chapman & Lindley 1980, Dunton and Schell 1986, Dunton 1990) by remobilisation of stored reserve carbohydrates. The high growth rates of *L. saccharina*, *S. dermatodea* and *P. palmata* measured here in June and the later decrease may be also a consequence of this seasonal change of physiological activities as observed for other Arctic Laminariales. Nutrient availability can also be an important abiotic factor controlling algal growth. In particular, *Palmaria palmata* might be affected at the level of photosynthesis, pigmentation and other biochemical processes by the a drastic decrease of nutrients in summer in the Kongsfjord (Bischof et al. 2002, Aguilera et al. 2002).

In addition to the above described exposure of algae to PAR the different species were also exposed to PAR + UVA and to the full spectrum by use of specific cut- off filters. Growth of deep-water species was sensitive to UVR compared to shallow-water species (Fig. 2).

Except for intertidal *Fucus distichus*, growth of all other studied species was significantly affected by UVA and UVB. In the case of the intertidal/shallow water alga *Acrosiphonia* sp., growth rate was strongly inhibited under total UV, as well as under PAR+UVA. In both intertidal species, the adaptation strategy to survive under exposed and stressful conditions is different. The morphology, i.e. a thicker thallus, better protects against high solar radiation such as in the brown algae *Fucus* or *Laminaria*. In these species, the fluence rate of the harmful radiation decreases strongly towards the inner cell layers (Dring et al. 1996). In contrast, in the case of *Acrosiphonia*, the growing apical regions of the alga are exposed to strong light, explaining the dramatic decrease of growth rates. The basal cells are well protected due to self-shading within the tuft and are able to grow out when the upper filaments are damaged.

Sublittoral species were already partially inhibited by UVA (320-400 nm; Fig. 2). This is probably due to the shown inhibition of photosynthesis by UVA (Hanelt et al. 1997b). There was no significant difference between exposure to full solar radiation or radiation deprived of UVA. A reason may be that only low UVB irradiance reached the sea surface during this time.

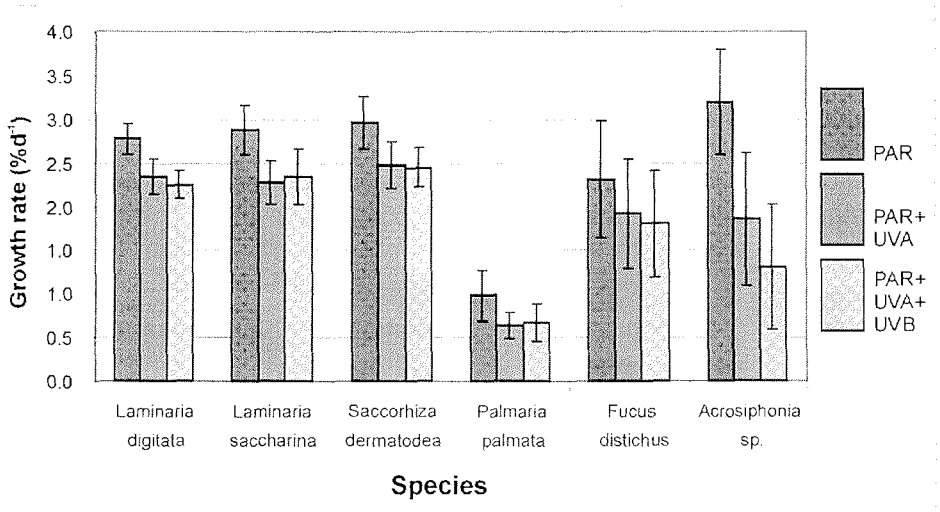

Fig. 2 Growth rate (% day^{-1}) of selected macroalgal species exposed to solar radiation in basins at sea level in July 1998. Experiments were run for three weeks. Plants were exposed to filtered UV radiation (PAR > 400 nm), filtered UVB radiation (PAR+UVA >320nm) and unfiltered solar radiation (PAR+UVA+UVB). Vertical bars indicate the standard deviation of 16 replicates per treatment (data taken from Aguilera et al. 1999b)

Laminaria species growing in the field at three different water depths were affected by the radiation penetrating into the sea (Fig. 3). No significant effect of UV radiation can be expected due to the strong attenuation of underwater

UVA+UVB irradiance (K_d for UVB = 0.66, UVA = 0.62, Aguilera et al. 1999a,b). However, there was a correlation between growth rates of *L. saccharina* and *L. digitata* and the depth (3, 6 and 9 m) as shown in Fig. 3. While for *L. saccharina* a decrease of 6.8 % of growth rate per meter depth was observed, growth rates of *L. digitata* decreased by 11 % per meter.

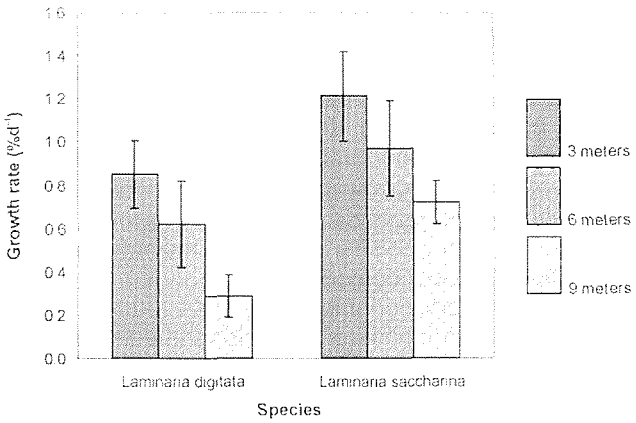

Fig.3 Field measurements of growth rate (% day^{-1}) of two *Laminaria* species at different depths. Vertical bars indicate the standard deviation of 7 replicates per treatment (data taken from Aguilera et al. 1999b).

Laboratory experiments under controlled conditions confirmed field data (Michler et al. 2002). These experiments were conducted with *Alaria esculenta* and *Laminaria saccharina* in a temperature regulated deep-freezer with mounted fluorescence-tubes. *A. esculenta* growing somewhat higher in the field than *L. saccharina* was much less sensitive to UVR (Fig. 4).

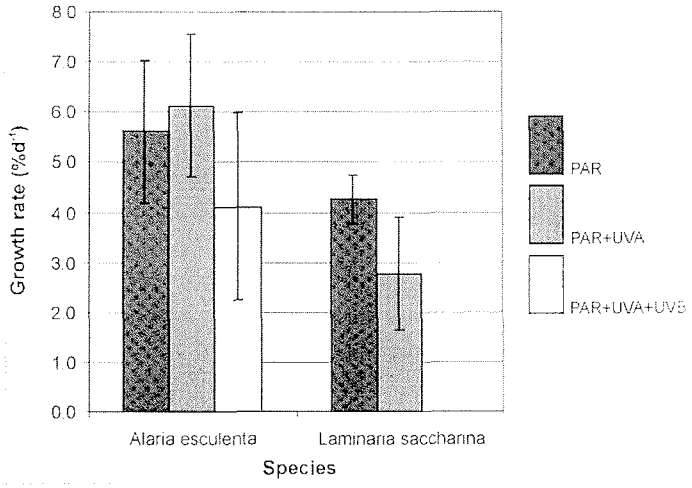

Fig. 4 Growth of two *Laminaria* species (% day^{-1}) at different artificial UV treatments (Q-panel UVA-340 fluorescent tubes, filtered by specific cut-off filters; 5 W m^{-2} PAR, 2.7 W m^{-2} UVA, 0.2 W m^{-2} UVB) at the beginning and after 28 days of incubations. Vertical bars indicate the standard deviation of 9-12 replicates per treatment (data taken from Michler et al. 2002)

Highest UV sensitivity was observed in the arctic–endemic deep water alga *Laminaria solidungula* compared to the other species from the upper to middle sublittoral. It is therefore reasonable to assume that a correlation between depth zonation and vulnerability to UV radiation exists. In the case of

L. solidungula not only UVB but also additional UVA (PAR+UVA treatment) caused a significant reduction in growth (Michler et al. 2002). In contrast, species like *Alaria esculenta* from the upper and middle sublittoral are regularly subjected to a certain dose of UVR, particularly of UVA (Hanelt et al. 1997b) and should therefore be less sensitive to combined effects of PAR+UVA. The measured differences in growth performances reinforce the conclusion of Hanelt et al. (1997) that even species which are already in culture for a long period of time retain a certain genetic adaptation to the light environment of their natural habitat.

The presented results give a new insight into the reaction of whole organisms upon exposure to UVR. More of these studies have to be performed to estimate the effects of UVR at the organism and community levels in order to predict the reaction of the whole ecosystem to enhanced UVBR due to stratospheric ozone depletion.

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UV effects on reproduction of brown algae from Kongsfjorden (Svalbard)

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Introduction

Recognition of the phenomenon of stratospheric ozone depletion and the resulting elevation of levels of UV-B radiation (UVBR) provided the rationale for studies on the effects of UV radiation (UVR) on terrestrial plants and marine micro- and macroalgae. Initially, macroalgal research focussed on the effects on mature individuals, in particular on the deleterious effects of UVR on photosynthesis and on DNA (Hanelt et al. 1997; Bischof et al. 1998, 2000, 2002; Karsten et al. 2001; van de Poll et al. 2002a, b; Bischof & Hanelt this issue; van de Poll & Breeman: this issue). Aguilera et al. (1999; this issue) and Michler et al. (2002) have documented the physiological impacts of UV on growth of macroalgae from Spitsbergen. Studies of the effects of UVR on the vulnerable early life history stages of polar macroalgae were stimulated by the need to assess the possible long term effects of increased levels of UVR on the persistence of species and their zonation, especially in the intertidal and upper sublittoral regions where UVBR can penetrate down to 8 m depth in clear waters (Bischof et al. 1998, Hanelt et al. 2001, Svendsen et al. 2002; van de Poll et al. 2002b).

Kelps belonging to the brown algal order Laminariales (Phaeophyceae) are important habitat-forming species in the Kongsfjord. A dense kelp forest grows on rocky surfaces between 1.5 and 13 -16 m depth and is structured by the perennial canopy species, *Alaria esculenta, Laminaria saccharina* and *Laminaria digitata*, together with the annual *Saccorhiza dermatodea* and at lower levels, *Laminaria solidungula* (Wiencke et al., this issue). Various other species of macroalgae including *Desmarestia aculeata* and the red alga *Palmaria palmata* comprise the understorey and many invertebrates are associated with the kelp forest (Lippert et al. 2001; Hop et al. 2002). Also potentially susceptible to the impact of UV radiation are the intertidal and upper subtidal assemblages of macroalgae that include the brown algae *Fucus distichus, Pilayella littoralis* and *Chordaria flagelliformis* (Hop et al. 2002; Wiencke et al., this issue).

Cell structure of brown algal propagules

Zoospores of brown algae are biflagellate motile cells a few microns in diameter and typically possessing one to several chloroplasts, a nucleus and several (phlorotannin containing) physodes (Henry & Cole 1982, Kawai 1992). In some species, e. g. in *Saccorhiza dermatodea*, the zoospores are phototactic and possess an eyespot. Zoospores normally lack a cell wall until after they have settled and attach to a surface. The physodes are arranged around the periph-

ery of the zoospores of *Laminaria* spp. and *S. dermatodea* as illustrated by Wiencke *et al.* (2004). The eggs of *Fucus distichus* are much larger than zoospores, with a diameter of 70-80 µm. The nucleus is centrally located. Physodes in fucoid eggs and zygotes are particularly abundant and are concentrated around the periphery of the cell, as in the zoospores (Schoenwaelder & Wiencke 2000, Schoenwaelder et al. 2003).

Impact of UVR on viability of brown algal propagules

The propagules of brown algae have been shown to vary in their susceptibility to UVR-induced damage. During three expeditions to the Koldewey Station, zygotes of *Fucus distichus* and zoospores of various brown algae were exposed for different time periods in the laboratory to an artificial radiation regime. UVR was generated by UVA-340 fluorescent tubes (Q-Panel, Cleveland, USA) and PAR was provided by daylight fluorescent tubes (Osram Lumilux Deluxe, L36W/12-950). The irradiances applied were: 7-33 µmol photons m^{-2} s^{-1} PAR (photosynthetically active radiation), 8.0-8.2 W m^{-2} UVAR, 0.9-1.3 W m^{-2} UVBR (Wiencke et al. 2000, 2004; Schoenwaelder et al. 2003). After 72 h of exposure

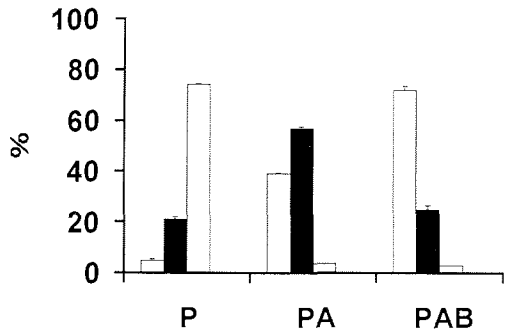

Fig. 1: Germination of zygotes of *Fucus distichus* after 72 h of UVR treatment (white bars: spherical, not germinated; black bars: germinated; stippled bars: one division; P: photosynthetically active radiation, PA: P + UVA-radiation, PAB: PA + UVB-radiation; data taken from Schoenwaelder et al. 2003).

to PAR most *Fucus distichus* zygotes had germinated and about 74 % had divided once (Fig. 1). With added UV exposure, the percentage of zygotes that failed to germinate increased to 40 % under PAR + UVAR and to 71 % under the full spectrum, including UVBR. UV treatments of zoospores of *L. saccharina, L. digitata, A. esculenta* and *C. flagelliformis,* collected from between 1 and 5 m deep in the Kongsfjord, had differential effects on viability as measured by the ability of zoospores to germinate after a 5 d recovery period cultivated in dim light (Wiencke et al. 2000). After 16 h treatment *L. saccharina* and *L. digitata* zoospores showed higher mortality (>90%) than either *C. flagelliformis* or *A. esculenta.* UVBR in combination with UVAR and low PAR (photosynthetically active radiation) was more damaging than PAR with UVA alone. A further study

(Wiencke et al. 2004) revealed a slight increase in the % germination of *A. esculenta* zoospores with longer recovery periods of 6-9 d following UVBR + UVAR + PAR treatment (Fig.2). In contrast to the other species studied, zoospores of *S. dermatodea* were found able to recover fully and germinate after 16 h UVB + UVA + PAR treatment, certainly the result of repair processes.

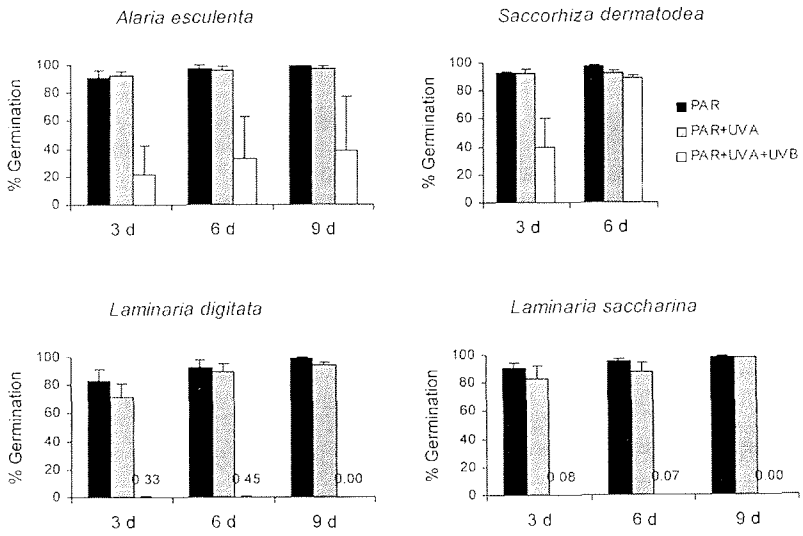

Fig 2 Germination of spores of *Alaria esculenta*, *Saccorhiza dermatodea*, *Laminaria digitata* and *L. saccharina* 3, 6 and 9 days after exposure to 16 h PAR and UV-radiation using cut-off filters (data taken from Wiencke et al. 2004)

Impact of UVR on DNA and photosynthetic efficiency of zoospores

The ability of zoospores of some species to recover from UV damage is presumed to be related to the repair of cyclobutane pyrimidine dimers (CPD), the common form of DNA lesions (Pakker et al. 2000a, b, Poll et al. 2002) and/or the turnover of damaged D1 protein in photosystem II (Xiong 2001). Wiencke et al. (2000) quantified DNA damage in *L. saccharina, L. digitata,* and *A. esculenta* zoospores by measuring the formation of thymine dimers after 4 h UV treatment. Whilst DNA damage caused by exposure to UVB was negligible in the shallow water species *A. esculenta*, it was significant in both species of *Laminaria*. In *L. digitata*, there was a linear relationship between DNA damage and the UVB-dose (BED_{DNA300}; Fig. 3). Wiencke et al. (2000) also measured the effect of 2.5 h UVB + UVA + PAR treatment on the photosynthetic efficiency of *L. digitata* zoospores using a pulse amplitude modulation (PAM) fluorometer. They showed that zoospores can photosynthesise at low light levels and that the optimum quantum yield (F_v/F_m) was lower than in young sporophytes. Exposure to UVA and UVB caused photoinhibition (Fig. 4).

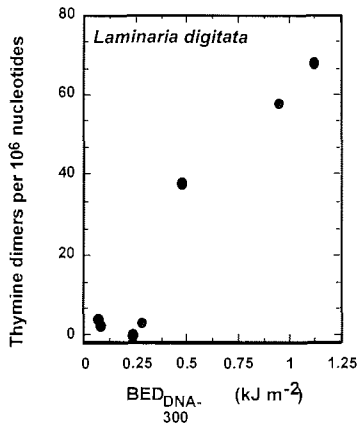

Fig. 3: DNA damage measured as formation of thymine dimers in zoospores of *Laminaria digitata* and the biologically effective dose (BED) of irradiances weighted using the action spectra for DNA damage (Setlow 1974; data taken from Wiencke et al. 2000).

The ability of brown algal zoospores to survive exposure to UV radiation may also be related to the presence of UV screening compounds, and/or to their capacity to synthesize such compounds in response to UV exposure. Physodes are vesicles containing phlorotannins that are highly characteristic of brown algal cells and present in zoospores. Phlorotannins absorb UV radiation (Ragan and Glombitza 1986), and we have demonstrated that the transmission of UVR through suspensions of zoospores of *Alaria esculenta, L. digitata* and *L. saccharina* from Kongsfjorden is strongly attenuated (Fig. 5). UV transmission decreased with the density of the suspen sion. Preliminary data using an integrating sphere indicate that only a small part of the decrease in UVR transmission is due to scattering, most of the UVR is

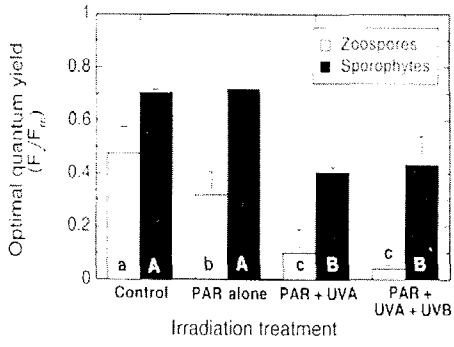

Fig. 4: Effects of PAR and UV radiation on photosynthetic activity (optimal quantum yield; Fv/Fm) of zoospores and sporophytes of *Laminaria digitata* after an exposure time of 2.5 h. Similar letters denote insignificant differences among means. The applied irradiation conditions were: PAR: 6.2-6.8 µmol m^{-2} s^{-1}, UVA: 5.6-7.6 W m^{-2}, UVB: 0.57 W m^{-2} (data taken from Wiencke et al. 2000).

absorbed by the zoospores. Of particular interest are the observations that exposure to UV radiation seems to induce phlorotannin synthesis in *S. dermatodea* and *A. esculenta*. In these species enlarged physodes were observed in zoospores after 16 or 20 h exposure to treatment with UVB + UVA + PAR (Wiencke et al. 2004). These are the two kelp species in which the zoospores show some capacity to recover after exposure to UVB strongly suggesting that the induction of phlorotannin synthesis is an adaptive response, and supporting the proposed UV-screening function of these compounds in brown algae.

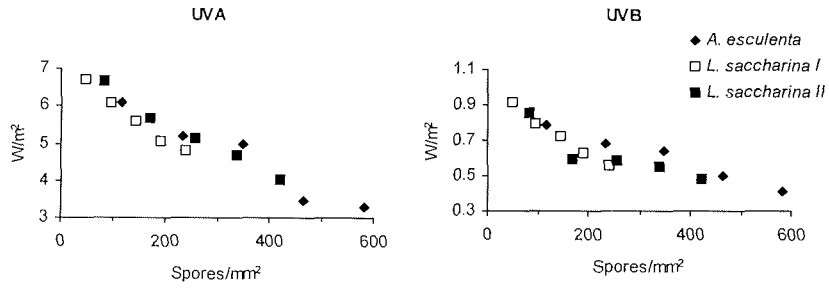

Fig. 5: Transmission of UVA and UVB by spore suspensions of Laminariales. The correlation coefficients range between 0.97 and 1.00, only the coefficient for *L. saccharina* II under UVB is lower (0.89; data taken from Clayton et al. 2004).

Ecological implications of differential responses of species to UVR

Wiencke et al. (2000) considered the likely ecological impact of incident solar radiation on zoospores in the upper subtidal zone of the Kongsfjord. They estimated the theoretical UV dose needed to cause DNA damage and suggested that between 0 and 5 m, 16 h exposure to natural irradiance was unlikely to cause DNA damage. However they noted that this exposure could impair germination and could potentially reduce the photosynthetic capacity of zoospores. The laboratory studies showing that the zoospores of the two shallow water kelps, *A. esculenta* and *S. dermatodea*, have the potential to acclimate to UV exposure (Wiencke et al. 2004), suggest that higher germination rates in these species might give them a competitive advantage in recruitment over other kelp species in the upper subtidal zone. It would be of considerable interest to know whether the propagules of other species of intertidal or upper subtidal brown algae such as *Fucus distichus*, *Pilayella littoralis* or *Chordaria flagelliformis* acclimate to UV exposure in the same way and whether this ability is common to all successful brown algae growing at higher levels on the shore. In addition, and perhaps the greatest challenge would be to investigate the responses of propagules under field conditions to confirm the relevance of results obtained in laboratory experiments.

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Different UVB-tolerance in herbivorous versus carnivorous amphipods from Kongsfjorden

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Introduction and objective

Over the last 30 years, polar environments, not only of the Antarctic but also the Arctic have received elevated levels of UV surface irradiation (UVR) due to stratospheric ozone depletion (Kerr & McElroy 1993, Groß et al. 2001). Although more prominent in the South, this is also an issue in the Northern hemisphere. Madronich et al. (1998) predict a maximal increase of erythemal (sun burning) UV-radiation to 22% over 1970s levels for Northern high latitudes. An increasing number of publications documents UVB-radiation to reach biologically relevant levels in Northern mid and high latitudes (Hunter et al. 1979, Björn et al. 1999, Browman et al. 2000, Williamson et al. 2001), which are expected to impact temperate and Arctic ecosystems, as elevated UV-levels are anticipated to persist over the next decades (IASC 1995, Madronich et al. 1998). Direct UV-photon interaction alters the chemical structure of biomolecules, and elevated oxidative stress in marine organisms has been detected following intense UVB-irradiation and is endangering terrestrial and aquatic organisms (Dunlap et al. 2000, Rozema et al. 2002). Mildly elevated UVB-irradiation increased mortality of Antarctic shallow water amphipods and caused elevated rates of lipid-peroxidation and impaired antioxidant enzyme activities (Obermüller et al. 2003). Exposure to natural surface UVB-levels increased mortality in Cladoceran populations (*Daphnia pulicaria*) from a temperate lake (Williamson et al. 2001) and reduced the reproductive success in *D. pulicaria* from subarctic fresh water ponds (Zellmer 1998). Moreover, natural surface UVB was shown to rupture the transparent animals' intestinal system (Zellmer et al. 2004).

The aim of the present study was to explore effects of atmospheric and elevated UVB-radiation levels on herbivorous and carnivorous/necrophagous (carn/necr) amphipods from Arctic Kongsfjorden. Carapax transmission was measured to approximate the impact of environmental UV-radiation on the animals' soft tissues. Irradiation experiments with a mild and a high UVB-dose, as compared to in-situ light climate, were performed to study UV-sensitivity of exposed animals. In particular, mortality rates and damage to biomolecules like lipids, proteins and DNA as well as protective mechanisms against photo-induced oxidative stress (antioxidant enzymatic systems, sunscreening substances) were investigated. A first set of results on UVB carapax transparency and photo-induced mortality of UV-exposed amphipods is presented here.

Material and methods

Solar UVB-radiation was measured with a 32-channel single-photon counting spectroradiometer installed on the roof of the NDSC-building at Koldewey station. Underwater light climate (0-5 m) in the fjord was recorded with a UVB-

spectroradiometer. Data are still being calibrated and processed and therefore not available to date. With maximal atmospheric UVB-intensities ranging between 0.8 and 1.2 W m^{-2} in July and August 2001 a moderate (0.4 W m^{-2}) and a high UVB-treatment (1.3 W m^{-2}) were chosen for laboratory experiments with amphipods. Experimental irradiation was carried out using white light- and UV-tubes (Q-Panel, type UVA 340) for moderate UVB-exposure and a sunshine simulator (SONSI), providing a solar-like spectrum (developed in the AWI Physics Department by H. Tüg and Fa. IsiTEC, Bremerhaven) for the high UVB-dose.

Two species of Gammarid amphipods were studied: the herbivorous *Gammarellus homari* (Gammarellidae) and the carn/necr *Anonyx nugax* (Lysianassidae). *G. homari* were collected between algae with a handnet between 0-5 m water depth at various stations along the coastline of Kongsfjorden (e.g. Nansen Bay, Hansneset, see Lippert 2003). The original habitats at the Southern coastline decline gradually to 12 m depth, and are colonized with medium and dense macroalgal communities. Hansneset, situated on the Western side of the island Blomstrandhalvøya in central Kongsfjorden is characterised by gradually (inner part) to steeply (outer part) declining rocky bottom, with mostly dense macroalgal communities (M. Assmann, pers. comm.). Adult *G. homari* were mainly associated with red algae (e.g. *Devaleraea ramentacea*), occasionally with brown algae, and could be found at the base of algal thalli. *A. nugax* were collected between 2-5 m depth with baited traps at London, a sampling site on the Southern side of Blomstrandhalvøya, where macroalgae are restricted to single drop stones and boulders. Animals were immediately transferred to the aquarium and kept at 6-8°C and 34 ‰ salinity prior experimentation, seawater being directly supplied from the cove. Only adult amphipods were used in the experiments.

Carapax UVB-transparency of *G. homari* and *A. nugax* was measured. Animals were dissected and the chitinous carapax cleaned from remaining tissue. The carapax was placed on a UV-transparent filter foil (295 nm cut-off filter) and transmission spectra were recorded in the sunshine simulator.

In a first series of experiments, UV-induced mortality was studied in irradiation experiments with Q-Panel-tubes (low dose), only. In each experimental set-up 20 – 33 adult amphipods were exposed in small aquaria (2l volume, 10cm depth) for 5 hrs daily, over 20 days to light intensities of: 0.4 W m^{-2} UVB, 3.7 W m^{-2} UVA and 5.7 W m^{-2} PAR (surface level), resulting in a dose of 1.44 kJ $m^{-2}h^{-1}$ UVB and an experimental daily dose of 7.2 kJ m^{-2} d^{-1}. Between each 5 h irradiation interval the animals received dimmed laboratory light, only (as the control set-up, see below). Over the entire 20 days of experimentation the animals were exposed to a maximal total dose of 144.0 kJ m^{-2} UVB during 100-irradiation hrs. This represents a mild dose (41% on average of atmospheric) compared to maximal surface UVB-doses of 2.88 – 4.32 kJ m^{-2} h^{-1} at noon during the experimental period when maximal UVB-intensities of 0.8 – 1.2 Wm^{-2} were measured. Average atmospheric daily doses for June and July 2000 were 36.6 and 22.3 kJ m^{-2} d^{-1} UVB, respectively (Hoyer et al. 2003). Assuming an attenuation of 53% for UVB per meter water column during the summer months (after Hanelt et al. 2001), the resulting average daily UVB-dose between 0 and 1m depth would range between 17.2 and 10.5 kJ m^{-2} d^{-1}. 100-irradiation hrs in the field would yield similar to higher doses between 135.4 and 203.0 kJ m^{-2} UVB in 1m depth than under experimental conditions. Thus, amphipods in our

experiments experienced 88% on average of 1m in-situ UVB-dose. In the laboratory, different cut-off filters settings for different wavelength ranges were employed: UVB+UVA+PAR (no filter), UVA+PAR (320 nm cut-off), and PAR (400 nm cut-off). Control animals received dimmed laboratory light only and no additional radiation. Where 3 replicate experiments were run means ±SD (standard deviations) are given. Where 2 or 1 replicate experiments were run single values are given (see legends of figures for details).
Herbivores were exposed without macroalgae to avoid shading effects. One group of herbivorous amphipods received algal food between irradiations, while the other was not fed. One group of carn/necr were fed little pieces of fish, while the other group was starved throughout the experimental duration of 20 days. Experiments were checked daily and dead animals counted.
In a second series of experiments, animals were exposed to a high UVB-treatment (1.30 W m^{-2} UVB, daily dose 18.72 kJ m^{-2} d^{-1}) as compared to maximal natural UVB-radiation (see above) amounting to a 35% increase on average of atmospheric UVB-dose. In both experimental UVB-dose-settings sub-samples were taken after 7, 12, 14 and 20 days for further analyses of the antioxidant enzymes superoxide dismutase and catalase, the lipid peroxidation status, ß-Carotene content, and content and composition of mycosporine-like amino acids (MAAs).

Results and discussion

Carapax UVB-transparency

Carapax transmission of adult *G. homari* and *A. nugax* was measured to approximate the impact of environmental UV-radiation on the animals' soft tissues. Figure 1 and 2 show transmission spectra recorded in the sunshine simulator. The following settings were compared: lamp spectrum without filter, spectrum below filter foil without carapax, and spectrum below filter foil plus carapax. Differences in carapax transparencies (as % of filter transmission) between herbivorous and carn/necr amphipods from Kongsfjorden are shown in Table 1.
Lower transparency, i.e. better shading against UVB and UVA was found in the Arctic herbivore *G. homari* compared to the carn/necr *A. nugax*. A higher degree of physical sunscreening may be necessary due to the preference of amphipods to associate with macroalgae, which means they are restricted to a certain water depth and light climate and dependent on the shading effect provided by the algae. Carn/necr amphipods can minimise UV-exposure time in shallow water by actively migrating to greater depths at noon. Carapax material was not further analysed for content of UV-protective substances or pigments.
In both amphipod species from Kongsfjorden carapax transmission is balanced in the UVB and UVA range. This holds as well for the Antarctic herbivorous species *Gondogeneia antarctica* and *Djerboa furcipes* (Tab. 2), which were investigated for UV-tolerance during two Antarctic expeditions in 2000 (Obermüller et al. 2003) and 2002 (Tab. 2).

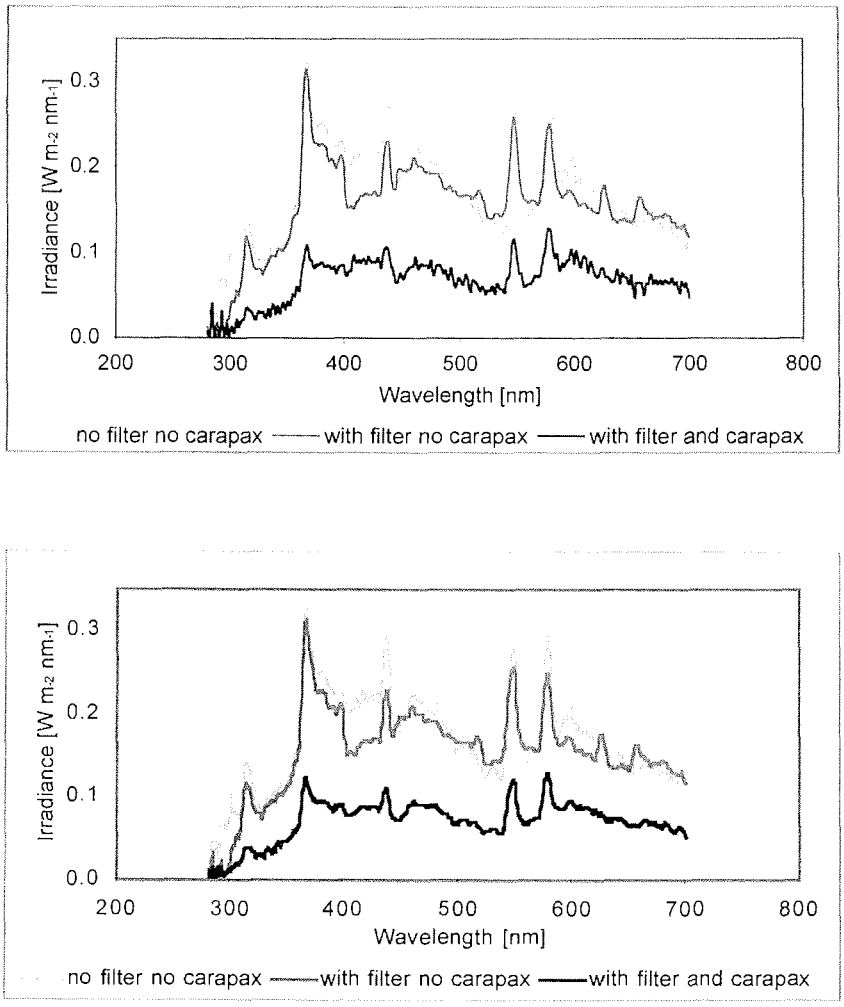

Fig. 2. Carapax transmission spectrum (295-700 nm) of carn/necr *A. nugax* (black line) recorded in the sunshine simulator.

Tab. 1. % Carapax transmission of lamp spectrum light between 295 and 700 nm of amphipods from Kongsfjorden, Arctic.

	% Transmission		
	UVB range (295-320nm)	**UVA** range (320-400nm)	**PAR** range (400-700nm)
G. homari, Arctic herbivore	37.5	36.3	45.6
A. nugax, Arctic *carn/necr*	41.4	41.3	47.4

Tab. 2. % Carapax transmission of lamp spectrum light between 295 and 700 nm of amphipods from Potter Cove, Antarctica.

	% Transmission		
	UVB range (295-320nm)	**UVA** range (320-400nm)	**PAR** range (400-700nm)
G. antarctica, Antarctic herbivore	56.4	58.5	61.2
D. furcipes, Antarctic herbivore	42.4	43.0	49.4

Carapax transparencies of *G. antarctica* are 20% higher in the UV-range compared to *G. homari* from Kongsfjorden, whereas transmission of *D. furcipes* is only slightly higher than in the Arctic herbivore and within the range of the carn/necr *A. nugax*. Both Antarctic species were collected at the same sampling site in the shallow rocky intertidal (0-2m) in Potter Cove, King George Island, where they are associated with macroalgae, which form moderate to dense communities. Despite higher carapax transparency (i.e. lower protection), *G. antarctica* is more agile than *D. furcipes* and actively swimming in the water column above and around the algae even during peak radiation at noon, thus being fully exposed to UV-radiation whilst active. Similar transmission values as Antarctic *G. antarctica* were also measured for the temperate North Sea amphipod *Chaetogammarus marinus*, where transmission was 9-22% higher (i.e. lower protection) in all spectral ranges in comparison to amphipods from Kongsfjorden (Obermüller et al. 2003).

UV-induced mortality

No mortality (100% survival) was found in herbivorous *G. homari* under almost any condition (Tab. 3). Together with the low UV-carapax transmission this reflects a high UV-tolerance of this species and supports its occurrence in various intertidal and subtidal habitats with moderate to dense macroalgal communities. In similar radiation experiments the intertidal Antarctic herbivore *G. antarctica* was slightly more sensitive. 98 and 89% of exposed animals survived a mild UVB-dose (Q-Panel-tubes: 0.38 W m^{-2} UVB, daily dose 6.82 kJ m^{-2} d^{-1}, i.e. 48% on average of in-situ dose) (Obermüller et al. 2003). Starvation further reduced survival of *G. antarctica* by 12% whereas in Arctic *G. homari* starvation had no effect on UVB-survival rates at the applied dose and data of starved and fed animals are shown together in Table 3.

Tab. 3. *G. homari*: Survival of amphipods exposed to a mild UVB-dose (7.2 kJ m^{-2} d^{-1}) during 20 days (20-33 individuals per experiment). Survival rate (%) of all individuals initially exposed. Data as means (±SD) for treatments "control" (3 replicates) and "UVB+UVA+PAR" (3 replicates). 1 experiment for treatment "UVA+PAR". Fed and non-fed animals shown together.

	0 days	10 days	20 days
control	100.0 (±0)	100.0 (±0)	100.0 (±0)
UVB+UVA+PAR	100.0 (±0)	100.0 (±0)	98.8 (±2.5)
UVA+PAR	100.0	100.0	100.0

In carn/necr Arctic amphipods of the species *A. nugax* both, UVB and UVA, led to reduced survival in fed animals (Fig. 4). Various authors have reported UVA to have positive and negative effects on plants and animals by stimulating photoenzymatic repair (PER), but at the same time contributing to the damaging effects of UVR (Williamson et al. 2001 and therein). Until day 7 UVA and UVB appeared equally damaging, where after subtraction of UVB clearly increased survival. As UVA was not selectively cut off, we cannot determine the respective contribution of PER, dark repair and photoprotection to overall UV-tolerance, as defined by Williamson et al. 2001. As a matter of fact, fed amphipods appeared more vulnerable to UVB than starved ones (Fig. 4).

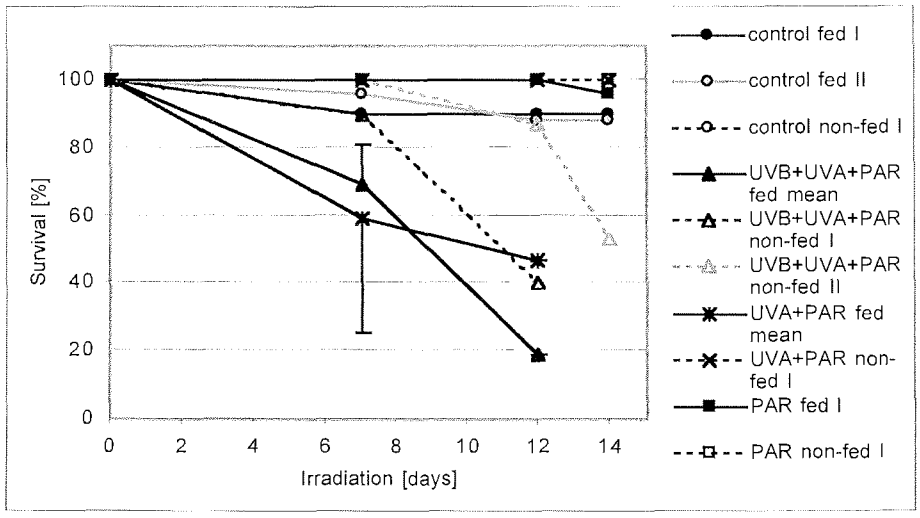

Fig.4. *A. nugax:* Survival of fed (filled symbols) and non-fed (open symbols) amphipods exposed to a mild daily UVB-dose (7.2 kJ m^{-2} d^{-1}) during 14 days (20-30 individuals per experiment). Survival rate of all individuals initially exposed. Data as means ±SD for treatments "UVB+UVA+PAR fed" (3 replicates) and "UVA+PAR fed" (3 replicates). 1 experiment for other treatments: "control fed I", "control fed II", "control non-fed I", "UVB+UVA+PAR non-fed I", "UVB+UVA+PAR non-fed II", "UVA+PAR non-fed I", "PAR fed I", "PAR non-fed I". Experiments "UVB+UVA+PAR fed", "UVB+UVA+PAR non-fed I", and "UVA+PAR fed" were stopped after 12 days due to poor condition of exposed animals.

Polar and sub-polar carn/necr amphipods have been reported to survive between one and several months (Sainte-Marie et al. 1989: *A. nugax*, St. Lawrence estuary, Canada, Chapelle et al. 1994: *Waldeckia obesa*, King-George Island, Antarctica) of starvation mobilising storage lipids, but also inducing metabolic reduction (Chapelle et al. 1994). This is an adaptation to fluctuating food supply in their natural habitat. Chapelle et al. (1994) recorded a decrease of oxygen consumption in *Waldeckia obesa* during starvation over 65 days and a dramatic increase to even higher consumption rates than those, measured in control animals prior to starvation, when the amphipods were fed again. Elevated post starvation rates lasted for 8-10 days. We conjecture, that a possible reason for the higher UVB-vulnerability of *A. nugax* might be the ad libitum feeding with fish and the ensuing high metabolic activity of the animals, which is bound to increase the metabolic production of reactive oxygen species,

and obviously has rendered the animals more susceptible to UVB damage. This is in keeping with the general finding that metabolic reduction can ameliorate production of oxygen free radicals and thereby confers higher stress resistance and longevity to an animal (further reading: Yoon et al. 2002). UVB-exposure of adult females of the copepod *Sinocalanus tenellus* significantly reduced gut pigment content, suggesting radiation to impact on feeding or digestion processes (Lacuna and Uye 2000). On the contrary, in *Daphnia pulex*, a frequently occurring cladoceran crustacean in shallow alpine and Arctic lakes, increasing the quantity of algal food had a positive effect on UV-tolerance, leading to increased survival (Zellmer 1996). In our experiments differences in mortality between non-fed and fed control amphipods amounted to 10% after 12 experimental days, where after no more fed control animals died. 100% survival was found only in non-fed *A. nugax* under all irradiation condition, except when UVB was included. We hypothesize that carn/necr amphipods are more sensitive to abiotic stress during intensive feeding and digestion processes.

Our preliminary results of the high UVB-dose experiments and UVB-induced effects on oxidative stress parameters support the findings presented in this study. The investigated carn/necr amphipods are more vulnerable to UVB-exposure than their herbivorous kin, which was indicated in the clearly differing survival rates of herbivores and carn/necrs (this study). Antioxidant enzyme activities of exposed amphipods seem to be maintained at the same level as in controls (catalase) or induced (SOD) in herbivorous *G. homari* under both high and low UVB-dose, whereas in carn/necr *A. nugax* antioxidant enzyme activities fail to be induced (SOD) or collapse (catalase). This reflects a high UV-tolerance of the herbivore species. The results of the two experimental series will be completed by a detailed analyses and comparison of the amphipods' biochemical defence systems to ameliorate photo-induced oxidative damage.

Our investigations will further evaluate the question whether carn/necr lack UV-absorbing sunscreens (MAAs), which herbivores extract from their macroalgal diet, (Obermüller et al. 2003), and thereby lack a vital part of protective defence against UV-induced damage.

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5. FEEDBACK MECHANISMS FROM THE BIOSPHERE TO THE ATMOSPHERE

Marine macroalgae from Kongsfjorden, Arctic - Comparison of halogenating activity and release of volatile organobromine compounds

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Introduction

Since the discovery that man-made volatile organohalogens are responsible for a massive loss of stratospheric ozone, these compounds have been under scrutiny (Anderson et al. 1991, Solomon 1990). To avoid a collapse of the protecting ozone layer against solar ultraviolet radiation, the production and consumption of man-made volatile organohalogens is now controlled by international regulations (The Montreal Protocol 1989). In line with the industrial emissions, the natural emissions of volatile organohalogens were investigated too and several marine and terrestrial sources of volatile organohalogens were discovered (Laturnus 2001, Laturnus et al. 2002). Extrapolations of global emissions of volatile organohalogens from natural sources into the atmosphere revealed sources strengths comparable to the industrial input (Laturnus 2003, Laturnus this issue). While volatile organohalogens are quite important in atmospheric chemical reactions (Solomon 1990), nothing is known so far why and how organisms produce these compounds. Suggestions to explain the function include chemical defence (Fenical 1975), antimicrobial properties (McConnell and Fenical 1977) and side production in the metabolic system (Küpper et al. 1998). An enzymatic mechanism via haloperoxidases is suggested to halogenate organic matter. However, whether volatile organohalogens are formed by these halogenation processes is still unknown. The present study compares the occurrence of halogenating activity as an indication for enzyme activity in marine macroalgae with the release of volatile organobromine compounds by macroalgae, and will contribute to identify missing pieces of the puzzle on the formation of volatile organohalogens.

Material and Methods

Macroalgae from Kongsfjorden, Arctic, were cultivated in unialgal cultures under growth conditions mimicking the temperature and day length conditions in their habitat. The algae were kept under photosynthetic active radiation (PAR, 20-80 µmol $m^{-2}sec^{-1}$) in 1-litre glass beakers containing filtered seawater from the North Sea enriched with nutrients (Wiencke 1990). The culture medium was changed weekly to avoid nutrient limitation.

To investigate the release of volatile organohalogens, whole algal thalli were used for the incubations. To study the release of volatile organohalogens from different parts of the algae, whole plants were cut into different sections by a

scalpel several days prior the incubation. Enhanced release of volatile organohalogens due to algal wounding was thereby minimised. Replicates of each algal species were placed without headspace in glass bottles filled with culture medium and exposed to photon fluence rates corresponding to the respective habit conditions of the algae. As controls, incubation bottles without algal samples were treated in the same way as the incubations bottles containing algae. After the incubation period, two 100 ml samples of the seawater were analysed by a custom-made purge-and-trap/cryofocusing system coupled to a gas chromatograph with electron capture detector. For identification and verification, the samples were analysed on a PoraPLOT-Q capillary column (length = 22.5 m, diameter = 0.53 mm, film thickness 20 µm). Temperature programme was set to 40 °C for 5 min, 5 °C/min to 180 °C, 180 °C for 10 min.

To determine the halogenating activity in macroalgae, the ability to oxidise bromine was assayed by spectrophotometry at 20°C by measuring the reduction in the absorbance at 278 nm of monochlorodimedone (ε = 19900 $M^{-1}cm^{-1}$) due to the formation of monochloromono-bromodimedone during bromination. Due to the temperature sensitivity of the haloperoxidases all steps of the algal preparation were carried out at temperatures below 5°C. Each algal sample was washed twice with cold distilled water for 30 seconds and blotted with paper tissue. The algal samples were then homogenised for 10 min in 10 ml 0.1 M KH_2PO_4/K_2HPO_4 buffer at pH 6. After centrifugation, crude extracts were immediately used to measure the ability to oxidise bromine (Laturnus et al. 1997). The reaction mixture contained 300 µmol KH_2PO_4/K_2HPO_4 buffer, 60 µmol potassium bromide, 6 µmol hydrogen peroxide, 0.3 µmol monochlorodimedone and 0.5 ml crude extract. The reaction was started by adding hydrogen peroxide. In all cases, blank tests were measured both without adding hydrogen peroxide to the reaction mixture, and with the deactivated extract (deactivation by heating the crude extracts to 70°C for 30 min) (Mehrtens and Laturnus 1997). One unit (U) of enzyme activity is defined for chlorination/bromination as the amount of enzyme capable of catalysing the halogenation of 1 µmol monochlorodimedone per minute (Morris and Hager 1966).

Results

A comparison of the halogenating activity and the release of organobromine compounds by different macroalgae is given in Figure 1. The release of organobromine compounds is given as amounts of bromine released and is calculated from bromoform and dibromomethane, two major compounds released by the algae investigated. The results clearly show a correlation between the occurrence of halogenating activity in the macroalgae and the formation and release of organobromine compounds by the algae. Phaeophytha and Chlorophyta are characterised by much higher halogenating activities and by high release rates of volatile organohalogens compared to Rhodophytas.

Halogenating activity was not distributed homogeneously in the algae. For example, higher activity was found in the blade of *Laminaria saccharina* (L.) Lamour. with a clear maximum of activity in the middle part of the blade (Figure 2). In contrast, holdfast and stipe showed considerably lower halogenating

activities. The release of volatile organobromine compounds from different parts of *L. saccharina* in general resembled the pattern of the detected activity, indicating that place of activity is identical with the formation and release of volatile organobromine compounds. The high release of volatile organobromine compounds from the top part of the blade may be due to tissue damages in this part of the alga.

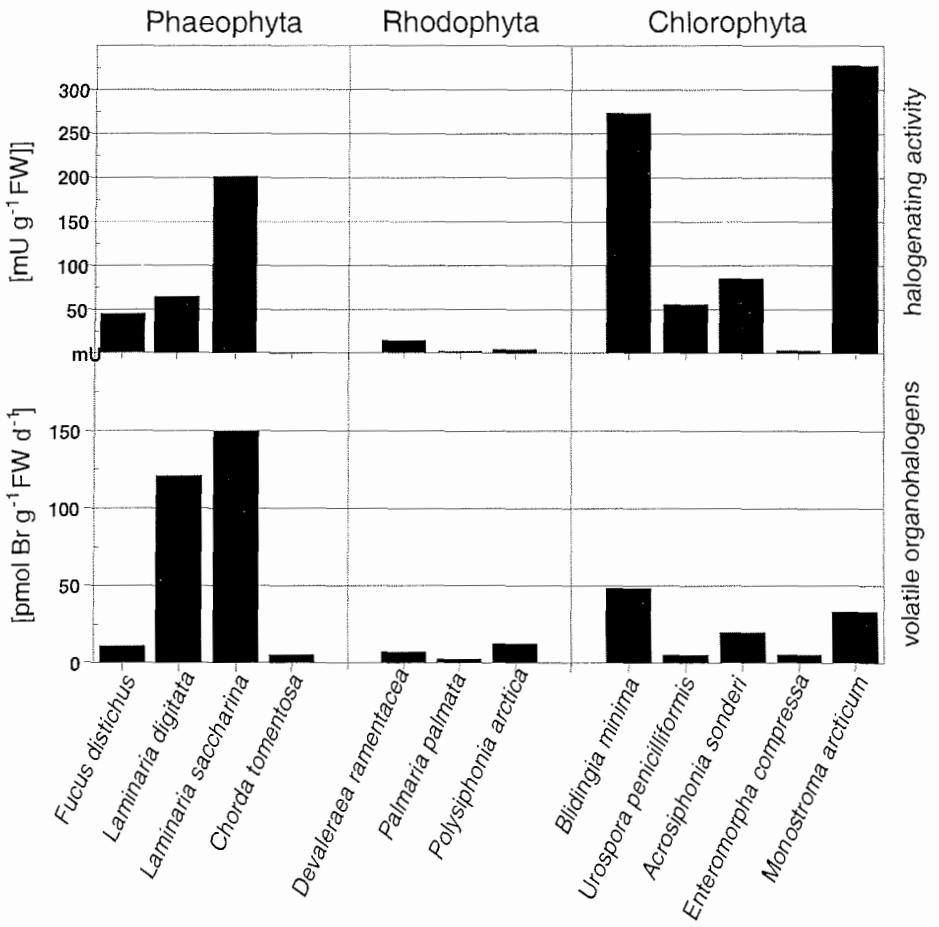

Figure 1. Comparison of release of volatile organohalogens by marine macroalgae and halogenating activity detected in macroalgae. The release of organobromine compounds is given as amounts of bromine released and is calculated from bromoform and dibromomethane, two major compounds released by the algae investigated (n=2, FW = fresh weight).

Discussion

The results clearly show that the ability to halogenate organic matter corresponds to the formation of volatile organohalogens. However, which mechanisms are behind the formation of volatile organohalogens is not yet

known in detail. In general, the formation of organohalogen compounds is based on an enzyme-controlled mechanism (Neidleman and Geigert 1986). Haloperoxidases, an enzyme group detected in a wide range of marine and terrestrial organisms (Yamada et al. 1985, Harper 1993) can catalyse the oxidation of halogens in the presence of hydrogen peroxide to form halogenated organic compounds.

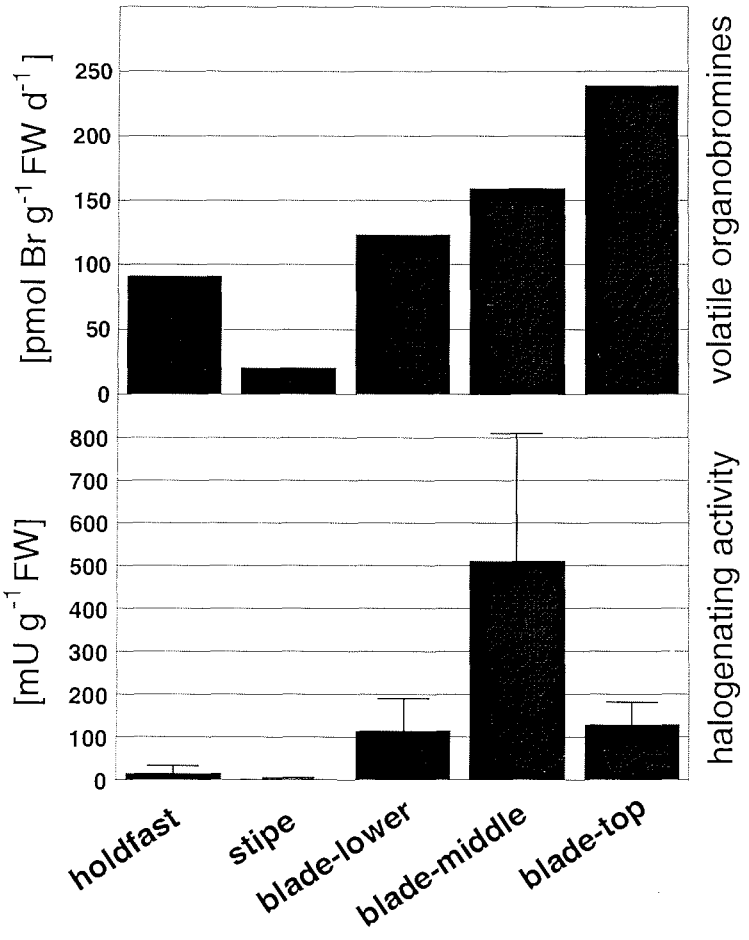

Figure 2. Halogenating activity and release of volatile organobromine compounds detected in different parts of the brown macroalga *Laminaria saccharina* (L.) Lamour. The release of organobromine compounds is given as amounts of bromine released and is calculated from bromoform and dibromomethane, two major compounds released by the algae investigated. (n=3, FW = fresh weight)

Recently, van Pée and Unversucht (2003) reported a novel type of halogenating enzymes called halogenases, which instead of using hydrogen peroxide require nicotinamide adenine dinucleotide (NADH). Halogenase is the reduced form of

nicotinamide adenine dinucleotide, a co-enzyme involved in biological oxidation-reduction processes.

Although the halogenation of organic matter in marine macroalgae is basically understood, the formation mechanisms for the single halogenated C_1 to C_4 compounds remain unknown. Metabolic pathways by which volatile organohalogens like bromoform or dibromomethane are synthesised were discussed by several authors (Theiler et al. 1978, Fenical 1975, Burreson et al. 1976). Intracellular halogenation of ketones present in algae followed by decay via the pH dependant haloform reaction can lead to the formation of polyhalogenated methanes like bromoform or dibromomethane. Significant linear correlations between the two compounds are an indication for this mechanism (Laturnus 1995). Another pathway may be the reaction of hypobromeous acid, an extremely reactive species, with organic matter to form volatile organohalogens. Hypobromeous acid can be formed by haloperoxidases located near the algal surface and then released into seawater (Wever et al. 1991). Mixed halogenated hydrocarbons like dibromochloro-methane or chloroiodomethane probably were also directly formed by macroalgae (Burreson et al. 1976) or by nucleophilic substitution of *e.g.* bromoform or diiodomethane with chloride ions present in the seawater (Class and Ballschmiter 1988). In algae, Laturnus et al. (1997) did not detect chlorinating activity necessary for the enzymatic formation of organochlorine compounds. Therefore, a direct incorporation of chlorine into organic matter may not be possible. However, Geigert et al. (1984) reported the formation of bromine and chlorine containing compounds in the presence of bromoperoxidase, suggesting that chloroperoxidases may not be necessary for the formation of chlorinated compounds. Halogenated C_2 to C_4 hydrocarbons are not accessible by haloform reaction. The formation mechanism of these compounds is still unknown. A suggestion is the enzymatic halogenation of alkenes (Geigert et al. 1984).

Methyl halides like bromomethane, apparently, were not formed by enzymatic reaction via haloperoxidases as no correlation between halogenating activity and methyl halide release was found for polar macroalgae (Laturnus et al. 1998). An earlier postulated mechanism involving dimethylsulfoniopropionate (DMSP) as a precursor (White 1982) is unlikely too as no correlation between methyl halide releases and DMSP concentrations in the macroalgae was found (Laturnus et al. 1998). At present, a third possibility based on the catalysis via a methyltransferase reaction is discussed (Wuosmaa and Hager 1990, Saini et al. 1995). A methyltransferring enzyme isolated from marine macroalgae and higher plants can catalyse the S-adenosyl-L-methionine-dependent methylation of the halides Cl^-, Br^-, I^- to the respective methyl halides.

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On the global importance of marine macroalgae as a source for volatile organohalogens – An extrapolation from screening studies of macroalgae from Kongsforden, Arctic

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The problem of stratospheric ozone depletion

In 1974, M. Molina and F. S. Rowland published a theoretical paper in Nature suggesting that man-made substances, such as chlorofluorocarbons (CFCs), introduced into the troposphere can be capable of rising unchanged into the stratosphere and can play a major role in the depletion of stratospheric ozone. Almost 10 years had to pass before scientific proof of this hypothesis was obtained through the observation of a massive loss of stratospheric ozone over Antarctica (Farman et al. 1985). The depletion of the stratospheric ozone layer causes an increase of ultraviolet radiation reaching the earth's surface and can lead to many types of damage on organisms and ecosystems (Johanson et al. 1995, Franklin and Forster 1997). To counteract the ongoing ozone depletion, today's effort mainly focus on reducing or omitting industrial emissions of volatile organohalogens. However, besides the industrial release also a natural input of volatile organohalogens was identified, whose sources hardly can be controlled by political regulations. Knowledge of the contribution of natural sources to the global emission of volatile organohalogens is essential to understand nature's part in ozone depletion, and to estimate the effect of global climate changes on the input of naturally produced volatile organohalogens into the atmosphere.

Industrial and natural sources of volatile organohalogens

Chlorofluorocarbons (CFCs) and methyl bromide are the first industrially produced volatile organohalogens under scrutiny. Due to their low chemical reactivity and long atmospheric life time, CFCs and methyl bromide can be transported into the stratosphere and act as a source for halogens involved in ozone destruction. However, other volatile organohalogens, such as methyl chloride (CH_3Cl), chloroform ($CHCl_3$), tetrachloroethene (C_2Cl_4), trichloroethene (C_2HCl_3), and dichloromethane (CH_2Cl_2), can be important too for stratospheric ozone chemistry due to the widespread use as industrial solvents, fuel components, and additives to manufactured products (Collins et al. 2001). Other sources are fossil-fuel combustion, incineration, pulp and paper manufacturing, water treatment (Keene et al. 1999), but also biomass burning for agricultural reason, as about 90% of today's biomass burning is induced by humans. Only a minor fraction is considered to be entirely natural, *e.g.* caused by volcanic eruptions and lightning (Lobert et al. 1999).

A possible natural formation of volatile organohalogens was first suggested in the mid seventies of the last century (Lovelock 1975). Since then a large variety

of volatile organohalogens was identified to be of natural origin (Gribble 2003). In the marine environment, the oceans are emitting large amounts of volatile organohalogens into the atmosphere (Lovelock 1975). Although only covering 29% of earth's surface, the terrestrial ecosystem equals the marine environment as a source for volatile organohalogens. Among the identified terrestrial sources are wetlands, peat lands, coastal salt marshes, tropical plants, rice fields, soil, termites, volcano and geochemical processes in the earth's crust (Laturnus et al. 2002).

Macroalgae as a source for volatile organohalogens

In the oceans, large amounts of volatile organohalogens were detected in areas with high biological activity (Manley et al. 1992). Lovelock (1975) first proposed that the large kelp *Laminaria* was responsible for high levels of methyl iodide, which he measured in a coastal region of Southwest Ireland. Until today, several macroalgal species from different climate regions were found to emit volatile organohalogens (Manley et al. 1992, Nightingale et al. 1995, Laturnus 1996). Screening studies conducted with marine macroalgae collected at Kongsfjorden, Arctic, revealed the emission of a large variety of volatile organohalogens (Laturnus 1996). Bromoform is the main compound emitted by macroalgae with up to 20 to 30-fold higher released amounts compared to other mainly released compounds such as methyl chloride and dibromomethane. Besides bromine-containing compounds also iodine and chlorine-containing compounds are released by macroalgae (Laturnus 2001). However, the released amounts are much smaller compared to volatile bromine-containing compounds, which in fact is surprising, especially when considering the abundant occurrence of chloride, compared to bromide, in the oceans. Apparently, the metabolism of most marine macroalgal species cannot incorporate chloride, perhaps due to its higher oxidation potential compared to bromide and iodide.

Global importance of volatile organohalogen emission by macroalgae

The discovery of a natural input of volatile organohalogens raised the question to what extent macroalgae contributes to the input of volatile organohalogens into the atmosphere. A comparison between the atmospheric input of chlorine originating from volatile organohalogens revealed a similar importance of industrial and natural sources (Table 1). Natural sources are even more important with respect to the atmospheric input of bromine and iodine, as the industrial input is negligible or does not exist. From industrial production data, the annual industrial input is estimated to 2500 Gg chlorine and 100 Gg bromine. Global extrapolations of results obtained from single sources investigated, lead to an annual natural emission of 2000 Gg chlorine, 1600 Gg bromine and 400 Gg iodine. Among the natural sources, the terrestrial environment resembles the marine environment in the annual emission of chlorine. In contrast, natural organic bromine and iodine are almost entirely of marine origin. The only so far identified terrestrial sources are insignificant (Laturnus 2003).

Table 1. Estimated industrial (ind) and natural (nat) emissions of volatile organohalogens.

source	CH_3Cl [Gg Cl yr^{-1}]		CH_3Br [Gg Br yr^{-1}]		CH_3I [Gg I yr^{-1}]		$CHBr_3$ [Gg Br yr^{-1}]		$CHCl_3$ [Gg Cl yr^{-1}]		other [Gg Cl yr^{-1}]		reference
	ind	nat	ind	nat	ind	nat	ind	nat	ind	nat	ind	nat	
oceans[c]		460		123		322		1230		303			Nightingale et al. 1995, Gribble 1999, Keene et al. 1999, Lobert et al. 1995, Laturnus 2001
biomass burning	640		25						2		62		Keene et al. 1999, Manö and Andrea 1994,
incineration	32												Keene et al. 1999
industry	7		52				24[g]		62		1567		Keene et al. 1999, Lobert et al. 1999, Itoh and Shinya 1994
fossil-fuel combustion	75										5		Keene et al. 1999
macroalgae[a]		<1		<1		<1		128		1			Laturnus 2001, Nightingale et al. 1995
microalgae[b]		3		3		36		66		20			Scarratt and Moore 1998, Gribble 1999, Sæmundsdóttir and Matrai 1998
soil[e]		112								178			Khalil et al. 1999, Keene et al. 1999
termites										89			Khalil et al. 1990
rice fields[f]										20			Khalil et al. 1998
peatland ecosystems		4		1		1				4			Dimmer et al. 2001
forest[d]										4			Haselmann et al. 2000
wetlands		34		4									Varner et al. 1999
tropical plants		639											Yokouchi et al. 2002
coastal salt marshes		120											Rhew et al. 2000
total (Gg halogen yr^{-1})													
- **industrial**	**754**		**77**		**-**		**24**		**64**		**1634**		
- **natural**		**1372**		**131**		**359**		**1424**		**619**			

[a]calculated with estimated global macroalgae biomass of 2.8×10^{14} g (Carpenter and Liss 2000) and release rates determined by (Laturnus 2001)
[b]calculated with estimated global microalgae biomass of 1.44×10^{12} g chl *a* (Behrenfeld et al. 2001)
[c]oceanic flux minus emissions from micro and macroalgae
[d]for Northern temperate forests
[e]global land area of 68.5×10^6 km^2 excluding polar areas
[f]calculated with estimated global rice field area of 1.45×10^{12} m^2 (Redeker et al. 2000)
[g]inland and coastal nuclear power plants, inland fossil fuel burning, desalination, domestic and industrial water (Quack and Wallace 2003)

The oceans are considered to account for around half of the annual natural input of chloroform, methyl chloride and methyl bromide into the atmosphere, and around 90% of the annual natural input of bromoform and methyl iodide into

the atmosphere. An extrapolation using emission studies performed with macroalgae from Kongsfjorden showed that the annual contribution of macroalgae to the global emission of volatile organohalogens is negligible (<0.1%). Only bromoform with approximately 10% is more important (Figure 1). However, recently microalgae have been suggested to be a source for volatile organohalogens too (Baker et al. 1999). On a global scale, microalgae contributed twice as much as macroalgae. However, macroalgae and microalgae together account for less than 10% of the estimated annual emission of volatile organohalogens from the oceans into the atmosphere. In some regions the oceans are acting as a sink for volatile organohalogens (Lobert et al. 1997), but even this cannot explain the missing sources. The transfer, for example, of methyl bromide from the atmosphere into the oceans is estimated to 110 Gg bromine (Lobert et al. 1995). Other unknown sources must exist. Possible sources could be oceanic sediments, bacteria, geochemical processes.

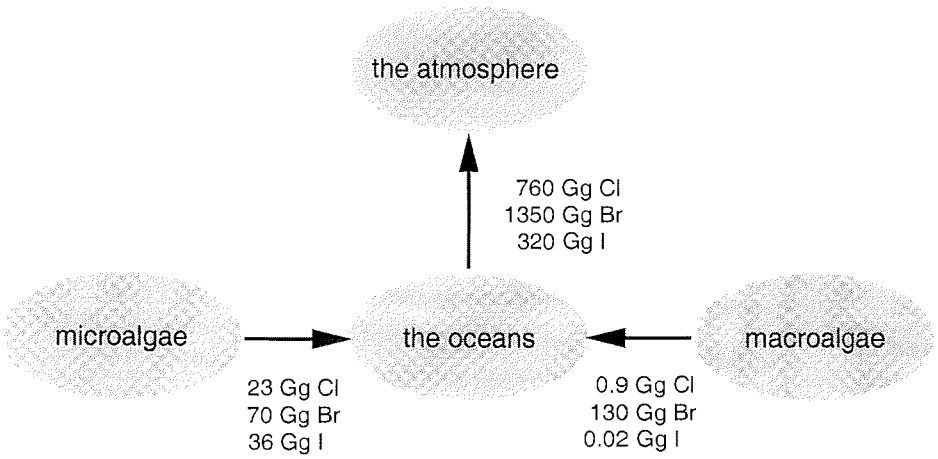

Figure 1. Contribution of two at present identified marine sources of volatile organohalogens to the input of organic halogens from the oceans into the atmosphere.

Influence of global climate changes

The importance of natural sources for the input of volatile organohalogens into the atmosphere may increase in future. Relevant compounds involved in ozone depletion, such as methyl halides, chloroform, bromoform, are emitted in large amounts by natural sources. Different to the industrial emissions, the natural part cannot be controlled by humans, but humans can influence it. Global climate changes as the result of human activities and uncontrolled eutrophication of the environment can effect the natural emission of volatile organohalogens. A study of macroalgae from Kongsfjorden, Arctic, Laturnus *et al.* (2000) revealed abiotic factors, such as temperature and nutrients, to

influence the release of volatile organohalogens by macroalgae. Recently, a study conducted on macroalgae from Kongsfjorden showed that elevated levels of solar ultraviolet radiation lead to an increase in the emission of volatile organohalogens by macroalgae (Laturnus *et al.* 2004). Although the regulations to reduce the input of CFCs and other man-made ozone depleting substance became effective (Tabazadeh and Cordero 2004), other factors like illegal production of ozone depleting substances (Spurgeon 1997) and large scale biomass burning may delay an early recovery of the stratospheric ozone layer. Furthermore, the warming of the troposphere at the same time leads to a cooling of the stratosphere and supports a further depletion of stratospheric ozone. A delay in recovery of the stratospheric ozone layer, however, means that elevated levels of ultraviolet radiation continue to reach the surface of the earth. Therefore, natural sources must be seen in a new light considering their source strength for volatile organohalogens, and must be included into future discussions on global climate changes.

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