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The Antarctic ecosystem of Potter Cove, King-George Island (Isla 25 de Mayo) Synopsis of research performed 1999-2006 at the Dallmann Laboratory and Jubany Station

Edited by Christian Wiencke, Gustavo A. Ferreyra, Doris Abele and Sergio Marenssi

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Introduction

In 1994 the Dallmann Laboratory was integrated into the Argentinian station Jubany on King George Island (Isla 25 de Mayo, 62°14' S 58° 31' W), as part of an international agreement signed between Germany and Argentina. The laboratory is jointly operated by the Instituto Antártico Argentino/Direccíon Nacional del Antártico and the German Alfred Wegener Institute for Polar and Marine Research in Bremerhaven. Since 1996 the Netherlands Organisation for Scientific Research (NWO) has participated in this cooperation and in the Dallmann Laboratory, presently the only tri-national research facility in the Antarctic Peninsula area. A new contract outlining the future development of the scientific cooperation at Dallmann Laboratory was signed between Germany and Argentina in April 2006. Starting in the International Polar Year (IPY) 2007/08, a limited number of places in the Dallmann laboratory are made available for internships of University students from different countries, to enable their participation in ongoing research projects.

View from Potter Cove to Jubany Station with the Dallmann Laboratory in front of the Three Brothers Hill (Tres Hermanos)

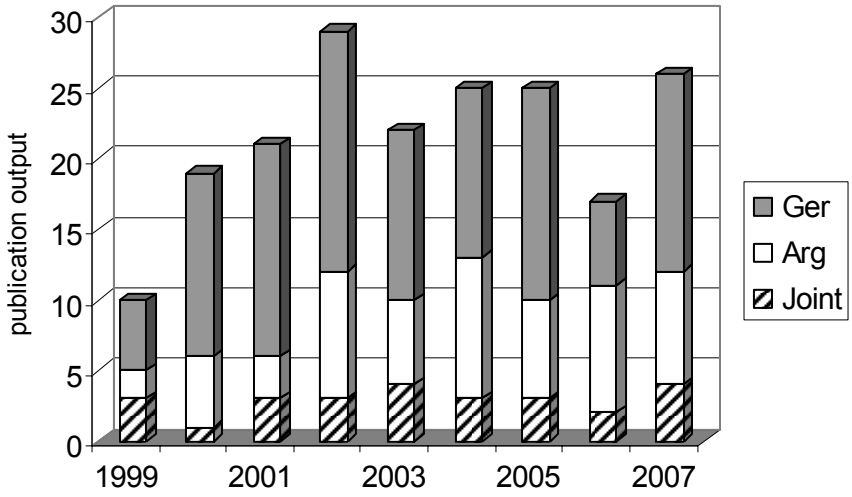
Named after the German explorer Eduard Dallmann, the Dallmann Laboratory houses 14 scientists during the Antarctic summer season and one overwintering scientist in winter. Research facilities include 7 wet and dry laboratories, a liquid nitrogen plant, a workshop and storage rooms. Communication with the rest of the world is possible via internet and satellite telephone. A biological sewage plant is connected for waste water treatment. Importantly, Dallmann/Jubany is a diving station, equipped with several boats for fieldwork and all necessary support to carry out safe diving operations in the Antarctic. This includes a decompression chamber for emergency treatment of diving accidents on King George Island.

Dallmann Laboratory is situated adjacent to Jubany Station at Potter Cove, a small inlet of Maxwell Bay (Bahía Guardía Nacional). It is surrounded by ice fields, glaciers and the prominent Three Brothers Hill, a tertiary andesic plug at the entrance of the cove. The marine environment of Potter Cove combines zones of glacier fronts, rocky shores and soft bottom areas, providing scientists with diverse habitats and ample opportunities to conduct research in different subsystems of the coastal communities in the maritime Antarctic. The area around the station offers working ground for geologists, geophysicists, glaciologists, hydrologists, soil scientists and terrestrial biologists. All in all, conditions in and around the base are optimal for scientific activities.

The main focus of research in the Dallmann Laboratory is on the communities of the marine terrestrial transition zone and the shallow water environment of Potter Cove. The ice-free areas in front of the Collins glacier are of interest to biologists and earth scientists. In the past, Jubany was one of the core stations of the SCAR-EASIZ programme, aimed at studying the bentho-pelagic coupling and the physiology and ecology of species and communities of the Antarctic pack-ice zone. During the International Polar Year 2007/2008 the Dallmann Laboratory is a core facility of the clicOPEN-Programme (IPY activity 34) which conducts co-ordinated research on climate change in the Antarctic Peninsula region and investigates the effects on the terrestrial and marine communities in an interdisciplinary and international collaboration. Moreover, the laboratory serves as basis for parts of the national Antarctic research programmes of Argentina, Germany and The Netherlands, directed at coastal research in maritime Antarctic.

Over the last 13 years a great number of individual projects have been performed at Dallmann Laboratory and Jubany Station. Many of these projects were planned and conducted in co-operation between our countries and involved a great number of master and doctoral students on both sides. This has resulted in many exciting new findings and initiated intensive co-operations between Argentine and German laboratories, involving not only the core institutions IAA and AWI, but several other universities and research institutions in both countries. A first summary of the results obtained between 1994 and 1997 was provided in the first Dallmann/Jubany Synopsis published in 1998 as Reports of Polar Research (299/1998). Beside numerous theses, over 190 new papers on research work from Dallmann and Jubany Laboratory have been published in international peer-reviewed journals since 1999 (see appendix at the end of this volume). We believe that 193 peer reviewed papers is a significant output for 8 Antarctic summer seasons in Dallmann-Jubany, where the Antarctic weather conditions restrict the “good sampling days” to a very limited number in each month and year. It means that on average 24 papers were published per field season, without considering thesis reports and numerous short contributions to meetings and symposia. However, there are always grounds for improvement. The graph below analyses the publication output of Jubany/Dallmann, indicating contributions from German (grey) and Argentinean (white) scientists and also showing as hatched part the proportion of Argentinean-German joint papers. Clearly the publication output on both sides has substantially increased since 1999. However, the number of joint publications is

surprisingly as low as 14% of the whole publication output. Further, those groups with the most intensive Argentinean - German cooperation over the last years also published the highest absolute numbers of papers. Obviously, therefore, cooperation increases the total number of publications, and we hope this can be a motivation in future work.



Number of German, Argentinean and jointly authored, peer-reviewed publications based on research in the Dallmann Laboratory and Jubany Station between 1999 and June 2007 covering the output from eight Antarctic field seasons. The list of all publications is found as appendix at the end of this volume.

Also, evidently young scientists on both sides were more open to the cooperation than established scientists, and we would like to encourage the oncoming generation of scientists to sustain and amplify their joint activities. The intensified scientific output at the beginning of the IPY indicates this to be a good opportunity for a fresh summary and to credit the recent progress in a second Dallmann/Jubany Synopsis, which we wish to present in the present volume of the Reports from Polar Research.

Chapter 1 addresses the environment of Potter Cove and links physics and earth science to biological research. Chapter 2 deals with the structure and function of the ecosystem. The ecophysiology of key organisms is dealt within chapter 3. Chapters 4 and 5 focus on the response of key organisms and communities to global and regional as well as to anthropogenic changes. Two of the available long term data sets are presented in chapter 6. We hope the synopsis will provide a useful baseline for future research and strengthen the scientific collaboration between the countries involved.

We also take this opportunity to thank the Argentinean crews maintaining the station and supporting work at the Dallmann Laboratory over the last 13 years. Moreover, we are indebted to the Argentine and German logistics departments for their perpetual strong support, in particular Guido Kleffel and Heinz Ahammer.

Further we would like to thank the Argentine and German diving teams, especially Max Schwanitz. Without the strong diving support much of the sampling and experimental work would never have been possible. The same holds true for the support by our technical staff, in particular Oscar Gonzalez and Richard Steinmetz who supported the technical part of the field work and the maintenance of the Dallmann Laboratory. Especially, we would like to remember Augusto “Alfa” Thibaud and Teófilo González, who lost their lives in September 2005 in Jubany.

Last, but not least we thank all authors in this synoptic volume for their contributions and the referees José Aguilera, Julia Boike, Fritz Buchholz, Anita Buma, Chuck Amsler, Graeme Claridge, Nestor Coria, Wilhelm Hagen, Walter Helbling, Dieter Hanelt, Elisabeth Helmke, Ulf Karsten, Rainer Knust, Jürgen Laudien, Eva Leu, Félix López-Figueroa, Hubert Miller, Markus Molis, Donna Patterson-Fraser, Franz Riemann, Bernd R. Schöne, Otto Schrems, Rod Seppelt, Michael Spindler, Dorothee Stübing, Sven Thatje, Dick Veit and Dieter Wolff-Gladrow for their constructive criticism.

Bremerhaven and Buenos Aires, in October 2007

Christian Wiencke, Gustavo A. Ferreyra, Doris Abele & Sergio Marenssi

1. THE ENVIRONMENT OF POTTER COVE

Two years of *in situ* UV measurements at Dallmann Laboratory/ Jubany Station

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1. Introduction

Solar radiation measured at the Earth's surface is subject to atmospheric absorption and scattering by air molecules, trace gases, aerosols and clouds. Of great importance for life on Earth is the photosynthetically active radiation (PAR, wavelengths: 400-700 nm) and ultraviolet (UV) radiation. UV radiation is sub-divided into UVC (<280 nm), UVB (280-320 nm) and UVA (320-400 nm). While the UVC radiation is completely absorbed in the atmosphere, some UVB can reach the Earth's surface. With the stratospheric ozone layer at an altitude of 15 to 30 km thinning (e.g. Nardi et al. 1999), more of the energetic and biologically effective UVB radiation can reach the Earth's surface and can damage living organisms (Environmental Effects Assessment Panel, 2006). Towards shorter wavelengths spectral UVB irradiance measured at the Earth's surface shows a steep decrease over six orders of magnitude (see Figure 1).

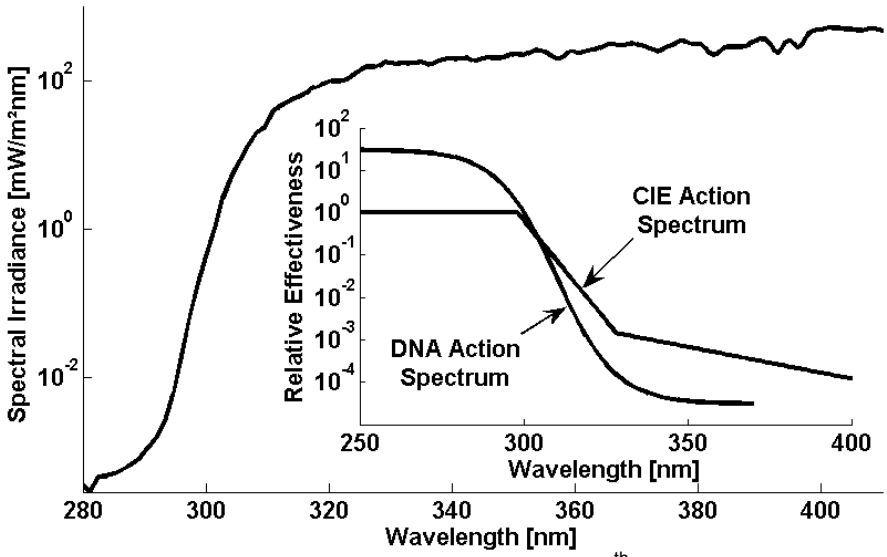

Figure 1: Spectrum of UV irradiance measured on 15th October 2005 aboard the research vessel *Polarstern*. The smaller picture shows the DNA and erythemal action spectrum. To obtain the biologically effective irradiance, the UV spectrum needs to be convoluted with the action spectrum.

Particularly organisms in Antarctica are exposed to enhanced UV irradiance every spring due to the transient loss of 70 to 80% of the stratospheric ozone, the so-called "ozone hole" (WMO, 2006). Enhanced UV radiation can penetrate deeper into the water column, thereby affecting marine organisms. Therefore it is of great importance to study the attenuation of UV in the water body. All kinds of particles in the water modify the radiation by scattering and absorption

processes. Due to refraction at the water surface, the apparent solar zenith angle under water differs from the one in the atmosphere.
At Dallmann Laboratory/Jubany Station (King George Island, Antarctic Peninsula), UV measurements have been carried out with broadband instruments since the 1990s (Gómez *et al.* 1997). A ground based UV spectroradiometer is in use during the summer seasons since 2002.

Here, we report UV and PAR measurements with broadband instruments on ground and under water for the summer season 2004/05 as well as ground based spectral measurements for 2004. Additionally, first measurements with a submergible multichannel UV spectroradiometer have been performed during two summers to characterize the seasonal variation of PAR and UV transmittance in the water column. We give a brief description of the instruments used, the measurement techniques, data quality control, and present results obtained during the austral summers 2003/04 and 2004/05.

2. Radiation detectors

Broadband sensors measure the radiation over a wide spectral range. Some instruments have a response similar to the erythemal action spectrum (McKinley and Diffey, 1987), others integrate the incident radiation without weighting. Broadband instruments are comparably cheap and easy to handle.
The advantage of spectrally resolved measurements of UV irradiance is that any type of action spectrum can be applied to the data. However, these instruments need careful calibration in the laboratory and in the field. Two major challenges have to be tackled when measuring spectral UV irradiance, which result from the properties of the solar spectrum: First, the wavelength calibration has to be very precise. According to Bernhard and Seckmeyer (1999) the misalignment of 0.1 nm in the UVB regime implies an error of up to 3.5% for the DNA weighted UV irradiance. UV data have to be corrected for possible wavelength shifts. A widely used method is the comparison of the UV spectra to an extraterrestrial solar spectrum (Slaper *et al.* 1995). Second, UV sensors have to be able to record the incident irradiance over six orders of magnitude. This is due to the strong ozone absorption of UVB irradiance in the atmosphere (see Figure 1). Thus, UV spectroradiometers have to be capable of detecting high intensities without saturating the detector and of suppressing noise and stray light well enough to give reliable data for the shorter wavelengths down to the detection limit.

2.1 Broadband sensors

A LiCor data logger (LI-1400, Li-Cor, USA) equipped with a flat-head cosine corrected PAR quantum sensor for air and underwater measurements (LICOR 190 SA and LI-192, respectively) was used to record PAR values at the surface (5 min intervals in summer 2004/2005) and under water. For the underwater measurements two sensors were used, one fixed at 1 m depth and the other lowered in 1 m steps. A Solar Light (PMA2100, Solar Light Co. Inc., USA) with a UVB (PMA2106-UW) and a UVA (PMA2110-UW) radiation broadband sensor was used for weekly radiation measurements, both at the surface and at certain depths under water in 2004/05.

2.2 UV spectroradiometers

The land based UV spectroradiometer designed by ISITEC GmbH consists of a separate UVA and UVB sensor. It was installed on the roof of the Dallmann Laboratory. As the solar UVA spectrum does not cover such a big range of orders of magnitude, it is sufficient to use a single monochromator for the UVA spectroradiometers. The detector consists of a 256 channel diode array. The UVB sensor is equipped with a Bentham DM 150 double monochromator and a 32 channel photomultiplier plate. The signals of all channels are recorded simultaneously, and 5-minute means were stored. For a complete description of the instrument we refer to Hanken and Tüg (2002).

The underwater measurements have been carried out with an underwater spectroradiometer, similar to the ground based instrument, mounted in a waterproof housing. The UVA sensor covers also the PAR region and was developed by Kruse. The diode array detector (MOS Linear Image Sensor S3901-265Q, Hamamatsu, Japan) is composed of 265 pixels. The spectral distance between two neighbouring channels is 1.6 nm, resulting in a wavelength range of 300-700 nm. The UVB sensor measures irradiance from 280 to 323 nm. The spectral distance between two channels is 1.35 nm. The power supply during field campaigns was realised by storage batteries. The underwater instruments were deployed from a small zodiac. For taking a vertical profile of UV irradiance in the water column, the sensor was usually put into the water in steps of 1 m to record data for one minute.

3. Calibration and correction of the spectral data

In contrast to the ground based spectroradiometer, the underwater ones are not temperature stabilized. Dark current and sensitivity of the instruments strongly depend upon temperature. Reliable absolute irradiance values can only be obtained by calibrating the instruments at the ambient water temperature. Otherwise only relative data can be calculated. The water temperature does not change much with depth (compare with Hanelt *et al.* 2004), so sensitivity and dark current are expected not to vary during the measurement time. The dark current of the instruments is determined before and after each field measurement by closing the optics with a black cover.

The spectral instruments provide raw data S in counts per second for each channel. The sensitivity R of each channel is determined in the calibration process, where a lamp with known emittance E is measured. Correcting for dark current DC we obtain $R = (S-DC)/E$ (1). With known sensitivity R, equation 1 can be rearranged to calculate the spectral irradiance $E = (S-DC)/R$ (2).

The underwater spectra obtained during the field campaigns 2003/04 and 2004/05 were corrected in the following way: Their wavelength shift was corrected by comparing with the Kurucz extraterrestrial solar spectrum (Kurucz, solar flux atlas). It was generally less than 1 nm. The data cannot be given in absolute values, due to missing calibration at the corresponding temperature, but after dark current correction they were normalized to the surface value. The UV irradiance at different depths is hence given in per cent of the surface value. In the following examples, the UV irradiance was integrated over wavelength for the different parts of the spectrum, UVB, UVA and PAR.

During the measurement time required to complete a profile, atmospheric conditions may change resulting in enhanced or decreased values of UV at

greater depths. In this case parallel ground based UV irradiance measurements can be used to correct for this effect. Even on almost cloud free days, which are quite rare at the Antarctic Peninsula, the changes of radiation due to thin, barely visible cirrus clouds are not negligible.

4. Area of investigation

Land based measurements were performed directly at Dallmann Laboratory/Jubany Station, King George Island, Antarctica, during the austral summer seasons 2003/2004 and 2004/2005. Three locations were chosen to perform underwater measurements (see Figure 1 in Zacher & Campana, this issue): (i) Peñón de Pesca (62°14'S 58°43'W; max. measured depth 14 m), (ii) the entrance of Potter Cove between location (i) and (iii) (62°13'S 58° 40'W; max. measured depth 20 m) and (iii) Peñón Uno (62°14'S 58°41'W; max. measured depth 7 m). Potter Cove is a bay opening towards the west. Due to a cyclonic circulation pattern, fresh water from the open ocean enters the cove via the measuring point Peñón de Pesca. Then the water passes the area of a glacier in the east of the bay (measurement point Potter Cove), and finally passes Peñón Uno (see also Roese and Drabble, 1998).

5. UV field measurements

5.1 Land-based UV irradiance

Highest daily UVA and UVB doses were observed in December whereas in the austral winter UV radiation does not reach the surface (Figure 2). A high seasonal variation and day to day variability depending on cloud cover was observed.

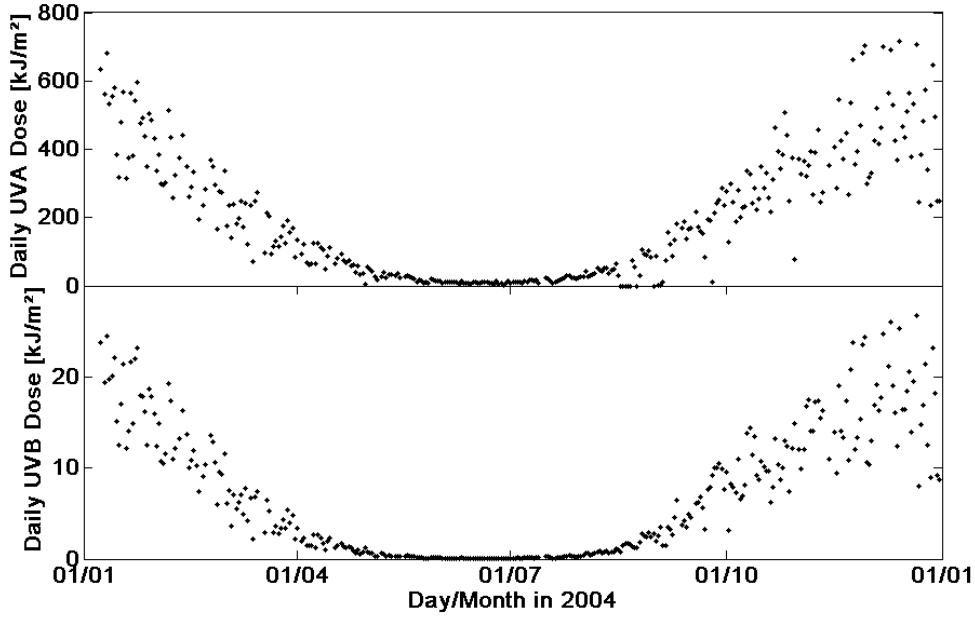

Figure 2: Daily dose of UVA (upper panel) and UVB (lower panel) irradiance measured with the ground based spectroradiometer at Dallmann Laboratory/Jubany Station in 2004.

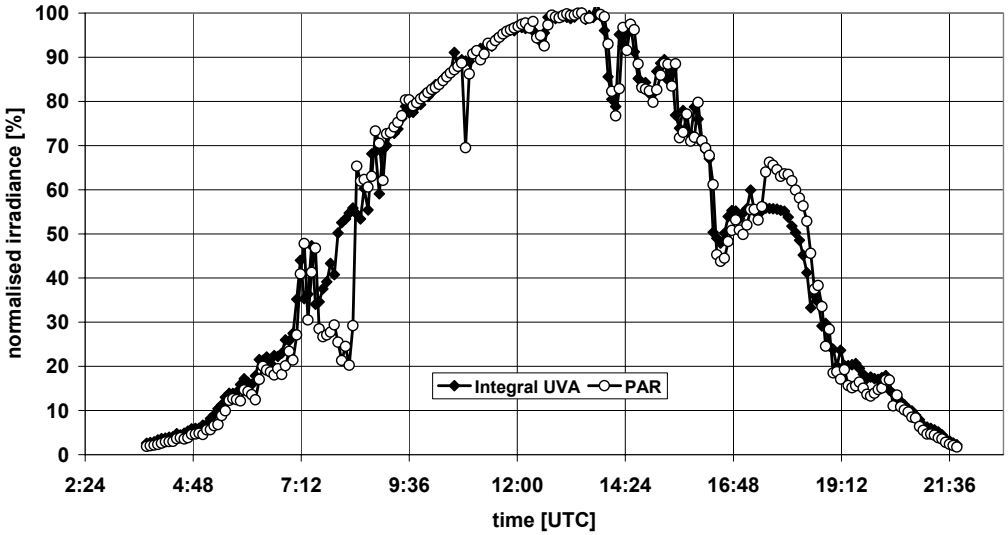

Figure 3: PAR and UVA measured at Dallmann Laboratory/Jubany Station on 14th December 2004 on a mostly sunny day. The values of each data set are divided by their maximum value to better illustrate the differences. Without clouds, both curves are similar. When clouds were passing, high differences in radiation (up to 35%) were detected by the two systems.

Also the PAR measurements (2004/2005) showed a high variability, both from day to day as within one day (Figure 3).

Table 1: 10% depth and k_d value of UVB, UVA and PAR ± S.D. at the three different sampling areas measured with broadband and spectral instruments.

Mean 10% depth [m], k_d	**Peñón de Pesca**			**Potter Cove**			**Peñón Uno**		
Broadband 10% depth	**UVB**	**UVA**	**PAR**	**UVB**	**UVA**	**PAR**	**UVB**	**UVA**	**PAR**
Nov/Dec 04	2.7 (±2.3)	5.5 (±4.9)	-	1.7 (±1.0)	2.6 (±2.1)	-	1.9 (±0.2)	3.1 (±2.0)	-
Jan/Feb 2005	4.3 (±1.9)	7.1 (±1.6)	10	1.7	0.8	-	2.1 (±0.8)	3.9 (±1.7)	3.5
Spectral 10% depth	**UVB**	**UVA**	**PAR**	**UVB**	**UVA**	**PAR**	**UVB**	**UVA**	**PAR**
Nov/Dec 2003	10	3.5	12.2	6.6 (± 1.3)	5.8	8.1 (±0.1)	4.5 (±0.1)	3.6	4.5
Jan/Feb 2004	4.9 (±3.9)	-	-	-	-	-	3.2 (±1.9)	-	-
Nov/Dec 2004	-	6.9	7.1	0.8	4.5	6.0	-	5.0	5.5
Jan/Feb 2005	-	11.1	14.0	-	-	-	-	4.2	5.5
Broadband k_d	**UVB**	**UVA**	**PAR**	**UVB**	**UVA**	**PAR**	**UVB**	**UVA**	**PAR**
Nov/Dec 2004	1.0 (±0.7)	0.7 (±0.4)	-	1.4 (±0.6)	1.0 (±0.4)	-	1.5 (±0.9)	1.2 (±1.1)	-
Jan/Feb 2005	0.6 (±0.2)	0.5 (±0.1)	0.4	2.4	2.3	-	1.0 (±0.4)	0.7 (±0.3)	0.8
Spectral k_d	**UVB**	**UVA**	**PAR**	**UVB**	**UVA**	**PAR**	**UVB**	**UVA**	**PAR**
Dec 2003	0.3	0.4	0.2	0.4 (±0.1)	0.4 (±0.2)	0.3 (±0.2)	0.5 (±0.2)	0.6	0.5
Jan 2004	0.7 (±0.2)	-	-	-	-	-	0.8 (±0.2)	-	-
Nov/Dec 04	-	0.4	0.4	0.6	0.5	0.4	-	0.6	0.8
Jan/Feb 2005	-	0.3	0.3	-	-	-	-	0.6	0.5

5.2 Underwater UV irradiance

As examples for the results of radiation profile measurements, the 10% penetration depth of radiation (± Standard Deviation; S.D.) as well as the diffuse vertical attenuation coefficient of downward irradiance k_d are reported. The k_d value is defined by the following formula (Kirk 1994):
$k_d = ln[E(z_2)/E(z_1)]*(z_1-z_2)^{-1}$ (3), where $E(z_1)$ and $E(z_2)$ are the respective irradiances at depths z_1 (0.1 m) and z_2 (2 m).
Low k_d values describe transparent water with little attenuation of radiation, whereas high k_d values mean turbulent water with a high extinction. Table 1 shows the mean 10% depth (± S.D.) at the three different study sites measured with the broadband and spectral instruments and the mean k_d value (± S.D.), respectively.

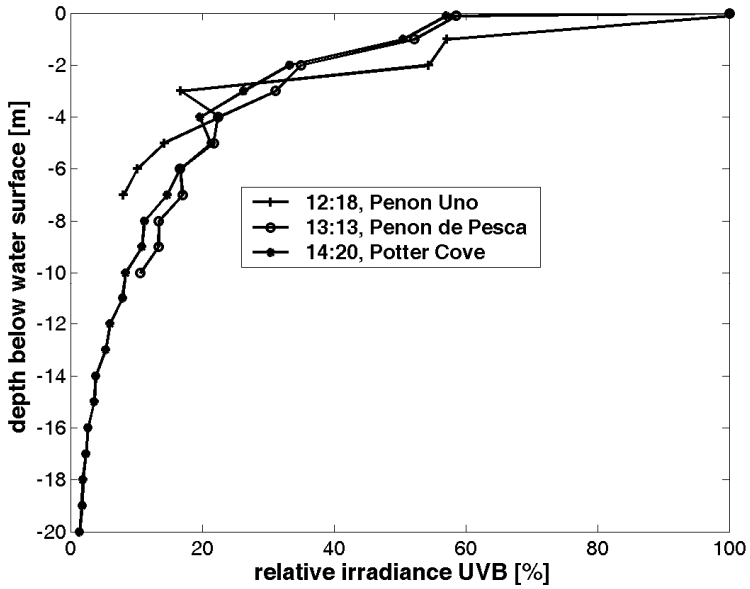

Figure 4: UVB irradiance profiles measured at 3 different sites on 29th December 2003, before the onset of glacier melting.

Figure 4 shows UVB profiles, measured at the three different sites on the same day, 29th December 2003, under clear water conditions. The profiles of Peñón de Pesca and Potter Cove show the same attenuation of radiation, the profile of Peñón Uno is characterised by variations due to changing radiation conditions.
Additionally, the profiles of relative PAR radiation were measured at Peñón de Pesca and Peñón Uno with two LiCor sensors on January 2nd 2005 (Figure 5). PAR is much more attenuated at Peñón Uno compared to Peñón de Pesca.

6. Discussion and recommendations for future measurements

As shown in Section 5.1, UV radiation measured in the Antarctic during the existence of the ozone hole is highly variable both from day to day and in the time scale of minutes. Figure 4 illustrates that even instruments operated at very close distance and averaged over the same time interval do not necessarily measure the same changes in radiation caused by fast moving clouds.
It is a difficult task to measure underwater UV profiles. Only on days with very low wind speeds (<10 m/s) it was possible to perform successful measurements. The measurements have shown that the penetration depth into

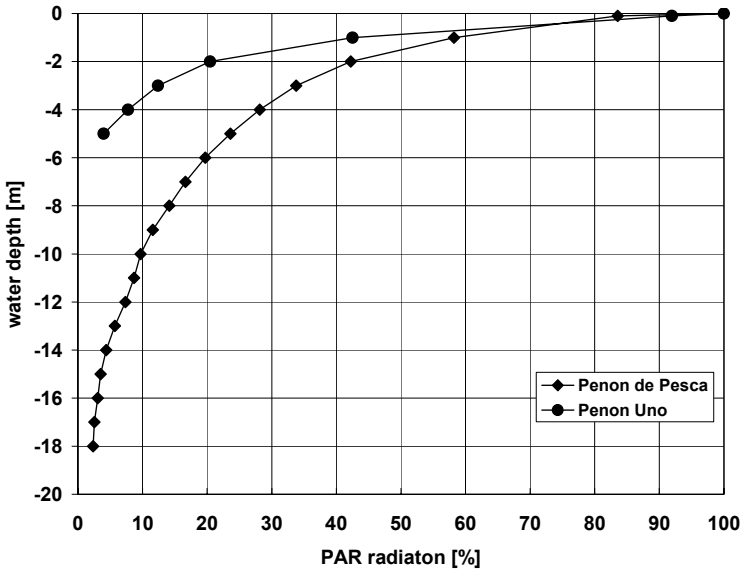

Figure 5: PAR profiles measured with two instruments at Peñón Uno and Peñón de Pesca on the same day, 2nd January 2005, after the onset of glacier melting.

the water is also subject to high variations (see Section 5.2). Those facts are important for biological studies of the effects of UV radiation on e.g. algae (Gómez *et al.* 1998, Zacher *et al.* unpublished).

The profile measurements performed at the three sites in spring before the melting process enriches the water with sediment showed a similar behaviour for both seasons, for UVA and UVB. The penetration of radiation into the water body was in the same order of magnitude with high uncertainties. In spring, before the end of December, the water was very clear at all places. With the onset of melting and the glacier bringing sediments into the water, it became more opaque during summer, especially at Potter Cove, near the source of the sediments, and at Peñón Uno, where the turbid water passes on the way out of the cove, whereas Peñón de Pesca was little or not affected by the meltwater. The penetration depth of radiation at Potter Cove was much less than at Peñón Uno for some days and the UV radiation at Potter Cove was already completely absorbed at 2 m depth in summer. The variability of UV profiles generally depends on water turbidity, mixing of the water with sediments from the melting and calving glacier as well as algae blooms (Vasilkov *et al.* 2005, Piazini *et al.* 2001). The 10% penetration depth of UVB and UVA in spring at our measuring sites were similar to other measurements in Antarctic waters reviewed by Tedetti and Sempéré (2006), and clearly higher than generally for coastal waters. The k_d values were comparable to values for clear waters reported by Smith and Baker (1981). This means that subtidal organisms in this area can be especially affected due to coinciding enhanced UVB radiation and very clear water conditions during spring.

The high uncertainties, sometimes up to the same order of magnitude as the measured value, are caused by the highly variable radiation conditions with time and the ratio of two values measured at different times. This effect can only be corrected successfully with a second sensor that is not subject to underwater changes, recording the radiation with the same time resolution at the surface. The need for such an instrument is demonstrated in Figure 3. A first example with two PAR sensors is shown in Figure 5.

Another problem for the spectroradiometers is the change in water temperature. In laboratory studies it was found that a change of the surrounding temperature by 1°C results in changes in the sensitivity of up to 7%, with differences for each channel. The large standard deviation is due to the few measurements and their high variability. To obtain statistically significant underwater UV irradiance data, more measurements are needed.
For future measurements, it is crucial to pay attention to the characterization of the spectral instruments and quality control of the measured radiation data as described in Section 3 to obtain reliable absolute values. For recording vertical profiles in the water, a second instrument at constant depth or at the water surface, very close to the profiling site and with the same time resolution should be used to monitor atmospheric radiation conditions, as presented here with two PAR sensors.

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Geology, tectonics and Ar-Ar ages of the magmatic dykes from Potter Peninsula (King George Island, South Shetland Islands)

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Introduction

Potter Peninsula is located at the southernmost extreme of King George Island (Fig. 1), stretching from 58°35.0' to 58°41.0' W and from 62°13.9' to 62°15.7' S. The unglaciated area comprises approx. 6 km^2, bordered by the Warszawa Icefield to the NE, Bransfield Strait to the SE, Maxwell Bay to the SW and Potter Cove to the NW.

Like in large parts of King George Island, the morphology on Potter Peninsula is predominantly characterized by a glacial landscape with offshore abrasion platforms, partly steep cliffs along the coast, and a rather smooth, hilly countryside in the interior. The most prominent morphological feature is Three Brothers Hill (196 m), a well known andesitic plug showing conspicuous columnar jointing (Fig. 2). It marks the final stage of activity of a Paleogene volcano, whose eruption products (lava flows and pyroclastic rocks) in combination with hypabyssal intrusions related to the volcanism, constitute most of the lithology observed on Potter Peninsula.

Among the first who carried out geological work in that area were FERGUSON [1921] and TYRELL [1921], who supplied short descriptions of the volcanic sequence. Later on, HAWKES [1961], BARTON [1961, 1965], SMELLIE et al. [1984] and BIRKENMAJER [1998] published more detailed geological and petrographic information, SMELLIE et al. [1984] also geochronological and geochemical data. Geological drafts and sketch maps of Potter Peninsula have been published by FOURCADE [1960], GONZÁLEZ-FERRAN & KATSUI [1970] and BIRKENMAJER [1998].

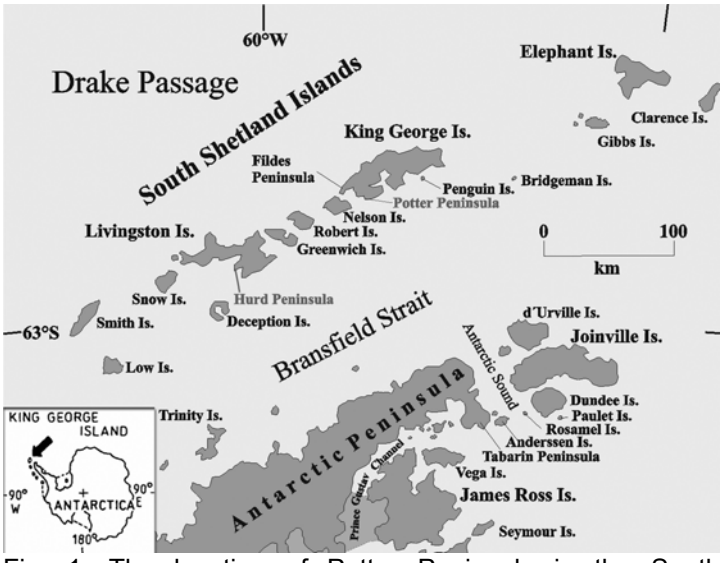


Fig. 1: The location of Potter Peninsula in the South Shetland Islands archipelago. Modified after VEIT (2002).

Geological frame

Potter Peninsula forms part of the downthrown Warszawa Block [BIRKENMAJER 1998]. The volcanic sequence cropping out here belongs to the King George Island Supergroup, with an observed local minimum thickness of approx. 90m [KRAUS 2005].

According to SMELLIE et al. [1984], the sequence can be referred to as part of the Fildes Formation introduced by these authors. Geochronological data from Potter Peninsula were published by WATTS [1982], who reports an Ypresian age (K-Ar, 50.6 ± 0.7 Ma) for Three Brothers Hill and Thanetian to Ypresian ages (57.9 ± 0.8 to 49.1 ± 0.9 Ma) for three andesitic lava flows. SMELLIE et al. [1984] obtained Ypresian to Lutetian K-Ar ages (49 ± 1 to 42 ± 1 Ma) for 6 basaltic to andesitic lava flows and hypabyssal intrusions, among them a Lutetian age (47 ± 1 Ma) for Three Brothers Hill.

Fig. 2: View towards SW to Three Brothers Hill (196 m), an Eocene andesitic plug showing prominent columnar jointing.

Three volcanic centers contributed to the volcanic sequence cropping out in the area: the former position of a stratovolcano is marked by the Three Brothers Hill plug, measuring about 500 m in diameter. Nearby Florence Nunatak, piercing the Warszawa Icefield about 4.7 km to the NE of Three Brothers Hill, is also a plug and marks the location of another extinct volcanic center. It is of basaltic andesitic composition and, like Three Brothers Hill, strongly columnar jointed. The remnants of a third but smaller stratovolcano are located at Stranger Point (Fig. 3). Today, this stratocone is completely eroded and only the reminders of the two feeding vents and the eruption products are left.

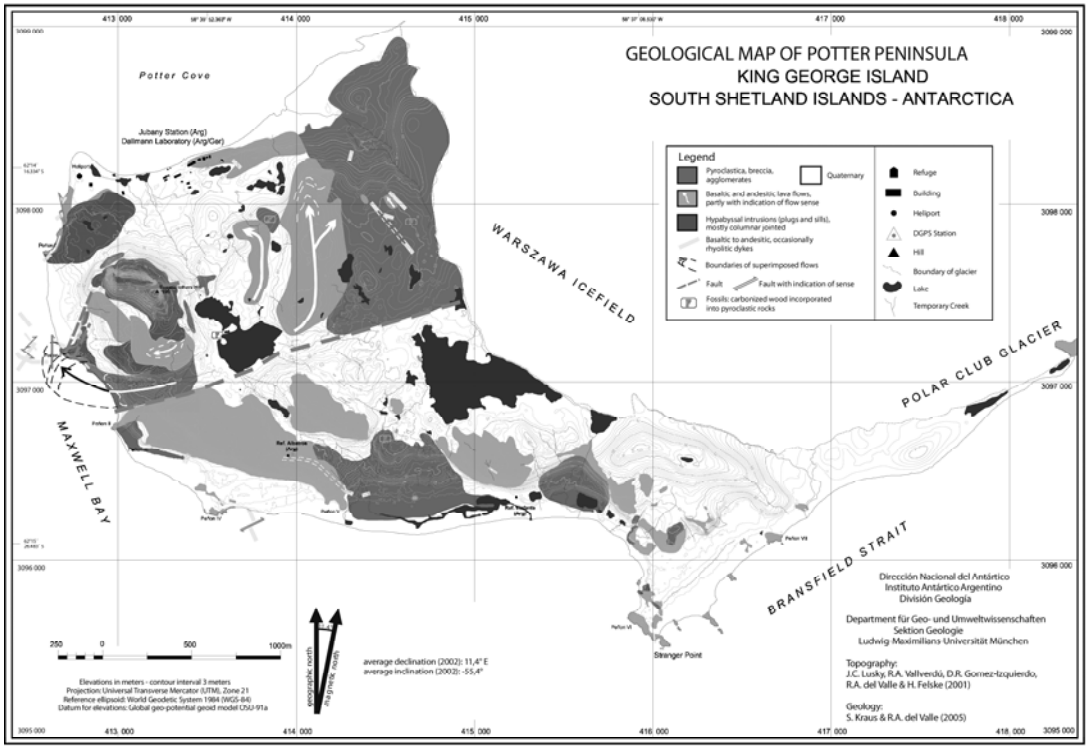


Fig. 3: Topographic and geological map of Potter Peninsula (King George Island, South Shetland Islands). Dyke thickness not scale appropriate. For high resolution color version please see: http://doi.pangaea.de/10.1594/PANGAEA.667386

The same applies to the Three Brothers Hill volcanic complex, which is eroded down to its deepest levels. Thus, the stratigraphically deepest units from the initial phase of volcanic activity are cropping out in some parts [KRAUS et al. 2000]. The lithology on Potter Peninsula comprises lava flows (~50%), pyroclastic rocks (ash-fallout, pyroclastic flow deposits, volcanic breccia and agglomerates, ~30%) and hypabyssal intrusions (dykes, sills and small subvolcanic intrusive bodies, ~20%).

Block faulting and subsequent tilting is evident everywhere on Potter Peninsula, though the individual blocks are tilted no more than 10-20° and without a prevailing direction. The prominent, NE-SW running fault separating Potter Peninsula in a northwestern and a southeastern sector was probably created during Late Cenozoic block faulting.

The dykes on Potter Peninsula

26 dykes crop out on Potter Peninsula, featuring a thickness between 30 cm and 10 m (average 3.68 m). They are far more abundant in the northwestern sector of the peninsula than in the SE towards Stranger Point (Fig. 3). Most

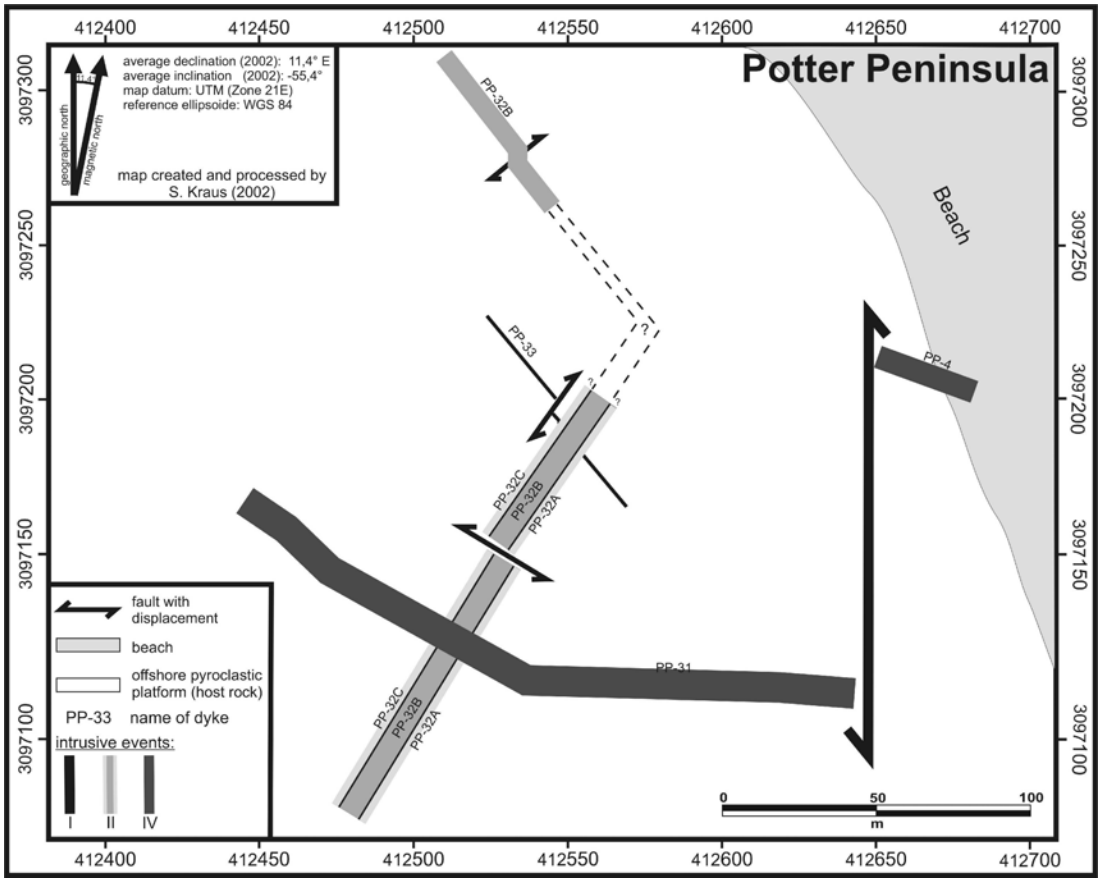


Fig. 4: Dyke system mapped at the western side of Potter Peninsula (Fig. 3). The system is located offshore, cutting an abrasion platform consisting of pyroclastic rocks. Accessibility is restricted to days with extremely low tide. The system comprises 5 dykes taking directions corresponding to intrusive events I, II and IV as determined on Hurd Peninsula, Livingston Island [KRAUS 2005]. Event III from Hurd Peninsula is not represented here. Note the multiple intrusion comprising dyke PP-32B (rhyolite, 7.6 m thick) flanked by two thin basaltic andesitic dykes (PP-32A and PP-32C, 1.0 - 1.2 m thick). Ar-Ar age determinations yielded same ages (Lutetian, approx. 46 Ma) for all dykes of the system, suggesting that the dykes intruded within a very short time, using different tectonic directions.

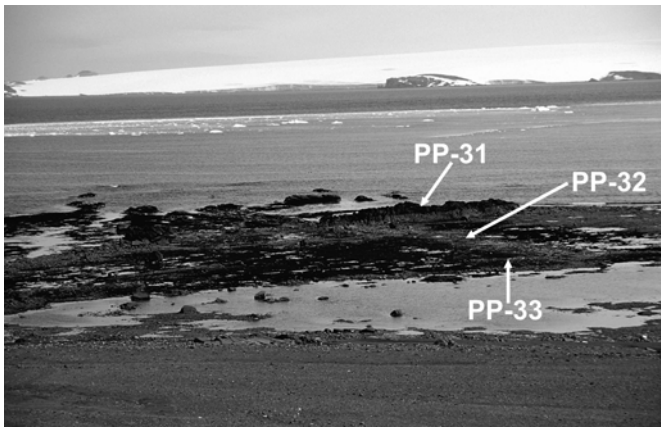


Fig. 5: View towards SW to the dyke system located offshore Potter Peninsula, cutting a pyroclastic platform. Barton Peninsula in the background.

of them are single, isolated dykes cutting the stratiform volcanic sequence, and can be traced from several meters length to up to 200 m (with interruptions). Like on adjacent Barton Peninsula, the only existing outcrop of a coherent dyke system allowing the observation of relative age relationships is an offshore pyroclastic abrasion platform near Peñón I (Fig. 3, 4 and 5). It is pierced by 5 dykes (some of them zigzagging), part of them forming a multiple intrusion (Fig. 4). The dykes are offset by three sets of faults. The oldest is dextral and strikes approx. 35-45°, the second sinistral striking approx. 120° and the third set is also sinistral, running exactly N-S (0° strike).

Most of the dykes are inconspicuous concerning their main characteristics and general appearance. The vast majority are of basaltic to andesitic composition, with mineralogies typical for a subduction related calc-alkaline suite. However, two outcrops deserve special attention, due to their unique appearance and features.

An especially spectacular example is an andesitic dyke cropping out in the northern part of Potter Peninsula near the border of Warszawa Icefield (414533 / 3098052, UTM, WGS 84, Zone 21E). Its orientation is 136/64 NE, featuring a thickness about 4 m; it can be traced over a length of approx. 60 m along a ditch approx. 1.8 m deep (Fig. 6). Here, the extensive pyroclastic rock sequence borders a small basaltic lava flow (Fig. 3). The dyke has intruded along this border between the two units. At approx. 150 m distance from the icefield, the dyke is located within an area from which the glacier retired only during the last 40 years. Thus, the ditch most probably represents the bed of a melt water creek which has fallen dry meanwhile. The water ran along the border between the dyke and the pyroclastic host rock, removing only the latter because of its lower resistance against erosion, thus


Fig. 6: About 60 m long ditch representing the bed of a meltwater creek now fallen dry. The left wall of the ditch is the outer surface of dyke PP-9. View towards NW.

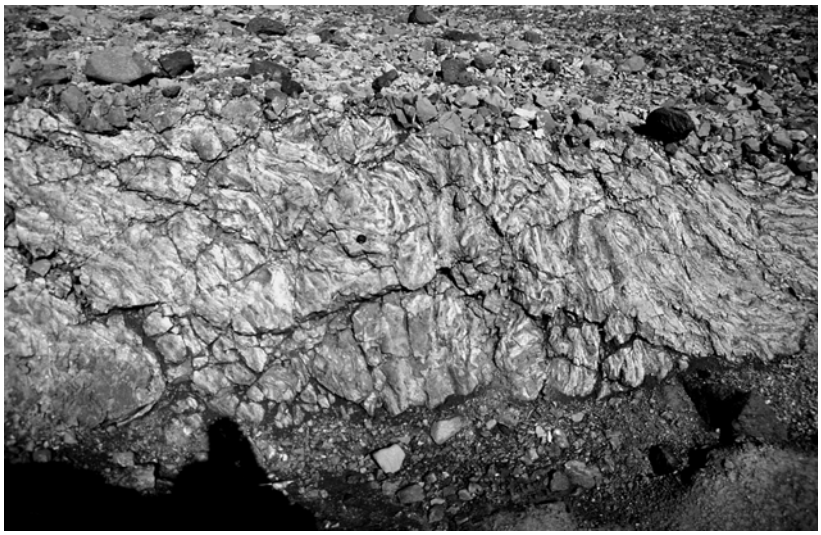


Fig. 7: Bulging structures on the outer surface of dyke PP-9, reflecting clearly the movement of the magma during intrusion. The dyke's chilled margin is greenish, fragments of the pyroclastic host rock baked onto the surface of the dyke are brownish. The ditch is approx. 1.5 m deep.

laying open the outer wall of the dyke over a length of approx. 60 m (Fig. 6).

Its appearance is characterized by bulging, more seldom globular structures and redbrown to greenish schlieren (Fig. 7). The bulges reflect clearly the magma's movement while intruding the fissure, mostly in a vertical but sometimes also subhorizontal direction. The color of the redbrown areas is due to fragments of the northeasterly lying pyroclastic host rock being baked onto the surface of the dyke, the greenish schlieren mark the chilled margin of the dyke itself, to a great extent consisting of secondary minerals like chlorite. Fragments of the pyroclastics are sometimes lined up in a string, also demonstrating the movement of the magma (Fig. 7). The dyke's approx. 4-5 cm thick chilled margin consists of a schlieren-like melange of greenish dyke- and redbrown pyroclastic material. Sometimes flame-structured amygdales of up to cm-size occur within this zone, often filled with a microcrystalline mineral of deep orange color, possibly zeolite. Within small geodes, this mineral sometimes forms dodecahedrons of up to 1 mm diameter. Further towards the dyke's interior, the color of the dyke rock changes to brownish-grey, then to grey. Small pores (< 1 mm) are aligned parallel to the dyke's outer wall and filled with calcite. They are aligned according to the orientation of the aforementioned bulges and thus reflect the magma's movements also in the dyke's interior. At about 15 cm distance from the dyke's surface, another zone of up to 1 cm big amygdales is visible, also partly filled with calcite. The bulk rock of the dyke consists of a light-grey, dense matrix hosting phenocrysts like pyroxene (up to 1.5 mm, euhedral, greenish), plagioclase (up to 2 mm, euhedral, whitish) and opaque minerals. Scarce amygdales of up to 2 cm diameter and irregular form are filled with chlorite and/or calcite.

A second outcrop is well worth mentioning, consisting of a multiple intrusion comprising three dykes. The outcrop is located at the shore SW of the Heliport (412683 / 3097998, UTM, WGS 84, Zone 21E), close to and possibly related to the dyke system shown in Fig. 4. A yellowish, rhyolitic dyke is sandwiched between two dykes of basaltic andesitic composition (Fig. 8), the orientation is 30/84 SE. The rhyolitic dyke is 4 m thick, shows tight cleaving and a smooth surface with small pores (mm-range) and single feldspar grains (< 0.5 mm). The rhyolite reacts with HCl, indicating presence of calcite; it bears accessory, mostly cubic pyrite (< 0.5 mm). The alteration rim is yellowish, 3-4 cm thick, and changes its color towards the fresher interior to grey-whitish. The contact with

the two enclosing basaltic andesitic dykes is not sharp, but a rather blurry, approx. 2-3 cm (max. 5 cm) wide transition zone showing a schlieren-like intermingling of the two magmas. Pyrite cubes (< 0.5 mm) appear more frequently in the vicinity of the contact but are restricted to the rhyolitic dyke. Pores, too, become more frequent towards the contact, indicating a stronger degassing towards the rim.

Each of the two flanking dykes (Fig. 8) is 1.6 m thick. Their contacts to the rhyolitic dyke exhibit sometimes a fine-grained darker banding about 1 cm thick but without glass. Mostly this margin is rather vague and in some parts missing, instead the aforementioned intermingling of the two magmas is prevailing. Plagioclase crystals are aligned parallel to the contact and sometimes arranged in a tile-like pattern. Like the rhyolitic dyke between them, these two flanking dykes are lacking a chilled margin at the contact with their acidic counterpart, being a strong hint on contemporaneous intrusion.

About 60 m to the S (412679 / 3097935, UTM, WGS 84, Zone 21E), a very similar situation occurs. Here, the orientation of the dykes is 50/80 NW, the rhyolitic dyke is only 3 m thick and the basaltic andesitic dykes each 1.6 m. A small, NW-SE running fault has cut and brecciated the dyke system.

At both outcrops, the rhyolitic dyke morphologically steps backward relative to the flanking ones and is also stronger jointed. This latter effect may be due to the considerable differences in acidity, resulting in a lower resistance against brittle failure of the rhyolite as compared to the basaltic andesite.

Two more outcrops on Potter Peninsula show the same situation of a rhyolitic dyke sandwiched symmetrically between two basaltic andesitic dykes (412529 / 3097149, UTM, WGS 84, Zone 21E and 413381 / 3096562, UTM, WGS 84, Zone 21E). The unique character of these outcrops has to be emphasized, because this type of multiple dyke intrusion has not been reported from anywhere else on the South Shetland Islands up to now.

Concerning the development of these remarkable intrusions, one explanation might be that the rhyolitic dykes intruded first, followed by tearing of the contacts to the wall rock during cooling. Subsequent intrusion of the basaltic andesitic magma might have occurred along these newly formed planes, accompanied by intermingling with the still not completely crystallized rhyolite. However, to our opinion this theory is not satisfying concerning the missing chilled margins of the rhyolitic

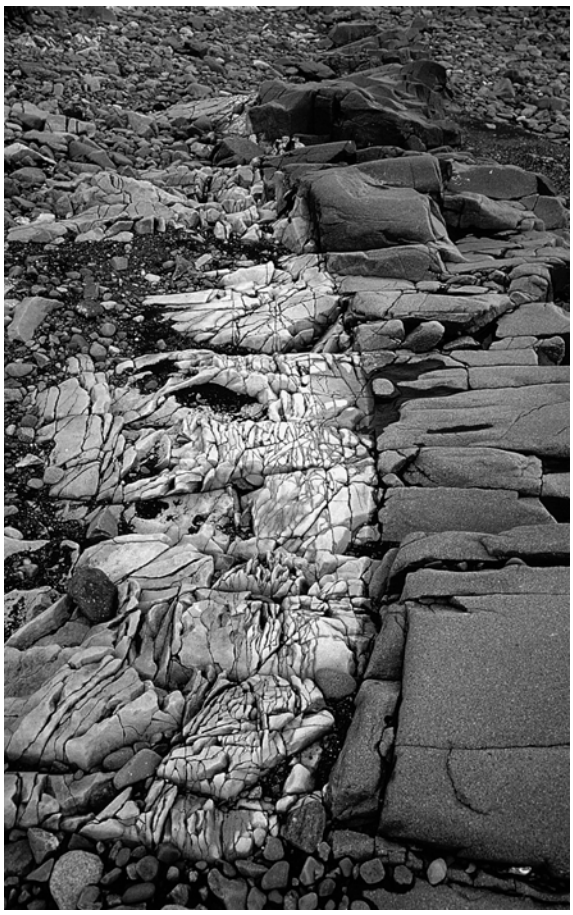


Fig. 8: A multiple intrusion, reflecting bimodal volcanism and maybe also bimodal flow. A rhyolitic dyke (left side) is sandwiched symmetrically between two 1.6 m thick basaltic andesitic dykes (one of them at the right side).

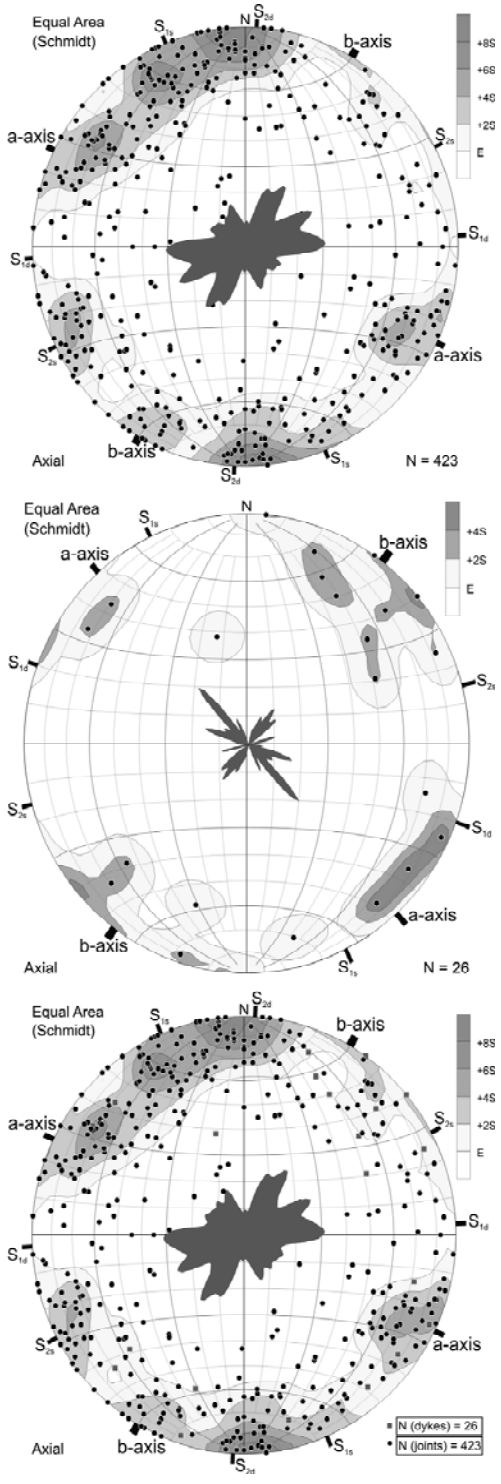
dyke and the schlieren-like intermingling of the two magmas, as especially the contacts of the rhyolite to the host rock should have cooled rapidly. Moreover, the outer surfaces of a dyke do often carry fragments of the host rock baked onto them (Fig. 7). According to the above theory, such fragments should be found along the contact between the rhyolite and the flanking basaltic andesitic dykes. However, this has not been observed at any of the outcrops.

A more comprehensive and maybe promising but yet unproven theory is bimodal flow [MCCLARREN 2003], requiring the contemporaneous intrusion of the crack by two types of magma, one of high viscosity and the other of low. The rhyolitic magma may have originated from the mush-zone of a differentiated magma chamber, whereas the basaltic andesitic material might have come from the chamber interior, both being pulled out through a crack in the chamber wall. When entering the fissure, the magma flow is probably rather chaotic, but the higher viscous magma (rhyolite) should, according to theory, soon become surrounded/sandwiched by the lower viscous, more basic material. Because of the much higher viscosity of the rhyolitic magma, mixing is rather unlikely. This phenomenon is well known to the petroleum industry, injecting water into oil pipelines in order to speed up the oil flow. In case of the dyke, the consequence would be that only the lower viscous (and hotter) magma is touching the host rock, whereas the rhyolitic magma remains insulated and does therefore suffer neither friction (which would lead to a slowdown) nor cooling. In other words, the more basic magma acts like a lubricant for the acid one. This process might allow a highly viscous rhyolitic magma to travel much longer distances than without presence of the more basic counterpart [McClarren 2003].

This second explanation appears plausible in this case, because the aforementioned schlieren-like intermingling and the missing chilled margins along the contacts between the basaltic andesitic and the rhyolitic dyke argue against a temporal gap between the intrusion of the two melts but instead for a contemporaneous one. Moreover, the position of the rhyolite sandwiched *between* the two basaltic andesitic dykes corresponds well to the above mentioned theory of the lower viscous magma acting like a lubricant, with the rhyolite placed in between.

However, this theory is not without weaknesses. In contrast to the mechanism observed in petroleum pipelines, it requires the contemporaneous injection of two liquids not only highly diverse in chemical composition but also in temperature. The difference should be several hundreds of degrees Celsius, and the question is what effect this might have during flow concerning the interaction of the two magma types. Another question is, if the conditions within a magma chamber really allow the contemporaneous injection of two such different magma types into a crack.

At least the occurrence of bimodal volcanism is indicated by the observed situation at the four outcrops, and probably related to the magma chamber which has fed Three Brothers Hill volcano. This assumption is supported by the relatively low distance (not more than 1 km) of all four outcrops to Three Brothers Hill (Fig. 3), furthermore by parallel trends displayed in certain geochemical diagrams [KRAUS 2005].



Abbr.	Explanation (with average strike)
b-axis	37°
a-axis	138°
S_{1d}	dextral first order shear direction: 110°
S_{1s}	sinistral first order shear direction: 154°
S_{2s}	sinistral second order shear direction: 75°

Abbr.	Explanation (with average strike)
b-axis	30°
a-axis	116°
S_{1d}	dextral first order shear direction: 87°
S_{1s}	sinistral first order shear direction: 157°
S_{2d}	dextral second order shear direction: 3°
S_{2s}	sinistral second order shear direction: 62°

Abbr.	Explanation (with average strike)
b-axis	30°
a-axis	116°
S_{1d}	dextral first order shear direction: 87°
S_{1s}	sinistral first order shear direction: 158°
S_{2d}	dextral second order shear direction: 3°
S_{2s}	sinistral second order shear direction: 63°

Fig. 9: Schmidt Net showing all joints measured on Potter Peninsula (A), the orientation of the investigated dykes (B) and a compilation of all tectonic data (C). Cooling joints within the dykes have not been plotted. The tables to the right of the stereograms summarize the principal tectonic directions of the respective net. For contouring, the Gaussian method 'K=100' has been applied. This method gives an expected count E, that is the same as the conventional 1% counting circle. The mean, or expected, value E is the count that should arise in each counting model if the data set was uniformly distributed. The weighting curve has a width at half-height of 8.1°. The contour levels are in multiples of s (standard deviation) above (or below) E. Poles to planes.

Tectonics

Due to the lack of folding visible in the field, no folding axis could be determined on Potter Peninsula. However, structural data obtained during extensive field work carried out on the sedimentary Miers Bluff Formation at Hurd Peninsula (Livingston Island) revealed a NNE-SSW striking folding axis and associated first and second order sinistral and dextral shear directions [KRAUS 2005].

The very similar pattern found on Potter Peninsula as compared to Hurd Peninsula in our opinion justifies the asignation of the different tectonic directions (Fig. 9) assuming a stress field similar as on Hurd Peninsula. A thus inferred b-axis strikes 30° and the corresponding a-axis 116°.

423 joints measured on Potter Peninsula, predominantly within the pyroclastic rocks and the dykes (cooling joints eliminated), reflect ac-planes oriented 116/87 NE, a dextral first order shear plane at 87/90 N (S_{1d}), a sinistral first order shear plane at 158/79 NE (S_{1s}), a dextral second order shear plane at 3/78 E (S_{2d}) and a sinistral second order shear plane at 63/80 SE (S_{2s}). All these directions correspond unexpectedly well with the stress regime determined on Hurd Peninsula (Livingston Island). Concerning the ac-, S_{1d}- and S_{2d}-directions, differences in strike are no more than 5°, whereas the sinistral first and second order shear directions deviate 13-14° from the corresponding values on Hurd Peninsula.

However, the average orientation of the dykes on Potter Peninsula deviates much stronger from the directions shown by the joints as well as from the directions observed on Hurd Peninsula. The Potter Peninsula dykes suggest a b-axis striking 37° and an a-axis oriented at 138° (Fig. 9), the latter deviating 22° clockwise from the corresponding direction as deduced from the joints. The same applies to S_{1d} (23° difference clockwise), and only S_{1s} is close to the direction shown by the joints (difference of 4° clockwise). At present, no convincing explanation can be presented for the differing behavior of the dykes.

Ar-Ar ages of the dykes

$^{40}Ar/^{39}Ar$ datings were performed on plagioclase separates of 5 dykes from Potter Peninsula. Sample preparation was carried out partly in Munich (Germany), partly at Stanford University (California, USA). Here, the measurements were carried out applying the stepwise heating technique. Sample preparation and the applied technique are described in detail by KRAUS [2005, the full datasets including all age spectra may be downloaded from http://edoc.ub.uni-muenchen.de/archive/00003827/). The coordination of the isotope derived ages to the geological time scale follows the International Stratigraphic Chart pub-

Table 1: Ar-Ar ages and other data of five dykes from Potter Peninsula. The close-lying ages reflect an intense, but short intrusive phase during the Lutetian. For further details and $^{39}Ar/^{40}Ar$ vs. $^{36}Ar/^{40}Ar$ isochron diagrams see KRAUS [2005].

Sample #	latitude (UTM, WGS84)	longitude (UTM, WGS84)	strike	dip	thickness	lithology	LOI (wt%)	Ar-Ar age (Ma)	MSWD
PP-32B	412529	3097149	35	76SE	7.6 m	rhyolite	4.08	**45.7** ± 1.2	184
PP-33	412546	3097200	140	60SW	0.7 m	trachyandesite	5.76	**46.43** ± 0.56	1.6
PP-11	414308	3098266	15	72W	4.0 m	andesite	3.05	**46.61** ± 0.37	5.8
PP-31B	412514	3097131	110	71N	6.2 m	basaltic andesite	2.76	**46.98** ± 0.62	10.6
PP-32C	412529	3097149	35	76SE	1.0 m	andesite	2.35	**47.19** ± 0.50	0.61

lished by the IUGS International Commission on Stratigraphy (ICS, website: www.stratigraphy.org).

A first order observation is the narrow time interval covered by the ages (Table 1). Dyke intrusion on Potter Peninsula seems to have been restricted to a short time between approx. 48 and 45 Ma during the Lutetian. A second order observation is that the dykes, without correlation to the ages, intruded along different tectonic directions. The two observations argue strongly for a short-lived, intense intrusive event producing dykes which took different directions, but all belonging to the same tectonic stress field. This means that the tectonic parameters changed only very slightly during dyke intrusion, but the overall stress conditions remained the same, an observation that has found to be true for the whole archipelago and for an even much longer period [KRAUS 2005].
The good correlation of these data with the ages published for the Three Brothers Hill plug indicates that dyke intrusion on Potter Peninsula was related to the volcano's final phase of activity, as was the formation of the plug.

Conclusions

1. Magmatic dykes are more abundant in the northwestern part of Potter Peninsula and around Three Brothers Hill than in the eastern part of the area.
2. All dykes belong to a calc-alkaline, subduction related suite, with lithologies ranging from basalts to rhyolites, but with andesites prevailing.
3. The time during which intrusion took place seems to have been restricted to a narrow interval between 47.2 ± 0.5 and 45.7 ± 1.2 Ma (Lutetian).
4. Dyke intrusion was most probably related to the final phase of activity of Three Brothers Hill volcano, an assumption strongly supported by the very similar ages published for the Three Brothers Hill plug, which marks the end of activity of the volcano.
5. A spectacular multiple intrusion (a rhyolitic dyke flanked by two basic ones) forms part of a dyke system piercing an offshore pyroclastic platform west of Three Brothers Hill.
6. The joint directions used by the dykes indicate a stable tectonic stress field during the complete time of intrusive activity, because all directions can be interpreted as belonging to the same tectonic regime. The use of different directions indicates that slight changes of the overall tectonic parameters led to different preferred directions, but within the same stress field. At least three different intrusive events are evident on Potter Peninsula.
7. Analysis of the structural data of the dykes and their host rocks shows, that the tectonic stress field prevalent on Potter Peninsula was very similar as in other parts of the archipelago (e.g. Hurd Peninsula, Livingston Island), and that only minor changes of this stress field occurred during the time of dyke emplacement.

Acknowledgements

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Appendix: SCAR accepted, Argentine and Chilean place names mentioned in the text.

SCAR accepted names	Argentine names	Chilean names
Bransfield Strait	Mar de la Flota	Estrecho Bransfield
South Shetland Islands	Islas Shetland del Sur	Islas Shetland del Sur
King George Island	Isla 25 de Mayo	Isla Rey Jórge
Livingston Island	Isla Livingston	Isla Livingston
Florence Nunatak	Yamana Nunatak	Florence Nunatak

Interaction between permafrost and groundwater on Potter Peninsula, King George Island (Isla 25 de Mayo), Antarctic Peninsula region

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Introduction

In this paper we describe the interaction between geocryological conditions and groundwater regimes on Potter Peninsula (62°17´ S, 58°40´ W) which is an icefree portion of King George Island (Isla 25 de Mayo), in the north-western Antarctic Peninsula (Fig. 1).

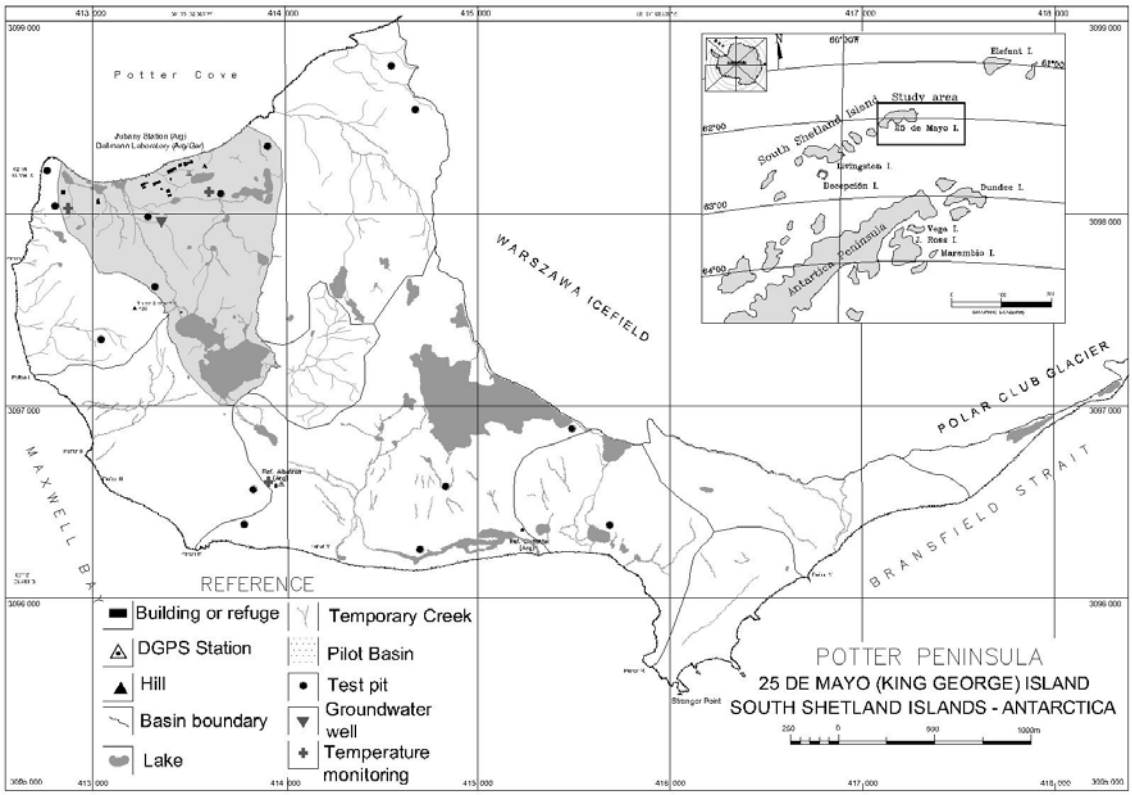

Fig. 1 Study area map and checkpoints

In this region, surface and sub-surface hydrological processes are observed to be strongly influenced by the present glacial retreat and active permafrost processes. Our aim is to contribute to the understanding of the response of Antarctic permafrost to a changing climate and hydrogeology in this maritime part of the Antarctic Peninsula.

Mean annual temperatures in this part of the Antarctic Peninsula range between -2°C and -5°C, whereas summer and winter means range between 0°C and +2°C, and -6°C and -10°C, respectively (King, 1994). Towards the north and west of the peninsula the air temperature has less influence on the sea-ice

(Reynolds, 1981). Snow accumulation measurements were performed in the Potter Peninsula area in the Matías Basin along a cross section profile during the spring and summer of 1992. Other snow studies were carried out in the high altitude area of Potter Basin on the boundary of the Warszawa glacier (Wunderle et al, 1998). These authors have been evaluating the influence of temperature and solar radiation on the hydrology, fundamentally during the ablation period. However, during the summer the discharge is independent of temperature and the discharges do not depend on the temperature (Silva Busso, 2003). The rainfall on the surface basin is the main water supply and during the melting season the area is subject to intense fluvial action.
A detailed study of the interaction between hydrogeology and geocryology has been performed at the Matías Basin in the Cove Potter area (near Jubany Station). In hydrogeological and geocryological studies we have assessed the relation between rainfall, snow melt and discharge of the Matías Stream that represents an example of subpolar wetland in the most north Antarctic regions.

The surface runoff and the groundwater hydrodynamics determine the water supply to the lagoon, habitat of a major part of the flora, lichens and mosses in the zone. So, our study gives a baseline for ecological investigations in the region. In previous papers, the cryogenic processes on Potter Peninsula area have not been studied in detail yet.

Permafrost conditions and cryogenic phenomena

Mapping of permafrost was based on field work and interpretation of satellite images and the aerial photos (scale, 1:20000). Additional information was obtained from existing topographic and geological maps (Braun and Grossman, 2002; Cañadas, 2003; Del Valle et al., 2004; Serrano et al., 2002).This information has been used for geocryological condition mapping on the ice - free area of the Potter Peninsula King George Island (Fig. 2).
Permafrost on the study area is comparatively warm (mean annual ground temperatures (greater than -2.0 ºC) and thin (less than 80 m). Assuming a homogeneous medium with a geothermal gradient of 0.03 ºC/m (considered as normal for continental Antarctic areas), the estimate of permafrost maximum thickness is 70-80 m in bottom moraines and terminal Holocene moraines, between 60 and 40 m in present moraine near of Warszawa glacier and less than 40 m in the fluvioglacial plain, gravity slopes (it is not solifluction slope) and wetland.
The beach zone with development of the sea water aquifer constitutes the permafrost limit of this area. The depth of temporal thaw is continued by climatic parameters and different lithology types related to the development of the surface and suprapermafrost water.
The active layer ranged between 0.5 and 0.8 m in the upper section of the bottom and lateral moraine, 1.0 and 1.5 m in the fluvioglacial plain and gravity slope and more than 2.0 m in the wetland, where the influence of surface and groundwater are more significant. The maximum thickness of active layer, more than 2.5 m, was observed near the coastline at 2 m a.s.l.
A particular characteristic of the permafrost and groundwater development of the Potter Peninsula is determined by the variety of periglacial landforms and cryogenic phenomena (Table 1).

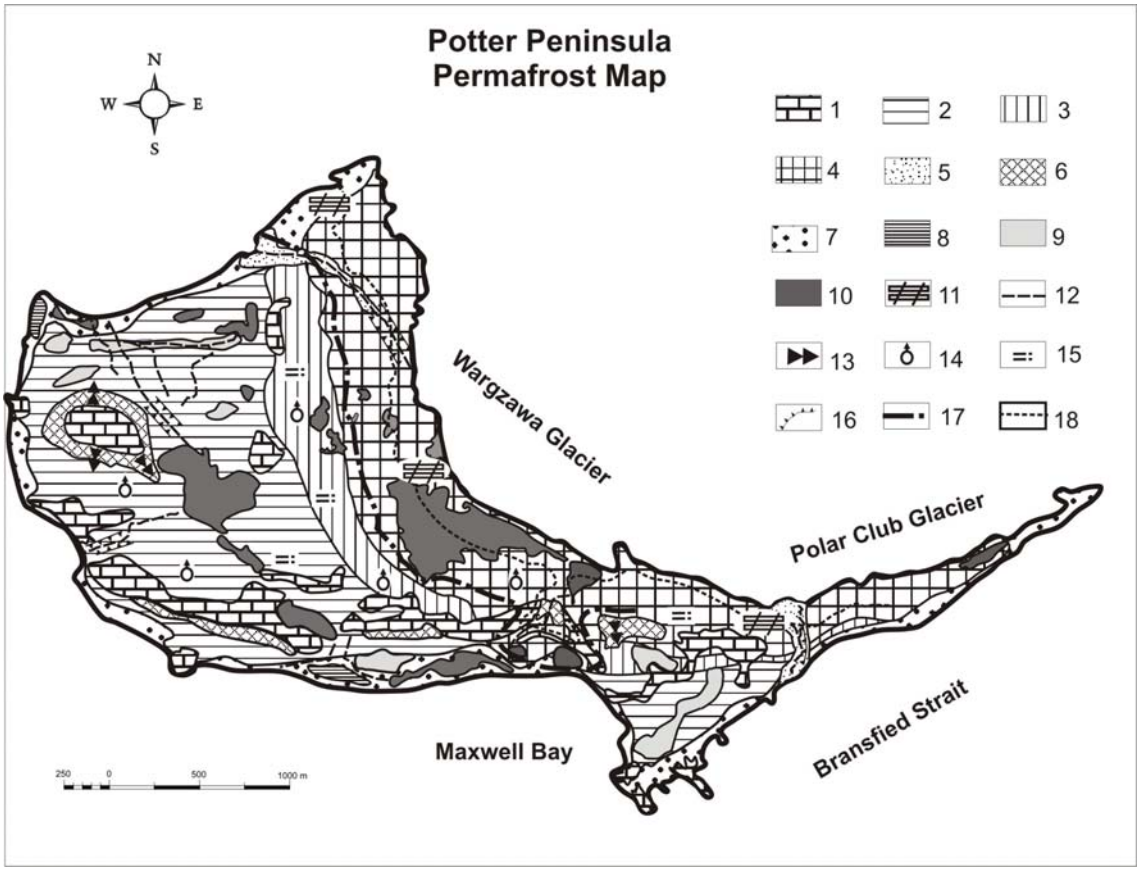

Fig. 2. Geocryological map of Potter Peninsula, King George Island. Geocryological and geomorphologic unities: 1 - Bedrock and cryoeluvium; 2 - Late Holocene terminal and bottom moraine; 3- Early Holocene terminal and bottom moraine; 4 - Present terminal and bottom moraine with buried ice; 5 - Present fluvioglacial deposits; 6 - Slope deposits; 7 – Beach; 8 – Paleo-beach terrace; 9 - Cryogenic wetland; 10 - Marginal and thermokarst lakes; 11 - Icing glade; 12 - Stream; 13 - Cryogenic landslide; 14 - Frost jacking; 15 - Sorted stripe; 16 – Thermoerosion scarp; 17 - Glacier margin at 1952; 18 - Glacier margin at 2002.

In general, all exposed ground surfaces in the ice-free area are subject to severe exogenic and cryogenic processes. The most common processes in the area are the frost jacking and sorted stripe, which are typical of different sediment types. The first one is observed on the present and Holocene bottom moraine with presence of the suprapermafrost aquifer. This process is typical of slope deposits and of wetlands too. The characteristic of the sorted stripe observed on the fluvioglacial plain, wetland and on the fine-textured bottom moraine are nets and stone polygon forms. The typical size of this patterned ground is between 0.5 and 1.0 m. The smaller pattern observed on wetland and fine clastic ground of the lake depressions is related to short-term diurnal fluctuations around the freezing point.

Ice-rich permafrost on slope deposits and lateral moraine with buried ice occurs generally as landslide events derived from local bedrock. Weathering and freezing actions of the bedrock outcrop are intense. These processes in addition to extreme wind action make up scree colluviums slopes at the foot of the vertical cliffs. Thawing of ice-rich permafrost with ground or buried ice leads to widespread development of thermoerosion processes on the fluvioglacial plain and thermokarst lake formation.

Geomorphologic units	Cryogenic conditions and phenomena	Surface and groundwater characteristics
Present bottom and terminal moraine	Permafrost new formation: icing, injection ice blister , frost jacking	Water stream, outlet of subglacial and suprapermafrost water
Holocene lateral moraine	Continuous pemafrost: cryogenic landslide, thermokarst	Suprapermafrost water, evidence of rock avalanche
Holocene bottom moraine	Continuous permafrost: frost jacking, sorted stripe	Outlet of interpermafrost water, temporal stream
Talus and gravity slope	Continuous permafrost; cryogenic landslide, frost jacking	Temporal stream, evidence of groundwater avalanche
Bedrock outcrop	Continuous permafrost: frost action, cryoeluvium formation	Suprapermafrost water
Fluvioglacial plain	Discontinuous permafrost: sorted stripe thermoerosion, injection ice blister, icing	Temporary stream, outlet of intrapermafrost water
Wetland	Discontinuous permafrost: sorted stripe, frost jacking	Puddle, outlet of suprapermafrost water
Beach	Permafrost limit, freezing temporal layer	Puddle and temporal channel, suprapermafrost water

Table 1. Distribution of cryogenic phenomena on the study area.

Relationship of permafrost to groundwater

The drainage network in the Matías Basin is discontinuous and poorly integrated due to the permafrost conditions and the changing fluvial processes. There is no erosion of glacial and fluvioglacial sediments yet. The Warszawa glacier does not contribute significantly to the drainage network and it has been concluded that fluvial discharge is mostly related to rainfall (Silva, 2003). During the summer periods, from 2002 to 2004, the temporally unconfined aquifer or suprapermafrost aquifer that coexists until late summer an even early autumn beginnings have been studied. We constructed a network of 30 monitoring wells (one every 0.031 km^2) in the suprapermafrost aquifer of Matías Basin.

The recharges conditions on the suprapermafrost aquifer have been evaluated from the daily measurements of static levels in several reference wells along the summer. The JB06 well has the longest recovered data series and it is located in the fluvioglacial plain. The observed increases and oscillations of the static water level have been correlated to the increases of rainfall in the same summer period (Fig. 3).

Daily oscillations of the water level exhibit a strong relation to the temperature only when subzero values suddenly occur. However, when the daily temperature averages are positive, rainfall is positively correlated to the water levels during some days. This circumstance shows a small local recharge, which comes directly from the rainfall. It is very probable that suprapermafrost groundwater supply also exists, in the axis basin direction on the Matías stream from the Matías lagoon. It is possible that the present basin area has an unconfined aquifer during the summer period. This aquifer has a groundwater flow integrated and it receives one direct recharge from infiltration of the local snow precipitations or the snow melting.

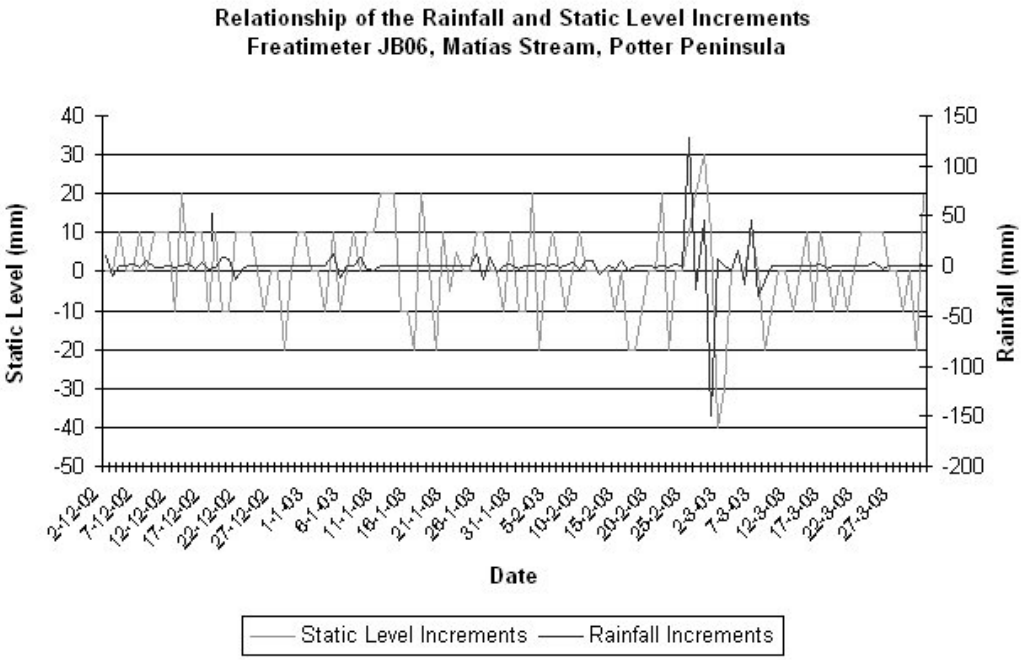

Fig. 3. Relationship between static level, rainfall and temperature. Matías Basin

It can be therefore demonstrated that there is a tendency to supply water from the unconfined aquifer onto the streams, especially on the lagoons and wetlands. An analysis of the groundwater gradient map in Fig. 4 and the piezometric level in Fig. 5 allows identifying the discharge areas and, qualitatively, the zones of greater permeability in the Matías Basin.

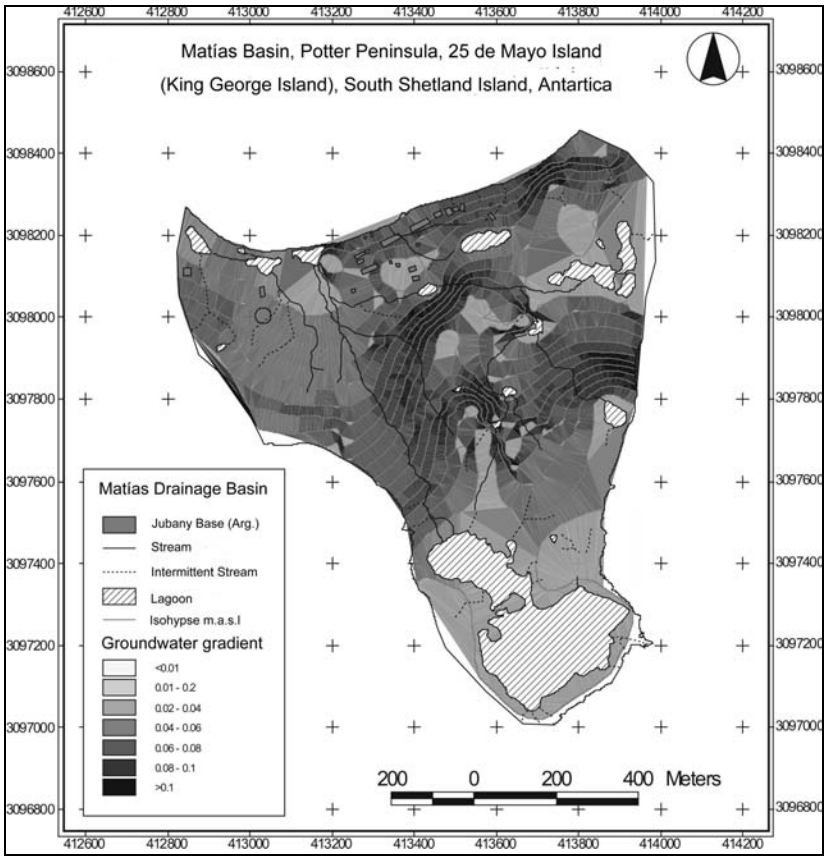

Fig. 4 Groundwater gradient map (adimensinal) of the suprapermafrost aquifer Matías Basin

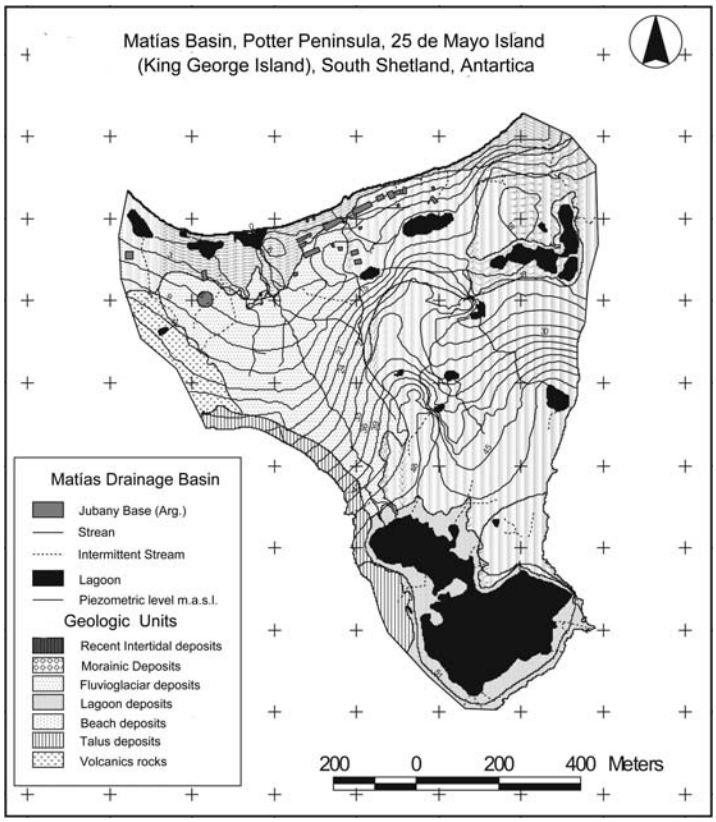

Fig. 5. Hydrogeology map of the suprapermafrost aquifer Matías Basin

The hydraulic gradients of the unconfined aquifer are between 10^{-1} and 10^{-2}. These values tend to coincide with the surface run-off and the groundwater flow in the active layer. This flow moves quickly through the ground thaw. Smaller values are related to the lagoon, wetland and the coastal zone on the limit of the permafrost area. Because of this, the lagoons are effluent. The local groundwater discharges at the coastline where groundwater resurgence phenomena or springs have been observed. This process can generate icing (freezing of the suprapermafrost or interpermafrost groundwater discharged in surface). In general, the fluvioglacial deposit areas and fissured volcanic rock outcrop areas present the lowest gradients, from which we infer they have the lowest permeability. The piezometric level and hydraulic groundwater gradient data show that the lowest gradients correspond to the lagoons and wetland areas. From this, it is deduced that the hydrodynamic balance of the lagoon in Potter Cove depends on the discharge groundwater flow from suprapermafrost and interpermafrost aquifers.

This interaction between the groundwater and the thaw layer during the summer has been proposed previously in Silva Busso and Fresina (2004). In the Matías Basin, from hydrochemical analysis of the surface water and groundwater samples carried out by Silva Busso et al., (2004), it was concluded that the stream and lagoons receive groundwater along the basin from the suprapermafrost aquifer and that the hydrochemistry characteristics confirm this circumstance.

Conclusions

The study area has a surface drainage system and subsurface suprapermafrost and interpermafrost waters related to drawdown and thickness of the active layer and local divergence of permafrost. In recent years, numerous geophysical studies were carried out in different parts of Potter Peninsula, particularly over the Matías Basin. The temporary nature of the hydrologic and groundwater regimes in this basin are related to the topographic slope and in particular to the recharge and discharge processes in relation with the local permafrost characteristics. The continuous and discontinuous permafrost presence and the meagre development of the active layer and cryogenic processes in the area during the southern summer accompany the development and trend of the hydrogeology processes. In the summer, the unconfined suprapermafrost aquifer has a hydrodynamic control on the hydrologic system across the study area and, particularly, on the wetlands. This characteristic is very typical in permafrost quaternary sediments and can be found also in other areas or islands of the South Shetland Islands.

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Iron, copper and manganese discharge from glacial melting into Potter Cove and metal concentrations in *Laternula elliptica* shells

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Introduction

Iron (Fe) is a transition metal and a micronutrient for plants and animals. Fe(III) is mostly insoluble and undergoes inorganic speciation to Fe(OH)x in seawater. To a major proportion, Fe(III) is bound in the suspended particulate matter fraction (>0.4µm particle size) in marine environments. Contrary, up to 99% of the dissolved Fe pool in seawater is reduced Fe(II) and is maintained in solution by complexation of organic chelators, stemming mostly from phyto-plankton exudates. This fraction is thought to constitute the "bioavailable" iron pool and its solubility in seawater increases at lower temperature and increasing concentration of the Fe complexing ligands. During daytime, Fe(III) undergoes complex photo-reduction mainly driven by UV-induced photochemical reactive oxygen species formation in oceanic and coastal surface waters (Croot et al. 2005). This photo-reduction process could be of major importance for bloom formation in natural and experimental Fe fertilization, as it maintains bioavail-able Fe(II) in solution within the euphotic zone.

The most significant sources of iron in seawater are dust deposition on the sea surface (Dierssen et al. 2002,) and, to a lesser extent, up-welling deep water over hydrothermal vents sites. In well mixed open ocean surface water, the concentration of dissolved Fe (<0.4 µm colloidal and complexed fraction) ranges at 0.1-2 nM (5.6-110ng l^{-1}) (Bucciarelli et al. 2001, Klinkhammer et al. 2001). In nearshore areas of the Antarctic Peninsula, sediment ablation from glacier melting, eroding the rock surface underneath the glaciers, can lead to Fe enrichment through transport of lithogenically derived sediment particles in coastal areas (Ahn et al. 1996, Dierssen et al. 2002). King-George Island (KGI) volcanic rock contains between 5 and over 7% Fe (Tatur et al 1999), and iron is washed to the sea in turbid melt water streams, visible all around the island. Most of the Fe in coastal seawater is bound to suspended sediment particles and the Fe concentration in suspended matter ranges between 0.2 and 318 µg iron l^{-1} in near shore areas (Ahn et al. 2004). Dissolved Fe concentrations of up to 10nM (560 ng l^{-1}) have been measured in coastal areas around the sub-Antarctic archipelagos, where natural Fe fertilization occurs through sediment erosion (Bucciarelli et al 2001, Chris Measures pers. comm.). Coastal melt water run-off at the Western Antarctic Peninsula increases during the summer months (November to February) when land glaciers melt water production increases due to higher air temperatures, and the process is predicted to inten-sify as climate warming in the area proceeds.

Marine filter feeders and surface sediment grazers in near shore areas ingest particulate Fe and other trace metals together with food particles. This uptake might increase as a function of glacier melt water release as the animals become virtually covered up with sediments. Fe is also sequestered into animal tissues from the dissolved Fe pool in the inhaled seawater, and recent exposure studies indicate this to be another source of potential importance for near shore fauna (Ahn et al. 2004, Gonzalez, Puntarulo, Abele unpublished results). Fe and other trace metals, taken up in the dissolved form, or ingested with filtrated particles, are incorporated into mollusc soft tissues and secreted from the mantle into the extrapallial fluid (located between mantle and clam shell). From the extrapallial fluid the ions are incorporated into the inner surface of the calcium carbonate shell (Tynan et al. 2005).

The present study investigates the concentrations of heavy metals, mainly Fe, copper (Cu), and manganese (Mn) in lithogenically derived particulate matter in Potter Cove, King George Island. Samples were taken in the inner cove, which directly receives melt water from Collins glacier. Further, 2 stations outside Potter Cove were sampled at about 2 km distance from the glacier front. Additionally, Fe, Cu and Mn and other trace elements were measured in the shells of the filter feeding soft shell clam *Laternula elliptica*, to see whether or not accretion in bivalve hard structures reflects the metal concentrations of the water column. The underlying idea is that metal accretion in shells of long lived bivalves could be used as proxy for the recent glacial melting process.

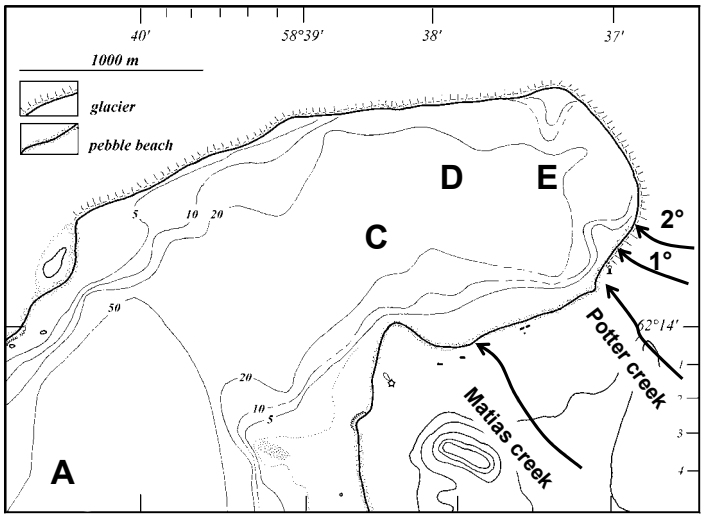

Fig. 1: Map of Potter Cove showing the sampling stations A, B, C, D in 2004. Creeks are indicated by arrows.
(Courtesy of G. Veit-Köhler, DZMB; Wilhelmshaven, Germany)

Material and Methods

Water sampling. Water sampling for the element analysis was carried out during 2 different field seasons. In December 2002, we sampled surface water and sediment trap material in the inner cove during one occasion, only. In summer 2004, stations C,D,E were sampled on a transect inside the cove at 2-weekly intervals and stations A and B were sampled outside Potter Cove once or twice during the whole season. Additionally, several melt-water creeks that transport sub-glacial sediments into the cove were sampled once or twice per month (Fig.1).

Water was sampled with a 5 l Niskin water sampler at different depths and stored in white plastic containers for filtration on the same day. Depending on the sediment content, 0.5 to 1.5l of the water was filtered onto MG 400 micro glass fibre filters (Munktell, Sweden) and washed in 10% HCl in aqua bidest prior use. Filters were folded using HCl-cleaned plastic tweezers and stored in HCl-treated Eppendorf cups. For each sampling up to 10 blank filters were stored to control contamination.

Sediment trap sampling: The automatic sediment trap (Fa. Isitec, Bremerhaven, Germany) was equipped with 100ml polyethylene flasks (3 x 125ml parallels/sampling interval) and programmed for 2-hourly sampling intervals over 24h on December 22nd, 2002. Following retrieval, 1 replicate was used for metal analysis and chlorophyll-a (Chl-a), 1 replicate for suspended particulate matter (SPM), and 1 replicate for C/N analysis. Organic and inorganic SPM fractions were measured gravimetrically. Two 50ml replicates were filtered onto preweighed GF/F filters, dried 24 h at 60 ºC, and weighed to the nearest 0.1mg. For Chl-a, a volume of 90 ml sample was filtered through Whatman GF/F filters and extracted 24 h in the dark at 4°C using 90% acetone and read on a Pharmacia Biotech Ultrospec 3000 UV/Visible spectro-photometer. Correction for phaeopigments was done according to Strickland and Parsons (1972). For metal analysis, 25ml of trap sample were filtered onto MG400 micro glass fibre filters (Munktell, Sweden) and stored as described above.

Metal analysis in bivalve shell material: Individuals of *Laternula elliptica* were collected at 10 m depth in Potter Cove fronting Jubany station. Shells of between 7 and 8 cm length were cut through the umbo with a diamond saw (Buehler, Isometh, Germany) and polished (see Philipp et al. this volume). Yearly growth rings, visible within the umbo, were analyzed for Mg, Al, Mn, Fe, Pb and U with Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS; 1064nm Nd:YAG Spectra Physics; Elan 6000 Perkin Elmer/Sciex). In the case that the diameter of the laser crater exceeded growth ring thickness, mean values of more than one growth ring were derived. As matrix matched standards were not available, no concentrations are provided in Tab. 2. Instead we normalized metal data to the Ca signal and calculated Fe/Mn and Fe/Cu element ratios in the shells for comparison to the field data.

Metal analysis: Filters of water and sediment trap samples were digested using 40%HF, 65% HNO3 and 70% $HClO_4$ (closed teflon beakers standing on a thermostat controlled heating plate). Completely digested filter samples were analyzed for Cu, Fe, Mn by inductively coupled plasma optical emission spectrometry (ICP-OES, Iris Intrepid (Type Duo), Thermo Nicolet GmbH, Germany).

Results

Water Column sampling and sediment trap material in December 2002

Metal concentrations in the particulate fraction of surface water amounted to 1.7 μM Fe (95.4 μg L^{-1}), 0.028 μM Mn (1.56 μg L^{-1}) (analyzes at wavelengths: 257, 259, 260 nm) and 0.004 μM Cu (0.26 μg L^{-1}). The Fe/Mn ratio was 61 and the Fe/Cu ratio 367.

Particulate Fe in sediment trap material correlated significantly with Mn and chlorophyll-a ($P<0.001$) (Chl-a not shown in Fig. 2) over the 24h sampling cycle. Fig. 2 shows that the Fe/Mn ratio remained at 55.8 ± 4.7 throughout the 24 h trap cycle, which indicates lithogenic origin (Fe/Mn of the earth crust 57.3, see www.webelements.de). Only 2 data points in the Cu measurements were above

detection limit and yielded an Fe/Cu ratio of 690, indicating an enrichment of iron in sedimenting particles, compared to the water column ratio. Additionally, SPM concentrations (dashed line) as well as tidal altitude (solid line) are shown. Peaks of particle sedimentation occurred around the tidal maxima, but were not consistent with iron or copper sedimentation maxima (not shown in Fig.2).

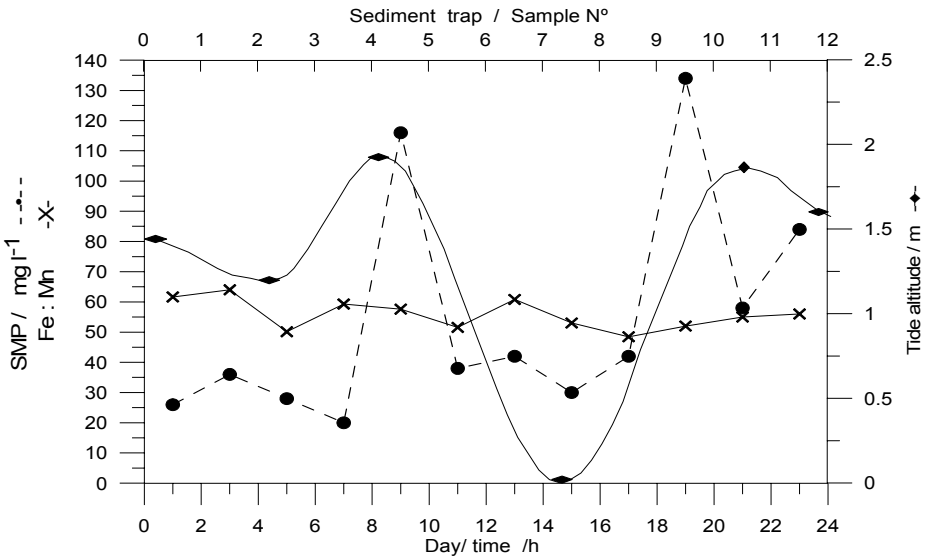

Fig.2: Ratio of particle bound Fe/Mn (**x**) in sediment trap samples; sediment particle flux (SPM) dry weight in mg∗L^{-1} (•) and the tidal altitude in m (♦) over 24 h on Dec. 22nd, 2002.

Metal concentrations in Potter Cove and in land run-off in 2004

Fig. 3 shows water column concentration depth profiles of particulate (> 4µm) heavy metals on 4 stations between the inside (E) and the outside (A) of Potter Cove on January 24, 2004.

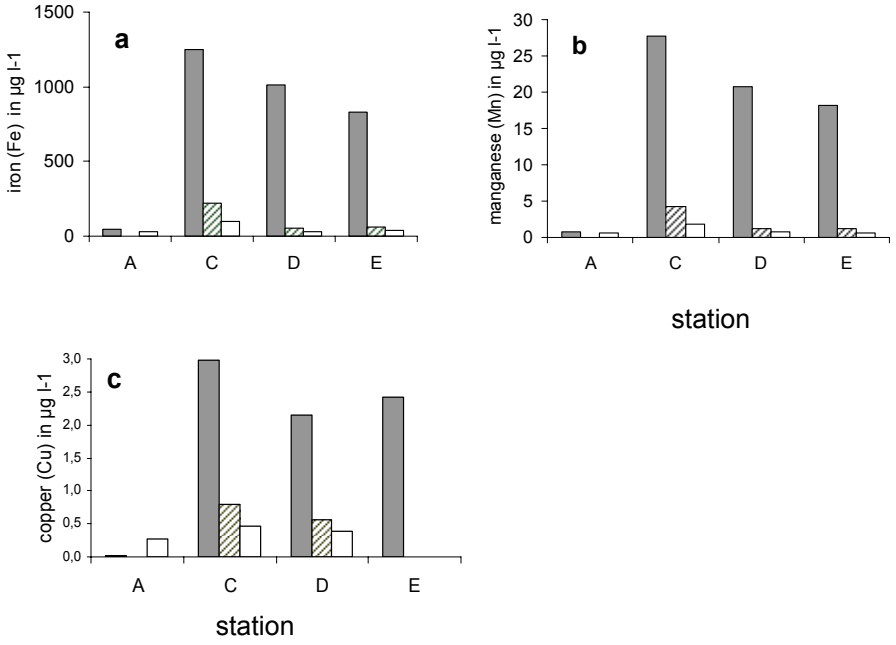

Fig. 3: Water column depth profiles of particulate (>4µm) heavy metal concentrations on a station transect in Potter Cove on January 2004. Column colours indicate water depth: grey 0m, hatched 15m, white 30m.

Station A at the mouth of the cove in Maxwell Bay was sampled only at 0 and 30 m water depth. Water column profiles of Fe (a), Mn (b) and Cu (c) displayed high surface values (0m) with concentrations decreasing dramatically at greater water depth (15 and 30m). Fe, Mn and Cu occurred at much higher concentrations in the inner cove (C,D,E) than at the mouth of the cove (A). The Fe/Mn ratio in the particulate fraction decreased 51,1± 12.5 at stations C,D,E in the inner cove to 45.9 ± 2.7 at station A outside the cove.

Table 1 summarizes the element composition in land run-off from creeks discharging into Potter Cove. Potter Creek as well as 1° and 2° Creek transport melt water from Collins glacier directly into the innermost part of the cove, whereas Matias Creek transports water from ice free areas at the bottom of the "Tres Hermanos" hill. Mn, Fe and Cu concentrations were dramatically higher concentrated in the effluents coming directly from the glacier underside. Especially in 2° creek the concentration of all three elements exceeded those in melt water from the ice free area at "Tres Hermanos" by the 10-fold. However, the ratios of Fe to the other 2 elements were higher in Matias creek water than in the melt water from the three glacial melt streams. These data provide a hint showing that melt water from glaciers and ice-free areas at Potter peninsula can be distinguished on the basis of the biogeochemical composition.

Table 1: Element composition (means of all samplings ± sd) as well as Fe/Mn and Fe/Cu ratio (means of all samplings ± sd) in melt water creek run-off into Potter Cove. Metal concentrations are in µg l^{-1}, number of samples taken from January to March 2004 were Matias Cr. n= 9, Potter Cr. n=9, 1° n=5, 2° n=5.

Creek	Fe/Mn ± s	Fe/Cu ± s	Mn ± s	Fe ± s	Cu ± s
Matias	39,3 ± 38,7	348 ± 318	15,9 ± 11,4	624 ± 419	1,79 ± 1,1
Potter	33,9 ± 28,6	303 ± 257	94,3 ± 60,1	3193 ± 1766	10,6 ± 6,8
Primero (1°)	35,2 ± 36,9	289 ± 306	89,5 ± 72,2	3153 ± 2113	10,9 ± 8,9
Segundo (2°)	24,0 ± 21,3	254 ± 222	256 ± 179	6129 ± 3371	24,1 ± 16,3

Element composition of bivalve shells

The element composition of 5 shells of *L. elliptica* is shown in Table 2. To account for variability in the surface texture of the samples, the laser energy, and the plasma conditions, all values were normalized to Ca (=relative intensity), which is homogenously distributed in the shell. Tab. 2 gives mean values of relative intensities and standard deviations of analysed elements. Whereas the Ca-normalized data of single elements cannot be compared to either sediment, or water column data, the Fe/Mn and Fe/Cu ratios can be compared between different data sets. Data represent means from measurements in yearly growth bands after year 5 of bivalve age. In the first 5 years, iron concentrations were over-proportionally high, correlating with the high respiration and growth rates in the young animals. After 5 years, iron concentrations in growth bands reached a steady state. Therefore, only analyses of growth > 5 y were included into the data in Tab. 2.

The ratio of Fe/Mn averages 24±0.9 and is lower than in water or SPM, which indicates less uptake of Fe or enrichment of Mn into the shells. Cu is also enriched in the shells of the Antarctic soft shell clam (Fe/Cu = 3.5 ± 0.1), whereas Fe was found rather down-graded compared to the high environmental levels (Fe/Cu 367 in surface water).

Table 2: Element composition in shells of 5 Antarctic soft shell clams collected in Potter Cove. Data are means of relative intensity of elements in growth bands >5y of bivalve age.

Laternula	Fe/Mn ± s			Fe/Cu ± s			Mn ± s			Fe ± s			Cu ± s		
1	24,8	±	1,8	4,85	±	0,41	0,056	±	0,002	1,39	±	0,09	0,285	±	0,016
2	17,4	±	1,4	3,29	±	0,29	0,017	±	0,000	0,30	±	0,02	0,091	±	0,004
3	20,3	±	1,2	2,58	±	0,13	0,034	±	0,002	0,70	±	0,03	0,270	±	0,008
4	35,7	±	3,2	1,29	±	0,09	0,016	±	0,001	0,57	±	0,01	0,439	±	0,030
5	22,0	±	1,2	5,25	±	0,24	0,034	±	0,001	0,84	±	0,03	0,160	±	0,004
Mean	24,0	±	0,9	3,45	±	0,12	0,031	±	0,001	0,76	±	0,02	0,249	±	0,007

Discussion

Fe, Mn and Cu are the three highly concentrated elements in the earth crust, and iron is specifically abundant (>5%) in KGI volcanic rocks, in glacier melt water, and in near shore surface water. Marine sediments in coastal areas contain > 2% (>20 mg g^{-1} dwt) of iron (Ahn et al. 1996). All three elements were highly concentrated in the glacier run-off from Potter creek, and in 1° and 2° creeks, alimented directly from the glacier underside. These creeks carried between 3000 and 6000µg l^{-1} of iron. Correspondingly high iron surface concentrations were found only in the inner Potter Cove area. At the Maxwell Bay stations A and B (B data not shown and similar to A), surface water concentrations of particulate iron were diluted to below 50 µg l^{-1} and so were the Cu and Mn concentrations outside the cove.

The on average Fe/Cu ratio for the earth crust is approximated to 927 (www.webelements.com), and the (sub-)glacial sands from KGI are within this range (Tatur et al. 1999). The Fe/Cu ratio was higher in sediment trap material (690) than in water column SPM (300) and decreased down to values between 1 and 5 in bivalve shells. The global ratio value for Fe/Mn is 57.3 (www. webelements. com), and this ratio was conserved in the water column and the sediment trap material, indicating both elements to be tightly coupled in sedimentary material. In contrast, lower ratios in shell material indicate preferential incorporation of Cu and Mn over iron into *L. elliptica* shell matrix. Indeed, both metals can substitute for Ca^{2+} ions in the calcium carbonate crystals (Tynan et al 2005). Cu appears to be concentrated in digestive gland (Fe/Cu 13-21) and gill (Fe/Cu 15) tissues (see Ahn et al. 2001). This indicates increased demand for Cu in the Antarctic soft shell clam. Copper containing hemocyanin (Hc) is the most common oxygen binding pigment in non-hemoglobin containing molluscs (Winzerling and Law 1997), and although not reported heretofore, it seems possible that copper containing blood proteins could exist in *L. elliptica*. Thus, low (and perhaps biologically irrelevant) Hc blood levels have been detected in other bivalves from the same area (*Yoldia eightsi*, Dewilde et al. 2003). Also Cu is the catalytic ion in iron-oxidases that oxidize ferrous Fe(II) to ferric Fe(III) for the binding to iron transport and storage proteins (Winzerling and Law 1997 for review).

Anyway, it seems that Mn and Cu, although occurring at less high concentrations in environmental samples are more concentrated in bivalve shells and tissues of the Antarctic soft shell clam. This may correspond to the relative toxicity of Fe, which is a more efficient Fenton catalyst than copper and induces formation of reactive oxygen species (ROS) in animal tissues, including very aggres-

sive hydroxyl radicals ($OH^{\bullet}$). We have shown that high natural Fe loads fuel lipid radical production in *Laternula elliptica* digestive gland samples (Estevez et al. 2002). And although these animals are adapted to the high iron levels in Potter Cove environments and should be tolerant of iron to some extent, they obviously limit uptake into their tissues to levels only twice as high as what we can measure in tissues from the European soft shell clam *Mya arenaria*. These latter animals come from low iron environments of the German Wadden Sea (Estevez et al. 2002). The strategy by which the animals eliminate iron could be via excretion with faeces and pseudo faeces (sediment particles aggregated with mucus and excreted without passing the alimentary tract) as shown by Ahn et al. (2004) for the Antarctic limpet *Nacella concinna* from the same Subantarctic island. In contrast, Cu was shown also to be taken up from the dissolved metal fraction over the gills in the mussel *Perna perna* (Yap et al. 2003), indicating that this element is less problematic with respect to ROS formation and of biochemical relevance for hemocyanin synthesis and iron oxidation in molluscs.

Therefore, less concentrated lithogenically derived metals such as Al, rather than Fe and Cu concentrations in growth bands of bivalve shells, could turn out to be useful markers for increased release of metals from melting glaciers in the Antarctic Peninsula region during the ongoing climate warming process.

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The potential ecological significance of dissolved and particulate matter in the water column of Potter Cove, King George Island (Isla 25 de Mayo), South Shetland Islands

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Introduction

Several studies with regards to primary production and particulate matter have been performed in the Potter Cove area, a shallow coastal environment characterized by low pelagic primary production and phytoplankton biomass during the whole year, and by a high load of suspended particles originated from terrigenous inputs and resuspension by winds (Klöser et al. 1994; Schloss and Ferreyra 2002; Schloss et al. 1999). The allochtonous inputs of particles mainly come from a glacier surrounding the cove, and from two freshwater streams (Matias and Potter). These freshwater inputs, together with the dominant wind stress conditions, define the typical structure of the water column in the zone, characterized by a shallow pycnocline (< 20 m, Schloss 1997). A conceptual model was developed by Schloss et al. (2002) aiming to explain the low phytoplankton biomass in the site, which show the characteristics of a typical HNLC (High Nutrients – Low Chlorophyll) system. Such a model hypothesized that the combination of the time scale of the physical factors (winds, freshwater runoff and ice cover) affecting both the radiation penetrating the water column and the depth of vertical turbulent mixing limit algal biomass accumulation.

It has been shown that freshwater streams in Antarctica may be a significant source of dissolved organic carbon (DOC) from terrestrial origin (Downes et al. 1986). However, very few data are available for Potter Cove (DOC) (Abele et al. 1999). It was suggested that DOC is a main sink of organic carbon in the ocean (Farrington, 1992). Part of the DOC reservoir is the primary source of energy for bacterioplankton respiration, therefore representing a significant component of the carbon cycle in marine environments. Moreover, photochemical reactions with ultraviolet-B radiation result in both, hydrogen peroxide formation as a photoproduct and low molecular weight compounds from the breakdown of the original molecules (Abele et al. 1999). This may enhance bacterial activity, thus modifying the dynamics of the carbon cycle. The aim of this research was to assess the concentration and variability of DOC and suspended particles in the water column at Potter Cove, a small fjord-like environment (50 m maximum depth) located in King George Is. (25 de Mayo Is., 62°14'S, 58°40'W).

Material and Methods

Field sampling was conducted during January-March 2000 at two stations, one located in the inner cove (S1) and the second one in the outer part of the cove (S2), from January 30 to March 30 2000. The sampling frequency was around 5 d, with a total of 12 stations. Only the results corresponding to S1 are shown here. Vertical profiles of temperature and salinity were obtained with a CTD instrument (ISITECH, Germany). Discrete water samples for analyses of particles (phytoplankton biomass as chlorophyll a (Chla), total suspended particulate matter (TSPM), percent of total organic matter (% TOM), particulate organic carbon and nitrogen (POC and PON, respectively)) and DOC were taken at 0, 5, 10, 20 and 25 m depth with Niskin bottles. Additionally, simultaneous samples were taken in the plume of the two main streams along the sampling period.

Determination of particulate matter:
For Chla measurements variable volumes of water were gently filtered onto Whatman GF/F filters and pigment extraction was done with 5 mL of Acetone 90% pro-Analysis, kept in darkness at 4°C during 8h and read with a spectrophotometer (Strickland & Parsons, 1972). TSPM was determined gravimetrically with an analytical balance, after filtering water onto pre-combusted and pre-weighted Whatman GF/F filters, and dryed at 60°C during 24 h. % TOM was estimated after combustion of the filters with the material at 500°C during 5 h, as the difference between the weight of the dry filters with the TSPM minus the filter with the combusted material. POC and PON analyses were performed with a Carlo Erba CHN particle analyzer, on samples filtered onto Whatman GF/F pre-combusted filters.

Determination of DOC:
DOC was measured by high-temperature catalytic oxidation with a Shimadzu TOC 5000 analyser equipped with a standard catalyst which consisted of Al_2O_3 particles containing 0.5% Pt. Samples were acidified with HCl, sparged for 10 min and 100 µL of the sample were injected on top of the 680°C catalyst and moved down by the carrier gas (pure oxygen) at a flow rate of 150 mL min^{-1}. The standard used for the organic carbon analysis was potassium hydrogen phthalate (Kanto Chemical Company) dissolved in Milli-Q water with UV treatment (Milli-Q 185 Plus, Millipore). The same water was acidified and used as blank. For further details refer to Skoog et al. (1997) and Kirchhoff (1997).

Statistical analysis
Regression equations and the corresponding correlation coefficients were computed using the Statistica version 7 software. Normality and homoscedascity of the data were tested to meet the requirements such type of analysis with the Kolmogorov-Smirnov and Levene tests, respectively.

Results and Discussion

CTD vertical profiles in general evidenced the presence of a shallow pycnocline, located between 5 and 10 m depth, as previously shown for the zone (Schloss, 1997). Such a strong density gradient can be explained by glacier and streams freshwater inputs. Vertical distribution of Chla, TSPM, POC and DOC closely followed the vertical distribution of density in the water column. Figure 1 shows a typical vertical distribution pattern of these parameters for January 30 2000.

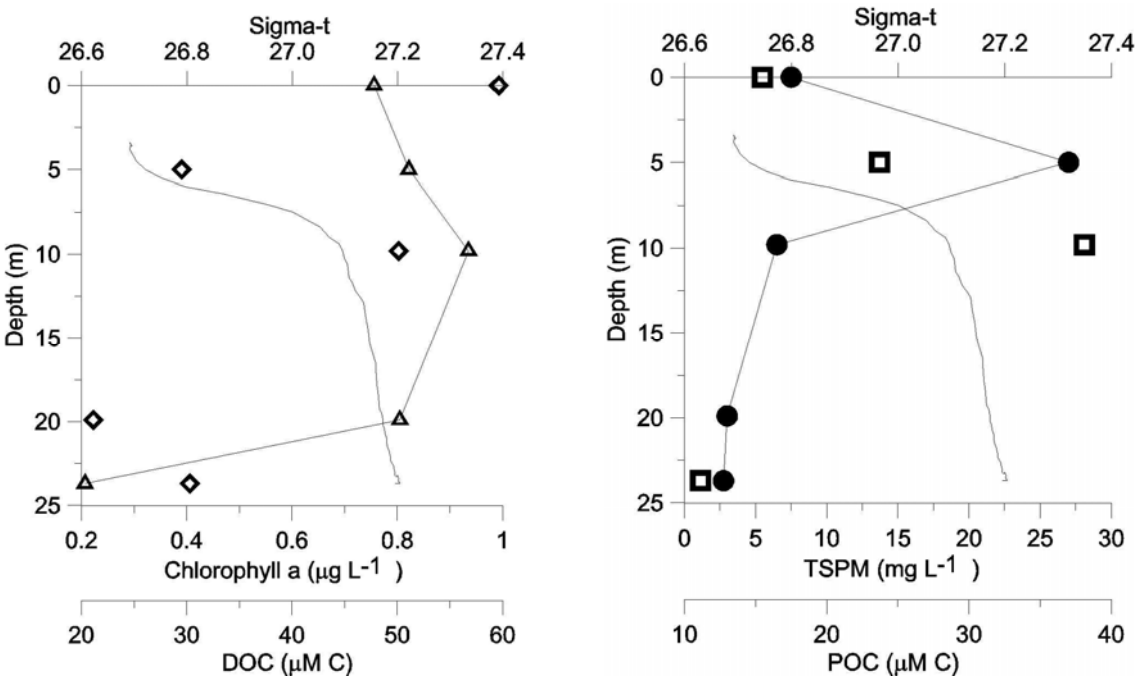

Figure 1. Vertical distribution of density (continuous line without symbols), Chla (µg L^{-1}, open triangles and continuous line) and DOC (µM C, open diamonds) (a) TSPM (mg L^{-1}, filled circles and continuous line) and POC (µM C, open squares) (b) during January 30 2000.

To describe the temporal evolution of the different parameters studied as well as the influence of the allochtonous inputs on their variability, the surface measurements in the water column of Potter Cove, corresponding to 0 and 5 m, were averaged and compared with the average values from the two streams. Chla concentrations in the cove were in the range of data previously reported for the area (Brandini and Rebello, 1994; Schloss et al. 2002), between 0.2 and 2 µg L^{-1}. They were similar to those in the plume of the streams, but higher during the periods of maximum phytoplankton abundance (Fig. 2 a). The C:N ratio (data not shown) varied from 6-7 during two maximum phytoplankton biomass events on 20 February and 13 March, respectively, and between 8 and 10 during the rest of the time. These values further coincided with relatively low wind stress periods (Fig. 2 b).

The TSPM concentration in the water column and in the plume of the streams was similar during most of the sampling period, at the exception of the first days of sampling, when incoming particles were clearly more abundant (Fig. 3 a).

The %TOM, however, was lower in the streams than in surface waters of Potter Cove from February 27 showing an increasing trend toward the end of the sampling, suggesting that allochtonous particles contained less organic carbon (Fig. 3 b).

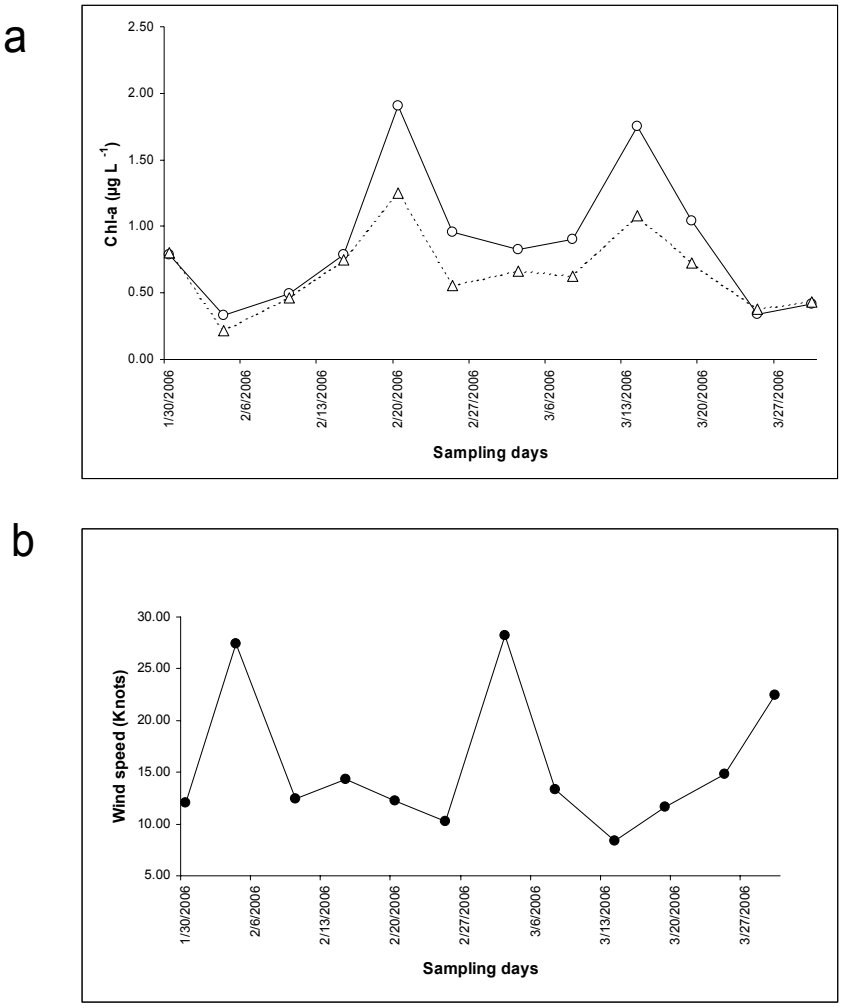

Figure 2. Chla (µg L^{-1}) in the surface of the water column (average of 0-5 m, open circles and continuous line) and the averaged values of the Matias and Potter streams (open triangles and dashed line) (a), and wind stress (filled circles and continuous line) (Knots) (b) measured in weekly intervals.

The amounts of POC and DOC entering the cove from both streams showed a high variability. No clear differences between the streams and the water column appeared in the temporal variation of POC, at the exception of a peak of ~40µM C on February 20 (Fig. 4 a). On the other hand, DOC concentrations at 0-5 m in the water column were higher than those found in the streams between February 10 and March 3, approximately. Such period roughly coincided with the first Chla maxima observed (Fig. 4 b).

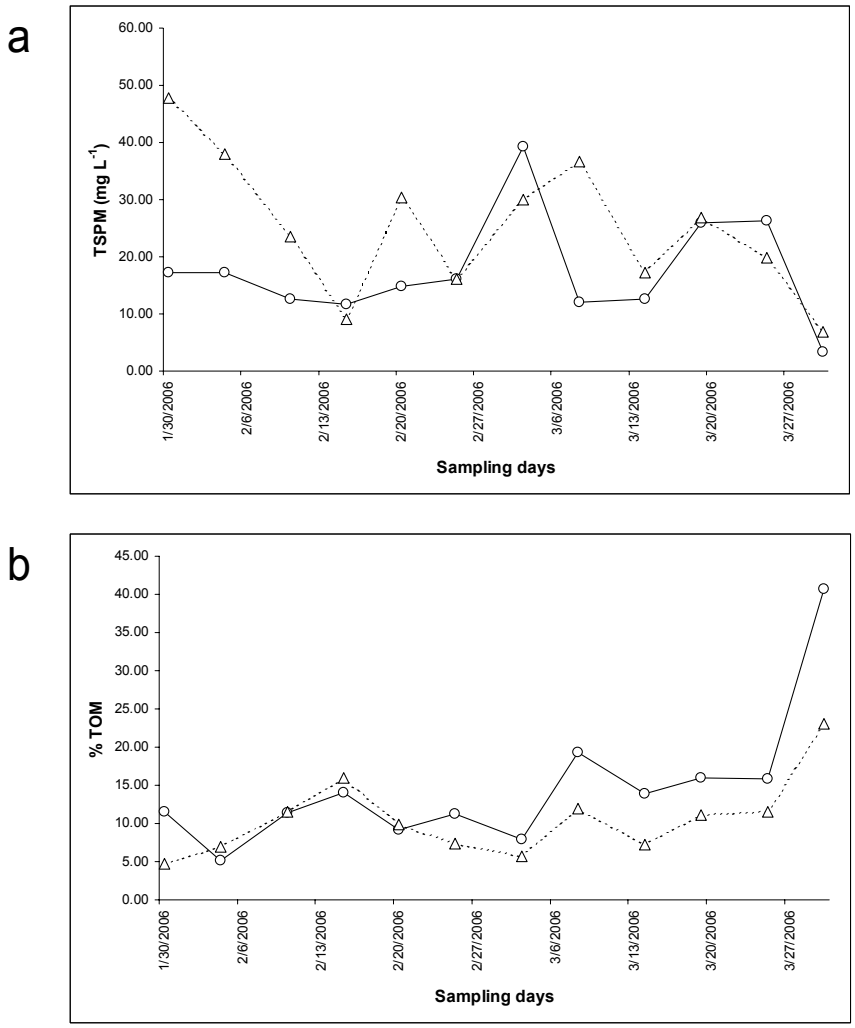

Figure 3. TSPM (mg L^{-}) in the surface of the water column (average of 0-5 m, open circles and continuous line) and the averaged values of the Matias and Potter streams (open triangles and dashed line) (a), and for the %TOM (same symbols) (b) measured in weekly intervals.

The relationships between the different studied variables were analyzed. Only those significantly related are shown here (Table 1). Chla in surface waters only correlated with PON, suggesting that particulate nitrogen in the water column mainly comes from phytoplankton. This was consistent with the inverse relationship between Chla and the C:N ratio. The same significant correlations were observed between these variables for particles in the streams. Furthermore, both Chla in surface waters and that entering the cove via the streams showed a strong correlation, suggesting the presence of significant inputs of algae from land. POC and TSPM in the water column were also directly related, suggesting that most POC originated from detritus more than from living particles. On the other hand, a direct relation was observed between %TOM from land origin and that in the cove, evidencing the transfer of organic particles via freshwater run-off to the sea. Moreover, the more the TSPM the less the %TOM in the streams, as shown by the significant inverse correlation between these variables. DOC in surface waters only correlated significantly with DOC in the streams, suggesting that the concentrations of such compounds observed in the water column of Potter Cove may in part originate from terrigenous sources.

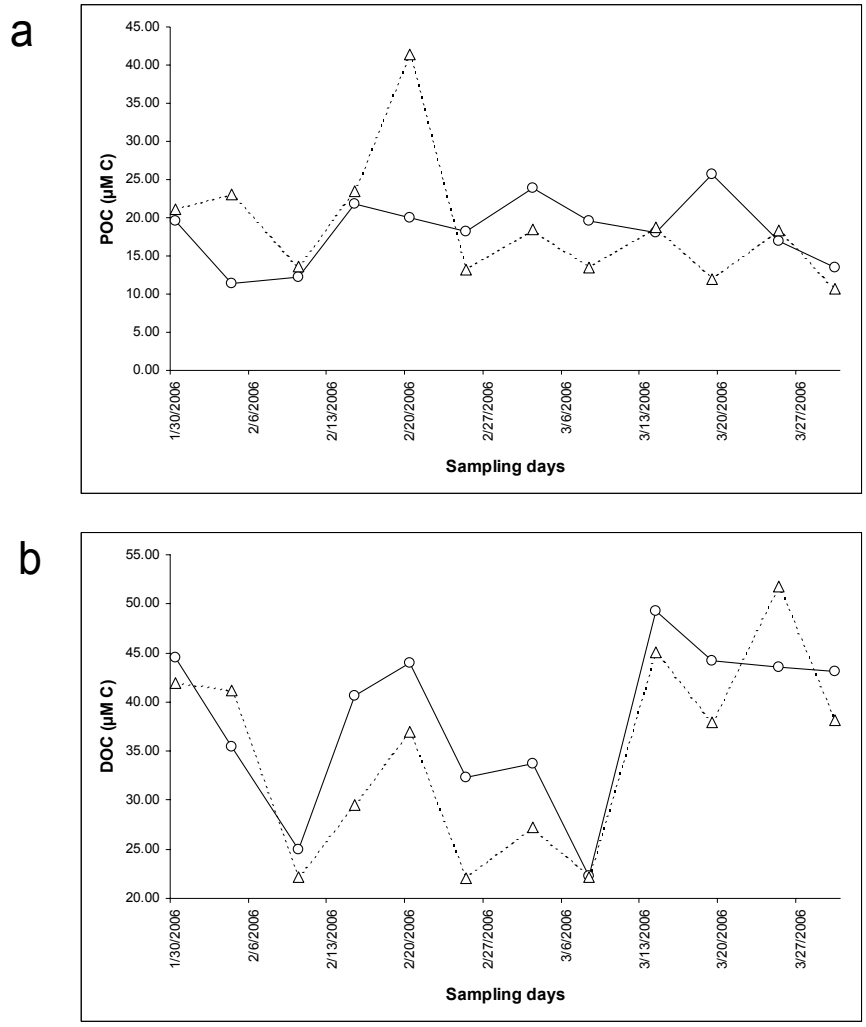

Figure 4. POC (µM C) (a) and DOC (µM C) (b) in the surface of the water column (average of 0-5 m, open circles and continuous line) and the averaged values of the Matias and Potter streams (open triangles and dashed line), respectively, measured in weekly intervals.

Reported DOC concentrations for the Southern Ocean range from 6 to 1000 µM C (Karl et al. (1996) and references therein). Values found in the area of Potter Cove during this research (~20-50 µM C) were within such range, and were closer to previous determinations done in the Bransfield Strait in December (between 52 and 102 µM C, Doval et al., 2002). Significant seasonal variability was observed in DOC in the area of the Ross Sea, with winter and summer values around 42 and 65-70 µM C, respectively (Carlsson et al., 2000). During the same study, winter and summer values of POC were 3 and 110 µM C, respectively. These differences were explained by large organic matter produced by photosynthesis during the growth period, particularly by the contribution of diatoms and *Phaeocystis antarctica*. Given that no bloom was observed during our sampling at Potter Cove, probably most of the total organic carbon (i.e., TOC, the sum of DOC plus POC) during this study originated from allochtonous inputs and resuspension from bottom sediments.

Regression equations	Probability
$Chla_{surf}$ = 0.61 PON_{surf} + 0.48	P < 0.001
$Chla_{surf}$ = -0.27 $C:N_{surf}$ + 3.16	P < 0.05
$Chla_{surf}$ = 1.63 $Chla_{str}$ - 0.20	P < 0.001
POC_{surf} = 0.26 $TSPM_{surf}$ + 13.92	P < 0.05
DOC_{surf} = 0.72 DOC_{str} + 12.47	P < 0.001
$Chla_{str}$ = 0.16 PON_{str} + 0.29	P < 0.05
$Chla_{str}$ = -0.19 $C:N_{str}$ + 2.33	P < 0.05
$\%TOM_{surf}$ = 1.52 $\%TOM_{str}$ - 1.42	P < 0.001
$\%TOM_{str}$ = -0.28 $TSPM_{str}$ + 17.61	P < 0.05

Table 1. Regression equations corresponding to the different variables which presented significant correlation coefficients. In the right column are shown the corresponding probability levels. The subscripts surf and str indicate surface and streams, respectively.

DOC concentrations were significantly higher than those corresponding to POC, averaging 70 % (± 6.9 %) of TOC. These estimations are consistent with determinations reported for winter and oligotrophic waters (Hansell and Carlsson 2001). The low phytoplankton biomass observed suggests that the biological activity in the water column of Potter Cove would be mainly heterotrophic, probably fuelled in part from a significant contribution not only from stream inputs but also from of macroalgae-released DOC. The outer part of Potter Cove present a complex macroalgal community with a high biomass (Quartino et al. 2001, 2005), with detritus losses from such stock accumulating in the inner part of the cove (Mercuri, pers. comm.). It has been shown that macroalgae represent a significant source of DOC and POC in coastal waters (Fischer and Wiencke 1992; Gutt et al. 1998; Duarte et al. 2005), which could support this hypothesis. On the other hand, these findings, together with the potential contribution of macroalgae to carbon dynamics in Potter Cove, reinforces the previous hypothesis by Schloss (1997) suggesting that the main energy source sustaining the profuse biomass stock of benthic filter feeders present in the sediments (Tatian et al., 2004) may be explained by macroalgal POC inputs. More research is needed on the above topics to assess the origin and fate of POC and DOC in the context of ecosystem processes.

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2. STRUCTURE AND FUNCTION OF THE ECOSYSTEM

Macroalgal assemblages related to abiotic factors in Potter Cove, King George Island (Isla 25 de Mayo), Antarctica

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Introduction

The outer side of Potter Cove (Isla 25 de Mayo, King George Island), South Shetland Is, Antarctica (62˚14'S, 58˚38'W) and the adjacent open coasts are colonized by extensive macroalgal assemblages which are an important energy source for benthic deposit and suspension feeders present in the inner cove (Tatián et al. 2004).

Previous studies in Potter Cove described the distribution of the macroalgae at the different depths and sites by scuba diving sampling and observations (Klöser et al. 1994) and also by underwater video documentation (Klöser et al. 1996). Although the video allows the monitoring of large areas it is not adequate for the estimation of species abundance, especially of the smaller species. Both methods are more likely to reflect spatial variability in the abundance of species with larger thalli than differences in the species composition of the flora (Klöser et al. 1994, 1996).

This paper summarizes the present knowledge (Quartino et al. 2001, 2005) about the distribution of macroalgal assemblages distribution related with the principal abiotic factors present in Potter Cove.

Materials and Methods

Six sites were sampled by SCUBA diving from January to March 1994, 1995 and 1996 at Potter Cove (Fig. 1). Three sampling units of $1m^2$ were placed at 0, 5, 10, 20 and 30 m along 26 transects perpendicular to the shore (total 130). All macroalgal individuals were removed from the substratum, except for the crustose algae. Field samples were carried to the Dallmann Laboratory at Jubany Station for identification and counting in order to obtain the density of each species.
The material was then fixed in 4% formaldehyde in seawater and transported to the Instituto Antártico Argentino in Buenos Aires. Dry seaweed biomass (g/m^2) was determined after drying algae at 60°C to constant weight. Voucher specimens were deposited at the Herbarium of the Museo Argentino de Ciencias Naturales in Buenos Aires (BAc).

The substratum at each sampling point was classified according to granulometry. Water temperature and salinity were obtained using a ME-ECO 219 micro CTD and PAR irradiance (400–700 nm range) was measured monthly at noon, at 0, 5, 10, 20 and 30 m depth at each sampling site during 1996, using a spherical underwater radiation sensor LI-192 SA. Daylength was determined at the date when the algal sample was taken. Phosphates and nitrates were determined from water samples using a 4-channel auto-analyzer Technicon II. A complete set of duplicate nutrient samples was taken at all sampled points in January 1996; values for other dates were estimated using historical data and interpolated values Schloss et al. (1998, 2002).

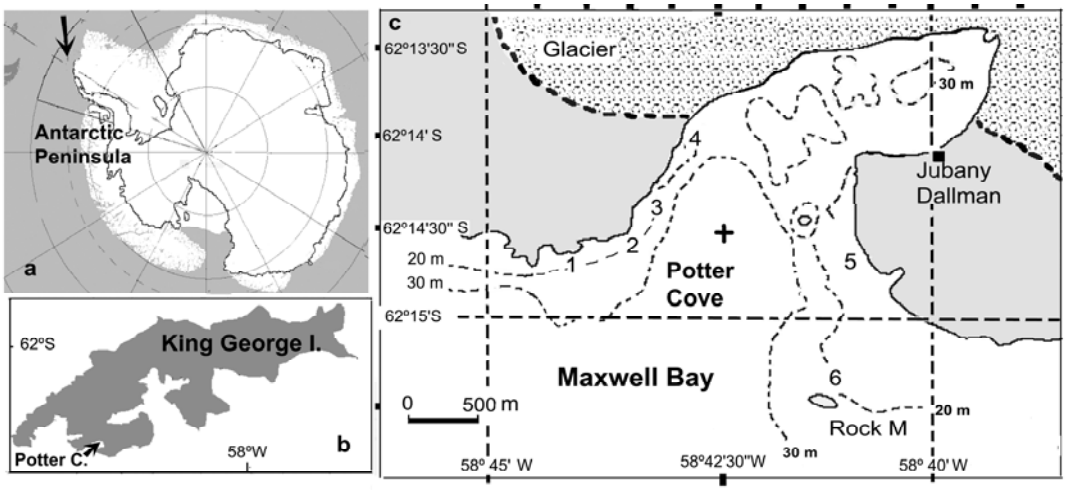

Figure 1. Map showing the location a- South Shetland Islands (Arrow) b- Potter Cove at Isla 25 de Mayo/King George Island (Arrow) c- Sampling sites at Potter Cove (Numbers mark the position of the six sites at the Cove)

Algal sampling units were grouped into clusters using incidence (presence-absence) and biomass data, with the K-means method (Hair et al. 1992, Legendre 2004). The Calinski-Harabasz pseudo-F-statistic was used as the stop criterion for the number of groups. This is a least-squares partitioning method allowing the division of a collection of objects into K groups (Hair et al. 1992, Legendre and Legendre 1998). The K-means program used (Legendre 2004) performs different data transformations and calculates the Calinski-Harabasz pseudo-*F*-statistic (Calinski and Harabasz 1974), because the Euclidean distance is not suitable for raw species abundance data involving zero abundance (Legendre and Legendre 1998). The Hellinger index is one of these transformations and it is recommended for clustering or ordination of incidence or abundance data (Rao 1995). Milligan and Cooper (1985) determined that the Calinski-Harabasz pseudo-*F*-statistic (CH) criterion was the best of 30 stop criteria tested to determine the correct number of groups. CH maximum value indicates optimal number of groups, corresponding to the most compact set. A validation of the results obtained with the K-means method when applied to the incidence data was informally done by comparing the results with the clusters obtained by the hierarchical agglomerative clustering UPGMA method based on the Ochiai index (van Tongeren 1995, Legendre and

Legendre 1998). In this case, the grouping process was stopped when the distance between successive clustering steps was maximal, as suggested by Hair et al. (1992). With the same objective, the results obtained with the K-means method when applied to the biomass data were also compared with the clusters obtained by UPGMA based on the Bray–Curtis index (with square-root transformation of the data).The indicator species of the groups determined with biomass data were identified with the indicator value index (IndVal) (Dufrêne and Legendre 1997). When quantitative (biomass or number) data are used, IndVal is calculated for each species i present in each group j as:

$$IndVal_{ij} = 100\ A_{ij}\ B_{ij}$$

A_{ij} is a measure of specificity whereas B_{ij} is a measure of fidelity of the species to the group. A_{ij} is defined as:

$$A_{ij} = N_{ij} / N_{i.}$$

N_{ij} is the mean abundance (biomass) of the species i across the sampling units in the group j, while $N_{i.}$ is the sum of the mean abundances of species i over all groups. The mean abundance in each group is used, instead of summing the abundances across all sites of a group, to remove any effect of the difference in number of sampling units in the groups. A_{ij} attains its maximum of 1 when species i is only present in group j.

B_{ij} is defined as: $B_{ij} = Nsu_{ij} / Nsu_{.j}$

Nsu_{ij} is the number of sampling units in the group j with species i present, while $Nsu_{.j}$ is the total number of sampling units in that group. B_{ij} attains its maximum of 1 when the species i is present in all the sampling units of the group j. A and B are combined by multiplication because they represent independent information about the distribution of species i. The index is maximal (100) when all the individuals of species i are present in all sampling units belonging to a single group. The indicator value of species i is the largest value of $IndVal_{ij}$ observed over all groups j:

$$IndVal_{\ I} = \max [IndVal_{ij}]$$

The IndVal program (Dufrêne 2004) randomly reallocates the sampling units in the groups to test the significance of the index. In this study, a significance level of $p < 0.01$ was used. For incidence data the expression of IndVal is the same as above, but with the correction of Dufrêne (2004), where N_{ij} is the number of sampling units of group j where the species i is present and $N_{i.}$ is the total number of sampling units where i is present.

The biomass groups obtained by the K-means method were related to the abiotic factors measured through a factorial correspondence analysis (CA). The use of CA with contingency tables is described in Legendre and Legendre (1998).The classes of the abiotic factors used in the CA were as follows. Depth (relative to mean low water level): D1 (≤ 5 m), D2 (6–15 m), D3 (≥16 m). Temperature: T1 (≤ -0.1˚C), T2 (0.0 to 0.9˚C), T3 (≥1.0˚C). Nitrogen (nitrate N+nitrite N): N1 (≤17.99 µM), N2 (18–24.99 µM), N3 (≥25 µM). Phosphates: P1 (≤1.99 µM), P2 (2.0–2.99 µM), P3 (≥ 3 µM). Salinity: S1 (≤33.49 psu), S2 (33.50–33.99 psu), S3 (≥34.00 psu). Daylength: L1 (≤14.9 h), L2 (15–17.9 h), L3 (≥18 h). Photon irradiance: IR1 (≤99 µmol m^{-2} s^{-1}), IR2 (100–499 µmol m^{-2} s^{-}

^{1}), IR3 (≥500 µmol m^{-2} s^{-1}). Substrate: SU1 (rocks >100 cm), SU2 (boulders 100–20 cm), SU3 (stones and pebbles <20 cm).
A general validation of the CA results was performed by means of a canonical correspondence analysis (CCA) (ter Braak 1986, ter Braak and Smilauer 1998) using square-root transformation of the original data, downweighting of rare species, preservation of the distance between species, and selection of significant variables ($p < 0.05$). Depth, temperature, nitrogen, phosphates, salinity, day length and irradiation were treated as quantitative variables. The substratum, which is an ordinal variable, was divided in six classes and treated as a quantitative variable following the suggestion made by ter Braak and Looman (1995). A previous detrended correspondence analysis (DCA) was done to calculate the gradient length and verify that the CCA was the right procedure (ter Braak 1995).

Results

The K-means method to the incidence data showed that the macroalgae species observed are best assembled into two main units. The indicator species of group 1 were: *Iridaea cordata, Adenocystis utricularis, Desmarestia menziesii, Ascoseira mirabilis, Curdiea racovitzae, Gigartina skotsbergii, Phaeurus antarcticus, Palmaria decipiens, Gymnogongrus turquetii and Monostroma hariotii.* The indicator species of group 2 were *Himantothallus grandifolius, Desmarestia anceps, Georgiella confluens, Ballia callitricha and Plocamium cartilagineum.* The same two main groups arise when these results were validated with the Ochiai index and UPGMA method.

K-means analysis was performed also using biomass data. The stopping rule of the Calinski-Harabasz pseudo-*F* statistic determined that the most compact set was composed of three groups. An internal validation of the method using the Bray & Curtis distance and the UPGMA algorithm showed also a main structure topology with three clusters, with 98.2 % coincidence in the groups composition after three isolated sampling units were discarded in the UPGMA.

The relationship between the three observed groups based on the biomass data and the abiotic factors measured was determined through factorial CA. The results showed that group A (characterized by *Iridaea cordata*, *Monostroma hariotii*, *Adenocystis utricularis* and *Enteromorpha bulbosa*) was mainly associated with rocky substrata, shallow water depths (0–5 m), high temperatures (over 1˚C), high irradiances (more than 500 µmol m^{-2} s^{-1}), intermediate to low salinities (below 34 psu), intermediate to high phosphate concentrations (over 2 µM) and low nitrogen concentrations (below 18 µM). This group included some species living in midlittoral pools and on rocks of the upper sublittoral zone.

Group B (characterized by *Desmarestia menziesii, D. antarctica, Ascoseira mirabilis, Curdiea racovitzae, Gigartina skottsbergii, Neuroglossum delesseriae, Phaeurus antarcticus, Gymnogongrus turquetii* and *Kallymenia antarctica*) was mostly associated with boulder substrates (20–100 cm grain size), intermediate

water depths (6–15 m), low temperatures (below 0˚C), intermediate irradiance values (100–499 µmol m^{-2} s^{-1}), intermediate to low salinities (below 33.5 psu) and intermediate nitrogen concentrations (between 18 and 24.99 µM).

Group C (characterized by *Himantothallus grandifolius*, *Desmarestia anceps*, *Georgiella confluens*, *Ballia callitricha* and *Plocamium cartilagineum*) was mainly associated with stones and pebbles (smaller than 20 cm), greater water depths (below 15 m), low and intermediate temperatures (below 1˚C), low irradiance values (below 100 µmol m^{-2} s^{-1}), high salinities (over 34 psu), high nitrogen concentrations (over 25 µM) and low phosphate concentrations (below 2 µM).

A CCA relating the biomass of individual species to environmental factors was performed to validate the above results that were based on groups. A DCA prior to the CCA confirmed that CCA was the appropriate procedure for this analysis (ter Braak 1995). Daylength and temperature were excluded from the analysis because they were not significant. The joint plot of species and environmental factors corresponds to the general CCA (Fig. 2). The environmental variables are represented by arrows. Environmental variables with long arrows are most strongly correlated with the ordination axes than those with a short arrow (ter Braak 1995). The cumulative percentage variance of the species data explained by the two first axes was 28.1 % and of 87.2 % for the species - environment relationship (Table 1).

Table 1. Summary of canonical correspondence analysis ordination.

Axes	1	2	3	4	Total inertia
Eigenvalues	0.756	0.215	0.063	0.038	3.458
Species-environment correlations	0.949	0.675	0.640	0.512	
Cumulative percentage variance of:					
species data	21.9	28.1	29.9	31.0	
species-environment relation	67.9	87.2	92.8	96.3	
Sum of all unconstrained eigenvalues					3.458
Sum of all canonical eigenvalues					1.113

In the plot, the indicator species of group A are associated with rocky shores, with the highest phosphate concentrations (over 2.04 µM), and with the lowest values for water depth (less than 15 m) nitrate concentration (less than 20 µM) and salinity (less than 33.9 psu). The indicator species of group B were associated with water depths of 15 m (weighted mean) or less, with substrata consisting of stones of 20–50 cm, with intermediate values of photon irradiance (weighted means 337.2 µmol m^{-2} s^{-1}), with low to intermediate phosphate concentrations (2.04 µM), with intermediate nitrate concentrations (20 µM) and with intermediate salinities (33.87 psu). The indicator species of group C were associated with substrata with sediments and rocks less than 20 cm in diameter and pebbles, with below-average irradiance and phosphate values, with high nitrate concentration, with above-average salinities (33.87 psu) and with large water depths (20–30 m).

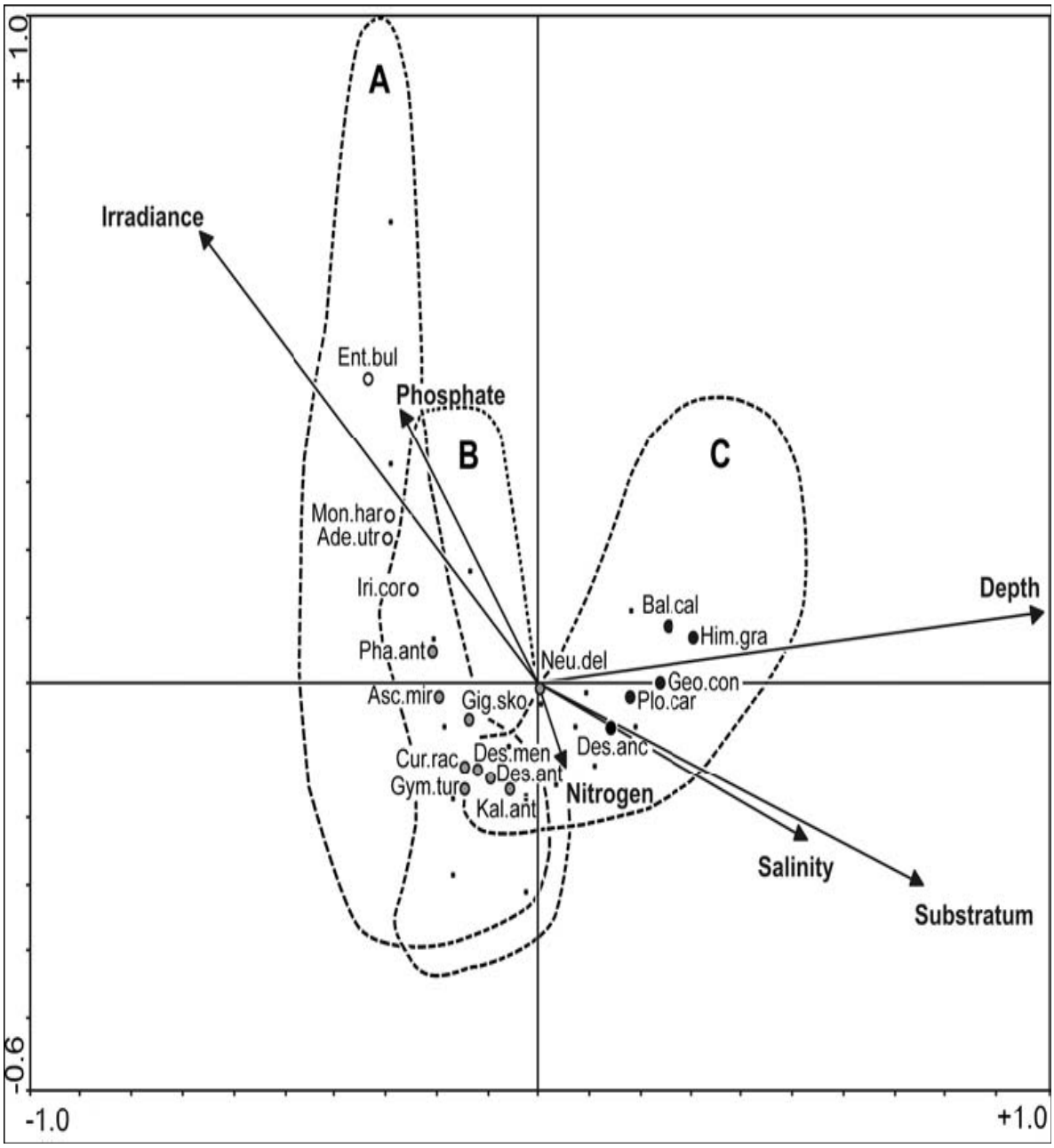

Figure 2. Canonical correspondence analysis (CCA) joint plot of species (biomass) and environmental variables. Indicator species of group A (white circles), group B (grey circles) and group C (black circles). Indicator species are abbreviated based on the first three letters of the genus and species names (see Table 1 for genus and species names). Points are nonindicator species. The areas A, B and C limited by dashed lines correspond to the distribution of the sampling units in the respective K-means groups. The arrows represented the environmental variables.

Discussion

The influence of an ecological factor may cause a qualitative change and the luxuriant development of one or few species, but may fail to cause any substantial change in the qualitative species composition of the biocoenosis; the result of such a quantitative change is called a facies (Pérès 1982). In Potter Cove two qualitative seaweed assemblages and three biomass facies were

found through alternative and robust clustering methods (K-means and UPGMA). The assemblages based on incidence data were one shallow assemblage (group 1) and one deep assemblage (group 2). Several species were probably overlooked, and these could include species of small size and also the crustose corallines documented by Klöser et al. (1994). The shallow qualitative assemblage (group 1) comprises two quantitative assemblages, which will be referred to as biomass facies or simply as facies. The first facies was characterized by *Iridaea cordata*, *Monostroma hariotii* and *Adenocystis utricularis* (group A) and was related to depth down to 5 m, high irradiance values and solid rocks; this group had the lowest species richness and highest biomass equality. The second facies (group B) was characterized by *Desmarestia menziesii*, *Ascoseira mirabilis*, *Curdiea racovitzae*, *Gigartina skottsbergii* and *Kallymenia antarctica* and was associated with depths of 6–15 m, large rocks and intermediate irradiance values. The second qualitative assemblage (group 2) was almost completely equivalent to a biomass-based group (group C). It was characterized by *Himantothallus grandifolius*, *Desmarestia anceps*, *Georgiella confluens*, *Ballia callitricha* and *Plocamium cartilagineum* and was mainly associated with depths below 15 m. Green (1976) advocates the use of incidence data in community delimitation, while many studies of biological impact assessment prefer quantitative data, as these are considered more sensitive to environmental variation. The analysis of our biomass data shows that differences between groups are mostly due to the presence of the large Desmarestiales and the analysis of density data stresses the variability of the most numerous and smallest species, e.g. members of the Rhodophyceae and Ulvophyceae, and the presence of numerous newly recruited thalli of large Phaeophyceae.

The species composition of assemblage A at Potter Cove is similar to the sublittoral fringe in Admiralty Bay (Zielinski 1990). This group is represented by only a few sampling units in the study at Signy Island (Brouwer et al. 1995) where it is characterized by *Iridaea cordata* and *Curdiea racovitzae*. It is almost absent at the inner site of Potter Cove (Quartino et al. 2001). In addition to the ice-abraded zone in the shallow sublittoral, two other macroalgal associations or zones have been traditionally recognized for Antarctica: a zone in the central sublittoral dominated by *Desmarestia anceps* and *D. menziesii* and a zone of *Himantothallus grandifolius* at greater depth (Heywood and Whitaker 1984).

Brouwer et al. (1995) classified fourteen lumped biomass samples from two sites at Signy Island (South Orkney) representing one exposed and one sheltered situation. Three of the groups at Signy Island (the one from exposed sites with *D. menziesii* and *D. anceps*, a second from sheltered sites with *Ascoseira mirabilis* and *Callophyllis atrosanguinea*, and a third with *D. anceps*, *Himantothallus grandifolius*, and *Kallymenia antarctica*) are equivalent when they are combined, to group B in our study. The group occurring in deep waters at Signy Island with *Himantothallus grandifolius* is similar to our group C. It is impossible to assess whether the dominance of *D. anceps* at intermediate water depths at Signy Island is dependent on exposure differences between the two sites, because at the sheltered site samples were only taken up to 12 m depth (Brouwer et al. 1995). At Signy Island *H. grandifolius* is often dominant on soft substrata at intermediate depths. At Potter Cove it became dominant at great

depths, on fine-grained substrata where *Desmarestia* species could not grow in the absence of hard substratum or boulders (Quartino et al. 2001).

Klöser et al. (1996) described a sublittoral fringe, an upper sublittoral zone with *Desmarestia menziesii* and *Ascoseira mirabilis*, and a *Himantothallus grandifolius* assemblage, which correspond to groups A, B and C respectively of this study. At vertical, calm sites they report *H. grandifolius* growing in the uppermost meters, as at Signy Island. They also describe a fourth belt dominated by *D. anceps*, between the *H. grandifolius* and *D. menziesii* belts. However, they point out the existence of more exposed sites where *D. anceps* grows deeper and yet other sites where the belt of *D. anceps* is suppressed by ice scouring. In our qualitative classification, *D.anceps* appears to be more associated with group 2, so it is possible that the fourth zone described by Klöser et al. (1996) corresponds to a facies of this assemblage, favored by hard substratum and moderate exposure conditions.

Chung et al. (1994) studied the sublittoral associations in Marian Cove (62˚13‘S, 58˚46’W), close to Potter Cove, along eight transects, using cluster analysis. They did not separate *Desmarestia menziesii* from *D. anceps*, neither did they give details about the composition of the associations, but they reported great quantities of *D. antarctica* and describe a characteristic zone dominated by *Palmaria decipiens*. Neushul (1964) also observed *P. decipiens (*as *Leptosomia simplex)* at very exposed sites in Half Moon Island (South Shetland). Chung et al. (1994) also report some stands of *Ascoseira mirabilis* replacing *Desmarestia* species. Klöser et al. (1996) point out that this species thrives under steady roller water movement and compares this observation with several former reports of its dominance under turbulent conditions.

Substratum, depth, nitrogen and phosphate concentration, salinity and irradiance were all related to the distribution of the groups and their indicator species in Potter Cove. Temperature and daylengths alone were eliminated by the CCA analysis as important factors, probably because the study was limited to the summer months. At Potter Cove, the greatest variability between qualitative seaweed assemblages were related to water depth, to the gradual change of substratum from hard to soft, to irradiance and to a lesser extent to salinity or temporary nutrient stratification. At the inner sites, drift ice scouring is considered a major disturbance factor for the benthic fauna (Sahade et al. 2004).

The outer Potter Cove coasts are characterized by an extensive underwater macroalgal forest on hard bottom, providing food to several benthic herbivores (Iken et al. 1998) and the habitat and shelter for different organisms (Momo et al. 1998). Macroalgae are important primary producers that contribute to the coastal food webs either directly (Iken et al. 1998) or indirectly, as they constitute a main source of particulate and dissolved organic carbon (Fischer and Wiencke 1992, Gutt et al. 1998; Duarte et al. 2005). In the Cove Cove, where pelagic primary production is low, both during winter and summer (Schloss et al. 2002) consequently**,** the rich benthic fauna present in the zone must probably depend on other food sources than phytoplankton (Tatián et al. 2004). The information supplied here provides the actual state of the macroalgal

assemblages at Potter Cove. Changes in the distribution limit of macroalgae may also entail important changes of trophic coupling in the coastal food webs.

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Macroalgal production and the energy cycle of Potter Cove

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Introduction

Coastal macroalgae carbon production seems to be an important food source to the benthic Antarctic communities (Iken et al. 1998; Huang et al. 2006). If not grazed, macroalgae die and decompose returning particulate organic matter and mineral nutrients to the system (Hanisak 1992). Zielinski (1981) observed that the seaweed decomposition process depends on the place where it occurs, the kind of thalli and the season.

Biomass data of Antarctic macroalgae are available from different Antarctic localities (DeLaca & Lipps 1976; Miller & Pearse 1991; Amsler et al. 1995; Brouwer et al. 1995; Klöser et al. 1996; Quartino et al. 2001). Growth rates of several Antarctic selected seaweed were determined in the laboratory by Wiencke & Dieck (1989); Wiencke (1990 a,b) and Gómez & Wiencke (1997). Production in the field can be estimated by differences in standing stocks between successive dates (Bellamy et al. 1973; De Wreede 1985; Israel 1995) or through the product of growth rates and biomass (Mann 1972).

An effort has been performed during the last years to achieve a deeper knowledge of the benthic system at Potter Cove (King George – 25 de Mayo Island, South Shetland, Antarctica) including studies of the macroalgal communities (Klöser et al. 1996; Quartino et al. 2005) and biomass (Quartino et al. 2001).

Macroalgae are especially abundant in hard substrata of Potter Cove; an important fraction of their production contributes to the soft sediments of the inner cove. The macroalgal detritus decomposes and is eaten by detritivores and suspensivores, supporting an important amount of the secondary production (Tatián et al 2004).

The present work intends to determine the importance of macroalgae to the matter and energy fluxes in Potter Cove, including making an energy budget and estimating the potential energy exportation from the cove to the open sea benthos and water column.

Site study and methods

Samples were taken from Potter Cove (62º 14’ S, 58º 38’ W); the study area is situated on the coasts at the mouth of the cove (Fig. 1). The bottom of the outer Potter Cove consists of hard substrate with macroalgal vegetation of changing

density. Detailed descriptions of Potter Cove have been presented earlier (Klöser et al. 1996; Quartino et al. 2005).
Biomass samples were obtained by scuba diving at six sites (Fig. 1), from January to March 1994, 1995 and 1996. Sampling units of 1 m^2 were placed along 26 transects perpendicular to the shore (total 128). All the macroalgae except crustose red algae were removed from the substratum. Seaweeds were separated, dried at 60 °C and weighed using electronic balances Mettler AJ (0.001-100g) and Sartorius PT6 (1 - 5000g) for biomass (g.m-2) determinations. Biomass data from two sites (Sites 1 and 2, Fig. 1) obtained during two summer seasons (1994 and 1995) and published growth rates (Wiencke 1990 a,b; Gómez and Wiencke, 1997) were used to calculate the macroalgal production of the five most abundant species.

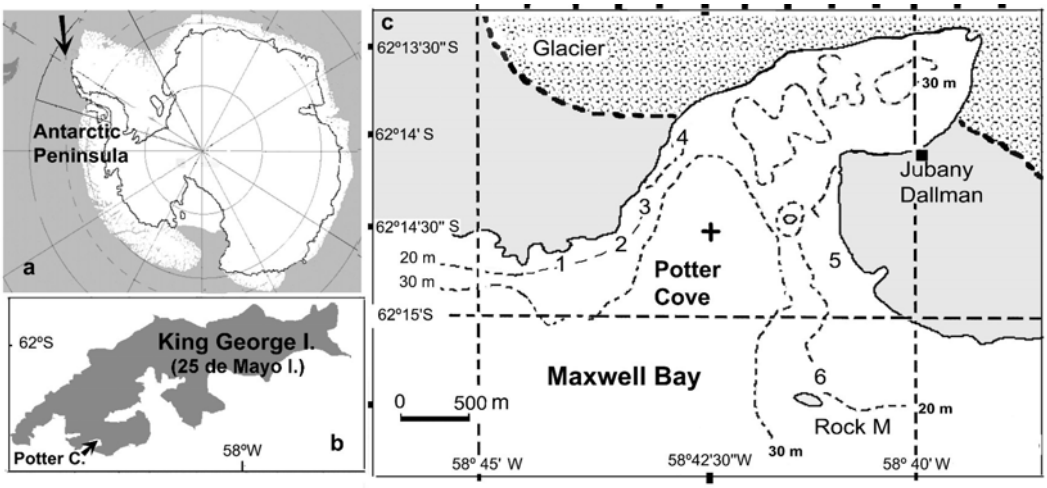

Figure 1. Study zone and sampling zones (numbered).

The average expected biomass (EB) of each species (i) during a month (m) was calculated for each depth (d) as:

$$EB_{i.m.d} = OB_{i;\ m-1.d}.\ e^{rt}$$

Where OB_{m-1} is the observed average biomass ($g.m^{-2}$) for the same depth at the previous month; r ($g.g^{-1}.d^{-1}$) is the daily intrinsic growth rate and t (days) is the period length.
The monthly average production of species i (P) ($g.m^{-2}$) at each depth in the period between two months was calculated as:

$$P_{[(m-1);\ m];\ i} = EB_{m;i} - OB_{m-1;\ i}$$

Where $EB_{m;i}$ is the expected biomass of species i (g. m^{-2}) at month m and $OB_{m-1;i}$ is the observed biomass (g. m^{-2}) at month m^{-1}. Areas with macroalgae at different depth intervals were calculated using the bathymetric charts of South Shetlands Islands H-137 (1:25,000) and Potter Cove n° H-711 (1:200,000) with an inset (1:10,000). The standing stock for each summer month was estimated as the sum of the product of the average biomass ($g.m^{-2}$) and the area (m^2).

The biomass flux (BF) to the ecosystem for the periods corresponding to increasing or stable biomass, was calculated as the difference of observed biomass and the expected biomass at the same date.

$$BF_{[(m-1);\ m];i} = EB_{m;i} - OB_{m;i}$$

These calculations were performed for Barton and Pinitos Points (site numbers X and Y in Fig. 1c), considered the main algal biomass sources for the cove. The period considered was from December to March throughout a 90 days interval.
Biomass data were transformed to energy values using the equivalence gave by Margalef (1981): 1 g of dry weight of algae = 18.75 Joules. In order to calculate the decomposed fraction we used two values for the decomposition rate: one is 0.0016 day^{-1}, estimated by Brouwer (1996); the other is the estimated by Pedersen et al (2005) for *Sargassum muticum*; despite this last value (0.016 day^{-1}) is too high for Antarctica, it is useful in this work because represents a possible maximum for decomposition.

Results and discussion

A total of 38 species was identified in the study area, within the sampling units. *Desmarestia anceps*, *D. menziesii* and *Himantothallus grandifolius* accounted for almost 80% of the biomass while *Iridaea cordata*, *Ascoseira mirabilis*, *Plocamium cartilagineum*, *Gigartina skottsbergii*, *Curdiea racovitzae*, *D. antarctica*, *Adenocystis utricularis* and *Palmaria decipiens* accounted for 15% of the biomass; the other 27 species provided only 5% of the biomass.
The total calculated area for the production zone is 2.5 10^6 m^2 containing a monthly average standing stock of 795.6 Ton for the summer period. The total produced biomass (dry weight) from December to March was 1168,6 Ton and the corresponding flux of biomass to inner cove in the same period was 1272.7 Ton.
Using these values, we calculated the total fluxes of algal biomass and energy in the cove (Figure 2). The values are only rough approximations but give us an indication of the importance of macroalgae in the matter and energy economics of Potter Cove.

The outer side of Potter Cove comprises an extensive community of macroalgae, which colonize the hard bottom and provide food to several benthic herbivores (Iken et al. 1998) and the habitat and shelter for different organisms (Momo et al. 1998). It has been shown that seaweeds are a main source of particulate and dissolved organic carbon (Fischer & Wiencke 1992; Gutt et al. 1998; Duarte et al. 2005) and provide a substrate for epiphytic communities (Dayton 1985). In Potter Cove pelagic primary production is low, both during winter and summer; consequently, the rich benthic fauna present in the zone probably depends on food sources alternative to phytoplankton (Tatián et al. 2004).

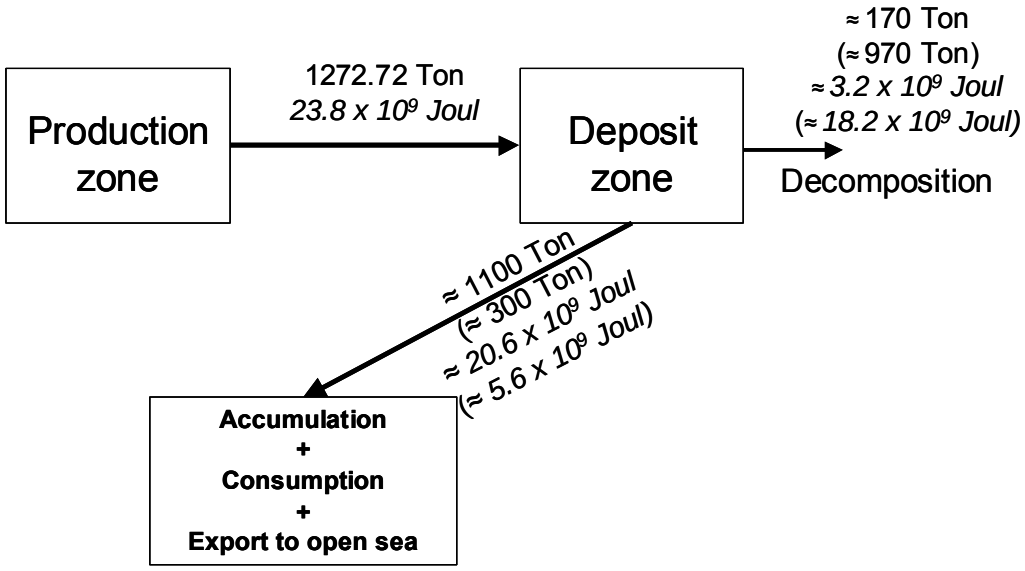

Figure 2. Macroalgal matter and energy budget in Potter Cove. Estimations were made using the decomposition rate given by Brouwer (1996); values in brackets were calculated using the decomposition rate given by Pedersen et al. 2005.

The mean wet biomass of Desmarestiales in Potter Cove, from 5 m to 30 m depth, ranged from 2400 $g.m^{-2}$ to 9600 $g.m^{-2}$, whereas in Anvers Island, from 5 m to 15 m depth, Amsler et al. (1995) reported values of 4500 $g.m^{-2}$ to 5200 g m^{-2}. As usual in the West Antarctica (Clayton, 1994) the Desmarestiales are the algae with the highest biomass at Potter Cove.

Large quantities of macroalgae are lost from the sublittoral by physical disturbances. Algae can sink to the hollows of the seabed, can be washed ashore, or drift in the sea. The detached and particulated macroalgae provide food for the filter feeders and detritivores (Fischer and Wiencke 1992; Gutt et al. 1998).

Duarte & Cebrian (1996) reviewed data from literature about the pathways for several marine primary producers, including macroalgae. They concluded that decomposition within the system is an important process for each macrophyte system (>40% of the net primary production), the herbivore pressure is significant for macroalgae only (>30%), export is significant (24–43%); and storage within the sediment is negligible for macroalgal communities.

Macroalgal biomass can become available to the fauna through different mechanisms of herbivory, or by thallus fragmentation and further bacterial degradation and by release of spores. In Potter Cove the limpet *Nacella concinna* feeds mainly on crustose red algae and *Ascoseira mirabilis*, while the isopods *Plakarthrium punctatissimum* particularly feeds on *Curdiea racovitzae* and *A. mirabilis* (Iken et al. 1998). Other herbivores such as the gastropod *Laevilacunaria antarctica*, the amphipod *Gondogeneia antarctica* or the fish *Notothenia coriiceps* are generalists and graze on a large variety of algal species (Iken et al. 1998); the same is observed for other amphipods (Huang et al. 2006).

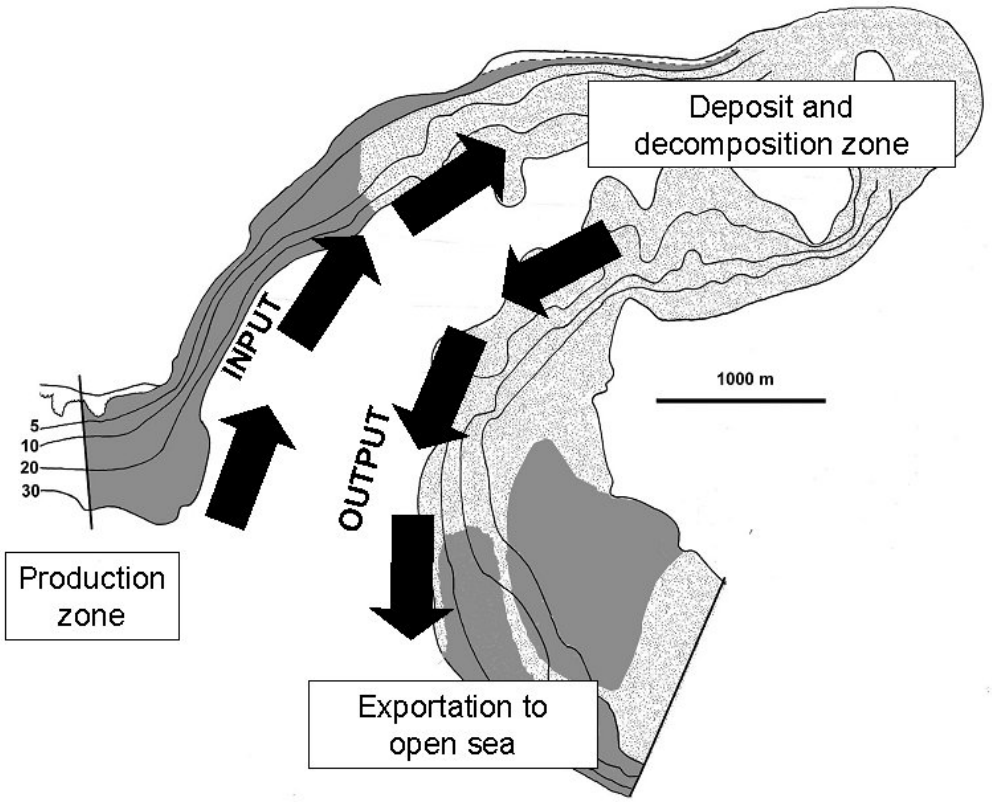

Figure 3. Main fluxes and processes in the dynamics of macroalgae in Potter Cove. Dark grey pattern indicates hard bottom, dotted grey pattern indicates soft sediments.

The horizontal flux of allochthonous material by currents reflected in the presence of macroalgal debris in the gut contents appears to be one of the main processes that ensure availability of organic matter to ascidians and the suspension feeder community in Potter Cove (Tatián et al. 2004). A significant macroalgal carbon input to soft bottom communities has been documented in Corbisier et al. (2004). According to Duarte et al. (2005) the excess production of the benthic compartment has two possible fates, to be stored in sediments contributing to burial therein or to be exported for use in the pelagic compartment. Following this idea, we summarize the dynamics of matter and energy fluxes in Potter Cove in Figure 2 and 3.

In conclusion, macroalgae are one of the main energy sources for Potter Cove, and probably support a large fraction of the secondary production of the benthos. Further studies are necessary in order to quantify more precisely the consumption and decomposition rates to make a finer balance. This work is only a start point for this objective.

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Mesozooplankton of Potter Cove: Community composition and seasonal distribution in 2002 and 2003

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Introduction

The zooplankton communities have been studied in several different regions of the Southern Ocean (e.g. Hopkins, 1985; 1987; Boysen-Ennen and Piatkowski, 1988; Hopkins and Torres, 1988, Hopkins et al., 1993; Pakhomov et al., 1997 a, b; Vuorinen et al., 1997; Carli et al., 2000; Pane et al., 2004). In the South Shetland Islands the interannual and seasonal variability of zooplankton has been investigated including coastal areas for more than 10 years (Menshenina and Rakusa-Suszczewski, 1992; Freire et al., 1993; Kittel et al., 2001).

Particulary in Potter Cove, zooplankton studies had been restricted to the population annual dynamic of the cyclopoid *Oithona similis* and *Oncaea curvata* and calanoid copepod species *Ctenocalanus citer* (Elwers and Dahms, 1998). Recently, the entire mesozooplankton community has been studied in detail (Fuentes, 2006) based on data from two year-round surveys. Some of the results of this study are presented in this work.

This study was conducted within the frame of a comprehensive zooplankton investigation that started in 2002 and is still going on as a monitoring program. The major aim is to detect possible changes in the zooplankton community in connection with global change. In the last few decades rising concern has been voiced about various aspects of regional climate change especially around the Antarctic Peninsula. In this sense, there have been dramatic changes in the abundance of some species zooplankton species (Atkinson et al. 2004) and non-native species reported for the first time (Thatje & Fuentes, 2003). Moreover, it is well accepted that ongoing plankton monitoring programs will be a key issue to identify future changes in marine ecosystems (Hays et al., 2005). We consider that due to its geographical location and the intense work being carried in the frame of the cooperation network between Argentina and Germany, Potter Cove is an ideal natural laboratory to test hypotheses concerning climate change.

Material and Methods

In 2002 and 2003, mesozooplankton was sampled weekly during summer and twice a week during winter at the inner Potter Cove (King George Island, South Shetlands, Antarctica, 62°14'S, 58°38'W) off Jubany Station.

Qualitative and quantitative surface samples from the upper water column (5 meters depth) were obtained by means of hand nets of 200 and 80 µm mesh size equipped with a calibrated flowmeter, and towed from an inflatable dinghy. When Potter Cove was free of sea ice (summer, autumn and end of spring of 2002 and the whole 2003) horizontal tows with hand nets of 200 and 80 µm mesh size were carried out for about 10 minutes over a distance of 500 m (measured with GPS). When Potter Cove was ice-covered (approximately from end of June to beginning of September 2002) samples were taken through a hole in the ice. The sampling hole was cut through the approximately 40 cm ice cover above a water depth of 12 m. In order to abide a sampling procedure comparable to the rest of the year, vertical hauls were taken from 5 m depth to the surface using the same nets (200 and 80 µm mesh size).

All samples were preserved with buffered 4% formaldehyde/sea water solution. Zooplankton was sorted using a Leica Mz 12.5 stereomicroscope, and samples were split when necessary, counted and identified either to the highest taxon or if possible to the species level. The abundances were expressed as Ind./100 m^3 due to the very low individual densities of some taxa and the different quantities of sea-water filtered in summer (50 - 70 m^3) and winter samples (2 – 15 m^3, with 15 m^3 in the majority of the hauls). For biomass measurements the individuals were frozen immediately after capture, then lyophilized and the carbon content of the animals calculated by means of a Carlo Elba CN Analyzer. The biomass is expressed in terms of mg of carbon per 100 m^3.

Results and discussion

68 mesozooplankton taxa were identified. Copepods represented the most diverse group with a total of 38 species (Table I). Within the study, two new zooplankton species were described: the harpacticoid copepod *Alteutha potter* Veit-Köhler and Fuentes (2007) and the narcomedusae *Jubanyella plemmyris* (Fuentes and Pagès, 2006) as well as some decapod larvae (*Emerita sp.* and *Pinnotheres sp.*) were found for the first time in Antarctic waters (Thatje and Fuentes, 2003).

Based on their presence in the samples, mesozooplankton species found in Potter Cove during the investigation period can be divided in two groups: the typical Antarctic coastal zooplankton species called “resident community” (R) and the species that come from external areas and entering the cove occasionally called “sporadic community” (S). The resident community is conformed mainly by small copepods and the sporadic community by euphausiids, salps and large calanoid copepod species (Table I).

Table I: Mesozooplanktonic taxa found in Potter Cove from February 2002 to March 2004.. R= resident species, S= sporadic species, NS= new species, FR= first record for Antarctica.

HYDROMEDUSAE
- **Anthomedusae**
- *Zanclonia weldoni* S
- **Leptomedusae**
- 1 species not identified S
- **Narcomedusae**
- *Solmundella bitentaculata* R
- *Jubanyella plemirix* NS, S
- **Scyphomedusae**
- *Diplulmaris antarctica* S
- *Desmonema* sp. 1 S
- *Desmonema* sp. 2 S

CTENOPHORA
- *Beroe cucumis* S

SIPHONOPHORAE
- *Diphyes antarctica* S
- *Dimophyes arctica* S
- *Sphaeronectes irregularis* S
- *Pyostephos vanhoeffeni* S
- *Mica micula* S

GASTEROPODA
- Bivalvia larvae not identified R
- **Pteropoda Thecosomata**
- *Limacina helicita* R
- **Pteropoda Gymnosomata**
- *Clione limacina* R
- *Spongiobranchaeae australis* R

COPEPODA
- **Calanoida**
- Calanoides acutus *S*
- Calanus propinquus *R*
- Calanus simillimus *R*
- Clausocalanus laticeps *R*
- Ctenocalanus citer *R*
- *Metridia gerlachei* R
- Metridia lucens *S*
- Stephos longipes *R*
- Microcalanus pygmaeus *R*
- *Paraeuchaeta antarctica* S
- *Paraechaeta* sp. S
- Rhincalanus gigas *S*
- *Scolecithricella minor* R
- *Scolecithricella dentata* R
- *Heterorhabdus austrinus* S
- *Gaetanus* sp. S
- *Temora* sp. S

COPEPODA
- **Cyclopoida**
- Oithona frigida *R*
- Oithona similis *R*
- *Pseudocyclopina* sp. R
- Oncaea curvata *R*
- *Oncaea* sp. R
- **Harpacticoida**
- FamilyHarpacticidae
- Family Ectinosomatidae
- Family Tibidae
- Family Peltidiidae
- *Alteutha potter* NS, R
- Family Porcellidiidae

Mormonilloida
- *Mormonilla* sp. S

EUPHAUSIDA
- *Euphausia superba* S
- *Thysanoessa macrura* S
- *Euphausia crystallorophia* S
- *Euphausia frigida* S

POLYCHAETA
- *Pelagobia* sp. R
- Family Syllidae
- Family Spionidae
- Family Alciopidae
- Family Lumbrineridae
- Family Tomopteridae
- Family Pelargidae

CHAETOGANATHA
- *Eukrohnia hamata* R
- *Sagitta gazellae* R
- *Sagitta marri* R

APPENDICULARIA
- *Fritillaria borealis* R
- *Oikopleura gaussica* R

SALPIDA
- Salpa thompsoni *S*
- *Ihlea racovitzai* S

DECAPODA

Zoea of
- *Chorismus antarcticus* S
- *Emerita* sp. FR, S
- *Pinnotheres* sp. FR, S

Considering the samples obtained from the 200 µm mesh size net, copepods were by far the most abundant mesozooplankton taxon contributing between the 97 % in 2002 (Table II) and 95 % in 2003 (Table III). Euphausiids (mainly

Euphausia superba) ranked second in abundance (1.6 % and 0.4 % in 2002 and 2003, respectively). All other taxa represented less than 0.1 % of the total abundance.

In the 80 µm mesh size net crustacean nauplii (mainly of small copepods) represented 30 % of total zooplankton in 2002 and 25 % in 2003. The total densities of mesozooplankton varied greatly among months but also between the two years studied (Table II and III, Fig. 1). In 2002, the annual mean abundance of mesozooplankton (115.451 Ind. 100 m^{-3}) was much higher than in 2003 and showed a maximum in winter (646.692 Ind. 100 m^{-3} in June and 474.095 Ind. 100 m^{-3} in July) when the cove was frozen. In 2003, the zooplankton abundance was relatively low (9.192 Ind. 100 m^{-3}) showing a maximum in autumn (53.997 Ind. 100 m^{-3} in May) and summer (10.682 Ind. 100 m^{-3} in March) (Fig. 1).

Most of the taxa showed high fluctuations in abundances ranging from near zero to values higher than 400.000 Ind. 100 m^{-3} (Table II and III). Due to the numerical dominance of copepods throughout the study total mesozooplankton distribution pattern reflected that of the copepods (Fig. 1), except when juveniles of *E. superba* were abundant in Potter Cove. The inverse relationship between the abundance of these two groups is evident in Fig. 1. The cyclopoid copepods contributed the largest fraction in both 2002 and 2003 (75 % and 84 %, respectively) followed by calanoids (21 % and 16.7 %) and harpacticoids (3 % and 1.4 %). Within the cyclopoid the species *Oithona similis* dominated representing almost 99% of this group. Within the calanoid, three species contributed more than 97% of all calanoids *Ctenocalanus citer* (42 % in 2002, 85 % in 2003), *Metrida gerlachei* (47% in 2002, 11% in 2003) and *Calanus propinquus* (10% in 2002, 5% in 2003). The other calonoid species showed very low abundances (≤1 %).

The abundances of all copepods reached the highest densities under the ice during the winter in 2002. The under-ice community was dominated by copepodite stages of the calanoids *C. citer*, *M. gerlachei* and *C. propinquus* and the cyclopoid *O. similis*. Other copepod species present under the ice in 2002 were the calanoid *Stephos longipes* and the harpacticoid *Alteutha potter* sp. n.. All the other copepod species occurred in very low numbers.

The highest abundance of some copepods species during autumn and winter has been observed also by Elwers and Dahms (1998), who found an increase of the total abundance of *Oithona similis*, *Oncaea curvata* and *Ctenocalanus citer* in this time of the year. The authors explained these high abundances with a possible increase of reproduction in summer but also with changes in the hydrography of adjacent areas due to the convergence of waters from the Weddell and Bellingshausen seas. We agree with these authors and add the idea that the ice cover contributes to a more stable environment in an area of intense water column mixing during most of the year (Schloss and Ferreyra, 2002; Fuentes,2006).

Table II: Annual mean abundance with ranges (minimum and maximum) and relative abundance of holoplanktonic and meroplanktonic groups of mesozooplankton in 2002.

N= 29	Abundance (Ind. 100 m^{-3})		Relative
Taxa	annual mean	range (min-max)	abundance (%)
Holoplankton			
Medusae	0	0 - 1	< 0.1
Ctenophora	2	0 - 18	< 0.1
Siphonophora	0	0 - 5	< 0.1
Polychaeta	150	0 - 447	0.1
Ostracoda	6	0 - 38	< 0.1
Pteropoda	165	0 - 946	0.1
Total Copepoda	112.433	26 - 470.824	97.4
Calanoida	23.980	9 - 201.346	21.3
Cyclopoida	84.179	9 - 434.692	74.9
Harpacticoida	3.251	6 - 28.500	2.9
Poecilostomatoida	50	0 -325	< 0.1
Nauplii	972	0 - 2.452	0.9
Amphipoda	639	0 - 5.615	0.6
Euphausida	1.864	0 - 14.986	1.6
Larvae	1.806	0 - 14.986	96.9
Juvenile	55	0 - 253	3
Chaetognatha	20	0 - 135	< 0.1
Salpida	82	0 - 880	0.1
Appendicularia	3	0 - 28	< 0.1
Mero/ichtyoplankton			
Polychaeta	28	0 - 28	< 0.1
Cirripedia	10	0 - 10	< 0.1
Decapoda	0	0 - 0	0
Echinodermata	578	0 - 411	0.50
Ascidiacea	328	0 - 288	0.3
Nemertina	41	0 – 29	< 0.1
Fish larvae	2	0 - 20	< 0.1
Total mesozooplankton	**115.451**		

The euphausiids, mainly *Euphausia superba*, were the second most abundant taxon. However, they do not occur continuously in the samples but show a distinct seasonal pattern of occurrence (Table IV). The early larval stages (calyptopes and early furciliae) were present mainly during the first months of the year and the juveniles became more abundant after September together with the late furcilia (VI) stage.

Mesozooplankton abundances in Potter Cove were in general highly variable and in coincidence with results by Kittel et al. (2001) for Admiralty Bay (King George Island). This variability might be influenced by the hydrography of the cove and the adjacent Maxwell Bay. This bay exchanges water with the Bransfield Strait which is in turn influenced by waters coming from the Weddell and

Table III: Annual mean abundance with ranges (minimum and maximum) and relative abundance of holoplanktonic and meroplanktonic groups of mesozooplankton in 2003.

N=56	Abundance (Ind. 100 m^{-3})		
Taxa	annual mean	range (min-max)	Relative abundance (%)
Holoplankton			
Medusae	2	0 - 5	< 0.1
Ctenophora	1	0 - 1	< 0.1
Siphonophora	1	0 - 1	< 0.1
Polychaeta	51	0 - 465	0.6
Ostracoda	0	0 - 1	< 0.1
Pteropoda	13	0 - 82	0.1
Total Copepoda	8.495	733 – 53.426	96.4
Calanoida	1.414	14 – 7.300	16.7
Cyclopoida	7.108	679 - 44.203	83.7
Harpacticoida	117	7 - 564	1.4
Poecilostomatoida	219	14 – 1.088	2.6
Nauplii	87	0 - 280	1.1
Amphipoda	21	0 - 47	0.2
Euphausida	33	0 - 150	0.4
Larvae	6	0 - 7	18.2
Juvenile	24	0 - 148	72.7
Adult	3	0 - 11	9.1
Chaetognatha	3	0 - 24	< 0.1
Salpida	0	0 - 1	< 0.1
Appendicularia	70	0 - 278	0.8
Mero/ichtyoplankton			
Decapod zoeae	0	0 - 4	< 0.1
Echinodermata larvae	3	0 - 18	< 0.1
Ascidiacea larvae	70	0 - 207	0.8
Nemertina larvae	2	0 – 8	< 0.1
Fish larvae	0	0 - 2	< 0.1
Total Mesozooplankton	**9.192**		

the Bellingshausen Seas. Hence, the mesozooplankton community composition in Potter Cove resembled that of the Bransfield Strait (Kim et al., 1991). More over, some zooplankton species found in Potter Cove samples have suggested the existence of other oceanographic processes like the intrusion of subantartic water masses (non Antarctic decapods larvae found in Potter Cove's waters, Thatje and Fuentes, 2003) and the upwelling of deep sea water masses (deep water Narcomedusae found in Potter Cove's coast, Fuentes and Pagès, 2006).

We agree with these authors and we suggest that the ice cover contributes to a more stable environment in an area of intense water column mixing during most of the year (Schloss and Ferreyra, 2002; Fuentes, 2006).

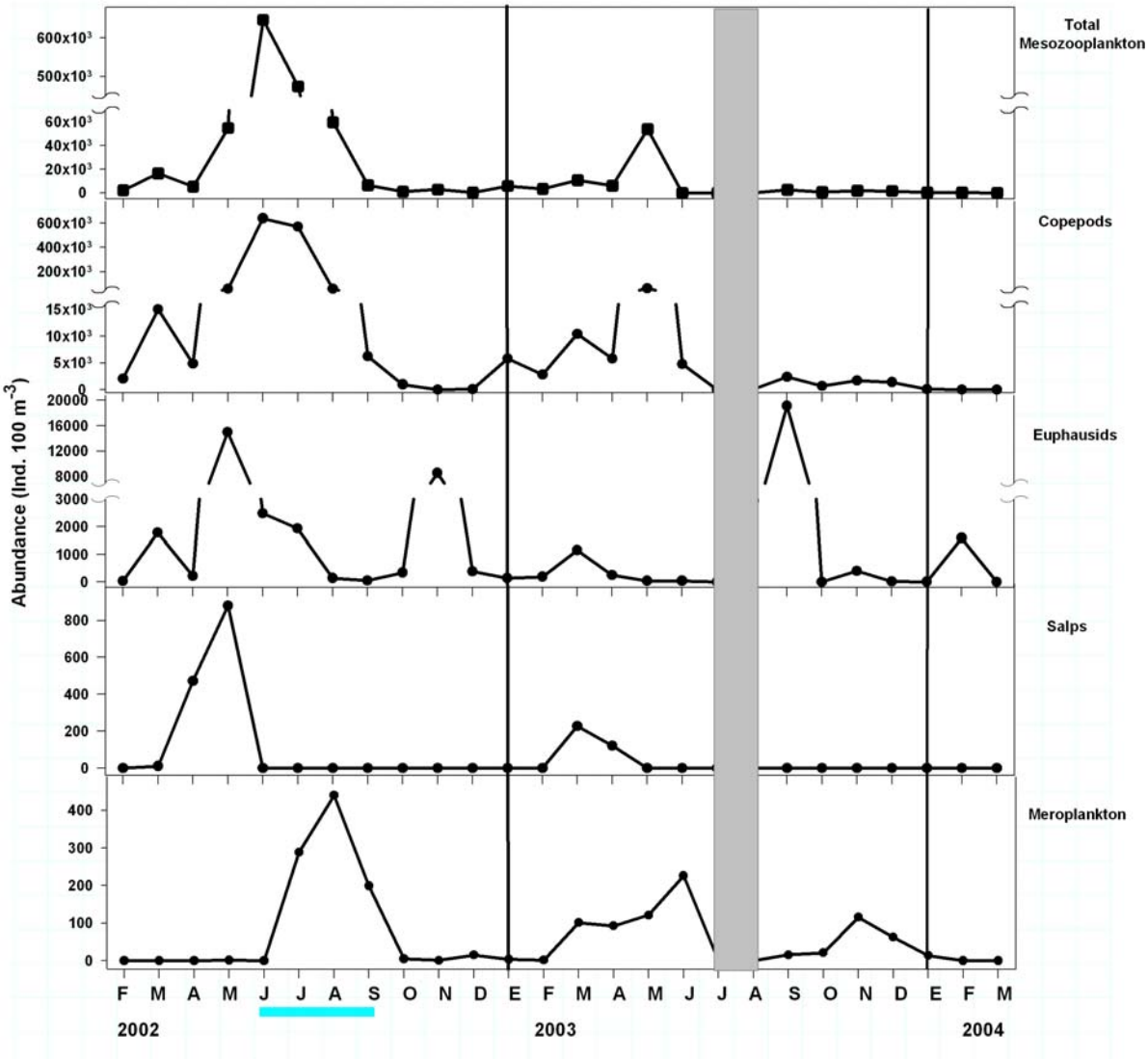

Figure 1: Seasonal abundance of the total mesozooplankton and selected groups of holoplankton and total meroplankton (grey areas: period with lack of samples). The bar on the X axis (year 2002) represents the period when Potter Cove was covered by ice.

Perspectives for the future

Given the importance of zooplankton in marine ecosystems as consumers, producers and prey and their role in the ocean elemental cycling and vertical fluxes (Marine Zooplankton Colloquium 2, 2001) it is surprising that there are relatively few long time-series of zooplankton data in Antarctica. Furthermore, zooplankton could be a particularly good indicator of climate change in polar marine environments. In this sense, the present work has generated a baseline of abundance and richness of zooplankton species, some of which could be considered indicators of certain environmental conditions. In future studies in the frame of the cooperation between Argentina and Germany, the continuation of the monitoring project is a key issue. Furthermore, and within the International Polar Year, we will focus on the effects of climate change on zooplankton populations. Through field sampling and laboratory experimentation the influence of ice melting and temperature increase (decrease of salinity, water transparency, increase in the concentration of suspended sediments) will be examined.

Table IV: Mean abundance and biomass of *Euphausia superba* in Potter Cove, years 2002 and 2003.

2002	Abundance (Ind. 100m^{-3})	Biomass (mg C 100m^{-3})	Stage
February	29	8,9	furcilia III
March	834	134,1	calyptopes I to III
	920	48,6	furcilia I
	20	263,8	juvenile
April	177	28,4	calyptopes I to III
	36	11,1	furcilia III
	2	26,4	juvenile
May	14.985	2.397,6	calyptopes I to III and furcilia I to III
June	2.281	699,6	furcilia III to IV
	192	2.532,5	juvenile
July	929	No data	furcilia IV to VI
August	144	No data	furcilia VI
September	50	No data	furcilia VI
October	228	114	furcilia VI
	112	168	juvenile
early November	7.378	3.689	advanced furcilia VI
	736	2.164,8	juvenile
late November	28	42,50	furcilia VI
	418	1.228,9	juvenile
December	369	1.324,7	juvenile
	20	No data	furcilia VI
Month of the year 2003	**Abundance (Ind. 100m-^{3})**	**Biomass (mg C 100m-^{3})**	**Stage**
January	120	1.832,4	juvenile
	20	575,4	adult
February	19	3,1	calyptopes I to III
	171	2.146,1	juvenile
early March	21	3,4	calyptopes I to III
late March	1.118	3.566,4	juvenile
	18	480,6	adult
April	249	794,3	juvenile
	4	106,8	adult
early September	124	No data	furcilia IV
	7.129	16.468	juvenile
	77	222,5	adult
late September	11.755	27.154,1	juvenile
	20	No data	furcilia VI
	8	23,1	adult
November	367	748,7	juvenile
	26	No data	furculia VI
	28	136	adult

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Morphometry of the developmental stages of *Calanoides acutus* and *Calanus propinquus* (Copepoda, Calanoida) from West Antarctic Peninsula waters

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Introduction

Calanoides acutus and *Calanus propinquus* are two of the most important calanoid copepod species in the Southern Ocean. They play a key role in food webs as plankton consumers and also as prey for large planktophagous organisms (Hopkins & Torres 1988, Atkinson 1995, Burghart et al. 1999, Pasternak & Schnack Schiel 2001). Several studies on biological and ecological aspects of these Antarctic copepods have been carried out to date (Hopkins 1985, Atkinson 1998, Huntley & Escritor 1991, Elwers & Dahms 1998). In addition, the morphology of adults is well known (Farran 1929, Hülseman 1996, Bradford-Grieve et al. 1999, among others). However, little is known about the morphometric features of the different developmental stages of these species. To our knowledge, the only study that reports values about the total length of CIII to CVI individuals of *C. acutus* and *C. propinquus* is that of Mizdalzki (1988). On the other hand, Zmijewska et al. (1999) studied *C. acutus* focusing on the effect of temperature on the size of its developmental stages in different Antarctic regions.

The distinction of *C. acutus* from *C. propinquus* is not an easy task due to the great morphological similarity between both species, particularly when they are at the same developmental stage. This becomes particularly complicated when the individuals are copepodites of the first developmental stages. Consequently, observations about the morphology of both species, such as those focused on the different length of antennae 1 and shape of the prosome's cephalic segment (rounded in *C. propinquus* and pointed in *C. acutus*) become useful tools for the identification of individuals of the two species. However, comparative analyses of the morphology of the different developmental stages are still pending.

In view of the above, the aim of the present study was to describe and to compare the morphological features of all the developmental stages of *C. acutus* and *C. propinquus* from the West Antarctic Peninsula waters in an attempt to facilitate their identification. Predictive regression models among the measured variables were also developed.

Methods

Individuals of *C. propinquus* were sorted from subsurface zooplankton samples collected in Potter Cove, Maxwell Bay (Guardia Nacional Bay), King George Is.,

South Shetland Is. during a period extending from February to April 2002. *C. acutus* individuals were sorted from the upper layer zooplankton samples collected from 15 coastal stations located on the West Coast of the Antarctic Peninsula during January 1998. Sampling was carried out by means of horizontal (Potter Cove) and oblique (Antarctic Peninsula west coast) tows at a 2-knot speed using a 200 µm mesh net aboard either 'Puerto Deseado' oceanographic vessel or a motor boat. All samples were preserved in buffered 4% formalin.

Identification and measurements of the six copepodite stages of both *C. acutus* and *C. propinquus* were carried out in the lab using a WILD M5 stereoscopic microscope. Total length (TL), prosome length (PL), urosome length (UL), and antennae 1 length (AL) were measured in 13 to 42 individuals at all developmental copepodite stages (C I to VI). A total of 131 *C. acutus* individuals and of 113 *C. propinquus* individuals were measured. Due to the scarcity of C I and C VI *C. propinquus* females in the samples collected, only 2 and 10 individuals were measured in each case.

The ratios between prosome length and urosome length (PL/UL), antennae 1 and total length (AL/TL), antennae 1 and prosome length (AL/PL), and between urosome length and total length (UL/TL), were calculated. Also, regression analyses were carried out on the data corresponding to both species using AL as dependent variable and either PL or TL as predictor variables. Regression lines and equations were developed according to the model yielding the best fit which was either linear or exponential depending on each case.

Results and Discussion

C. acutus mean TL varied between 1081.53 µm (sd 106.53) and 5269.33 µm (sd 310.74) in C I to C VI females whereas *C. propinquus* mean TL ranged from 1176.67 µm (sd 108.38) to 5432 µm (sd 251.07) at the same developmental stages. These values show a great similarity in size between both copepods (Table 1). On the other hand, a marked difference in AL was observed between the two species, ranging, in *C. propinquus*, from 1405 µm (sd 408.60) in C I to 5274 µm (sd 356.28) in C VI females, and, in *C. acutus*, from 888 µm (160.48) in C I to 5372 (sd 247.63) in C VI females. These results indicate that antennae 1 were shorter in *C. acutus* than in *C. propinquus* with the exception of C V and C VI females, in which they were of similar length (Fig. 1). PL and UL were similar in the two copepods (Fig. 1). However, it was observed that *C. propinquus* prosome was slightly larger in C I, C V and C VI females whereas it was smaller in C II to C IV individuals than *C. acutus* prosome at the same developmental stages. The *C. propinquus* urosome was shorter with respect to that of *C. acutus* in C I to C III copepodites and longer in C III to C VI females. These features were also observed in the PL/UL ratio of both copepods (Fig. 2). TL was smaller in *C. propinquus* C II to C IV than in *C. acutus* at the same developmental stages but it was greater in C V to C VI females (Fig. 1).

The UL/TL ratio in both copepods showed UL to be around 20% of TL, though except for C I, it was greater in *C. propinquus* than in *C. acutus*. AL/PL and AL/TL ratios were higher in *C. propinquus* than in *C. acutus* at all developmental stages, except in the case of C V and C VI females (Fig. 2).

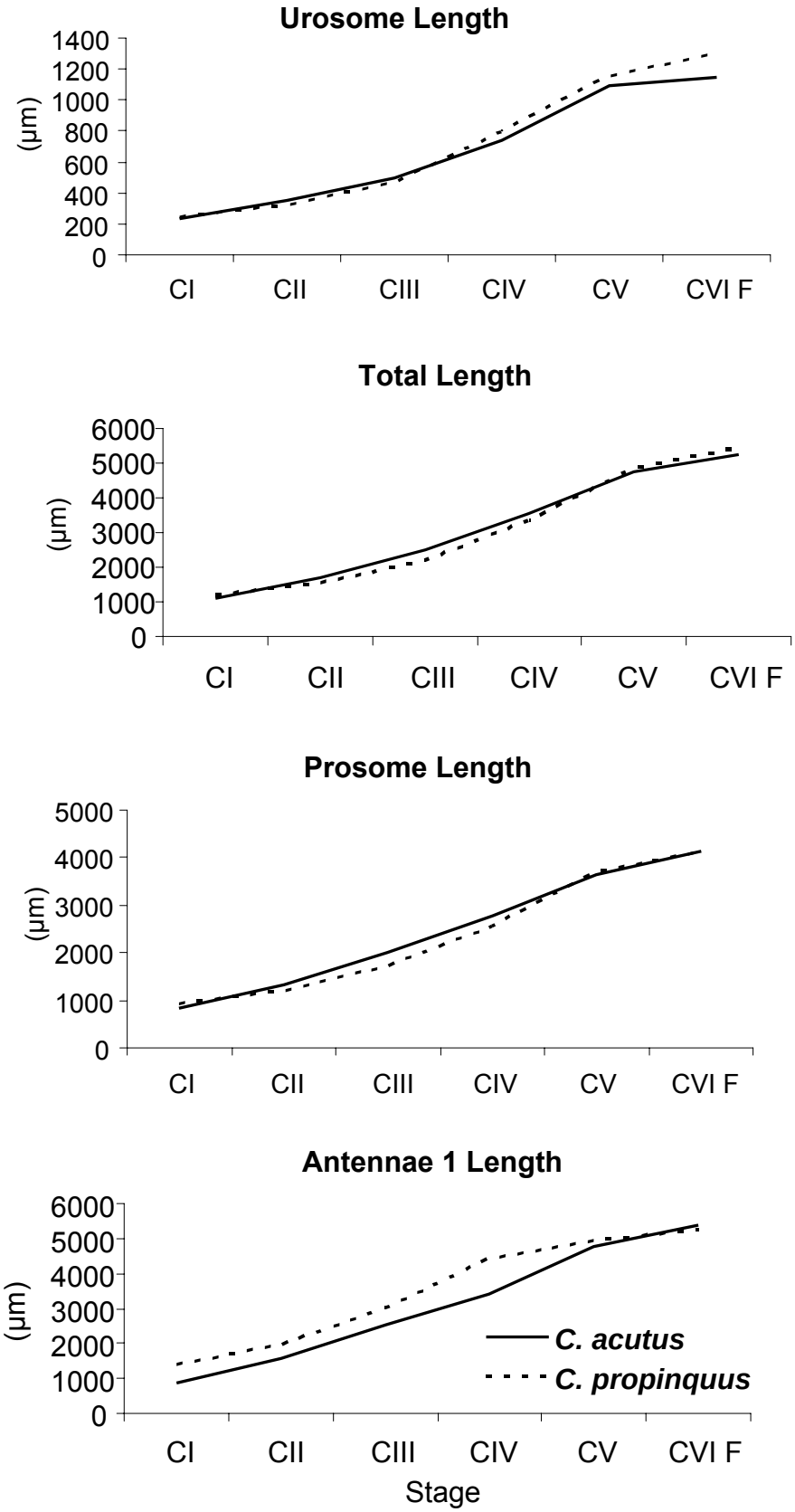

Fig. 1. Graphics of mean values of the urosome, total, prosome, and antennae 1 length measured in C I to C VI females of *Calanoides acutus* and *Calanus propinquus*. SD and range values as in Table 1.

Table 1. Mean, SD, and range corresponding to the variables measured at all the developmental stages (C I to C VI) of *Calanoides acutus* and *Calanus propinquus* copepods from Antarctic waters. N: number of measurements, PL: Prosome length, UL: Urosome length, TL: Total length, AL: Antennae I length.

***Calanoides acutus* - West Coast Antarctic Peninsula**													
Stage	N		PL (µm)			UL (µm)			TL (µm)			AL (µm)	
CI	33	**844.85**	76.00	720 -1000	**236.36**	45.13	160 - 360	**1081.21**	106.53	840 - 1280	**888.00**	160.48	800 - 1000
CII	32	**1330.94**	89.92	1240 - 1520	**353.75**	43.24	280 - 480	**1684.69**	105.00	1520 - 1880	**1589.23**	97.69	1400 - 1800
CIII	30	**2010.67**	114.92	1720 - 2320	**494.67**	53.94	400 - 600	**2505.33**	168.86	2120 - 2800	**2544.00**	192.74	2120 - 2800
CIV	13	**2788.00**	113.22	2600 - 2920	**740.00**	57.35	640 - 800	**3528.00**	112.82	3200 - 3600	**3437.50**	186.53	3200 - 3600
CV	15	**3652.73**	353.30	3320 - 4360	**1105.33**	213.34	720 -1400	**4758.06**	566.64	4120 - 5400	**4857.33**	579.60	4000 - 5400
CVI F	30	**4121.33**	211.00	3680 - 4400	**1148.00**	99.74	960 -1400	**5269.33**	310.74	4680 - 5800	**5372.00**	247.63	4800 - 5680
***Calanus propinquus* - Potter Cove (South Shetland Is.)**													
Stage	N		PL (µm)			UL (µm)			TL (µm)			AL (µm)	
CI	6	**936.67**	115.53	840 - 900	**240.00**	48.99	260 - 280	**1176.67**	108.38	1100 - 1180	**1405.00**	408.60	1100 - 1200
CII	30	**1196.00**	117.62	1640 - 1100	**329.60**	58.63	240 - 440	**1525.60**	152.04	1360 - 2000	**1984.78**	367.25	1600 - 3200
CIII	42	**1725.00**	117.19	1560 - 1960	**474.00**	49.09	360 - 600	**2186.00**	101.84	2040 - 2400	**3021.00**	204.97	2400 - 3400
CIV	30	**2561.33**	131.40	2400 - 2760	**790.67**	29.12	720 - 840	**3352.00**	146.46	3120 - 3560	**4440.00**	312.96	4000 - 4800
CV	33	**3707.88**	131.40	3600 - 4400	**1159.39**	29.12	1000 - 1240	**4867.27**	146.46	4600 - 5600	**4940.74**	312.96	4400 - 5200
CVI F	10	**4128.00**	168.44	3880 - 4400	**1304.00**	82.62	1200 - 1480	**5432.00**	251.07	5120 - 5880	**5274.00**	356.28	5200 - 5880

Table 2. Mean, SD, and range of ratio values between each pair of morphometric variables. N: number of measurements, PL/UL: Prosome length/ Urosome length, UL/TL: Urosome length/ Total length, AL/TL: Antennae length/ Total length, AL/PL: Antennae length/ Prosome length.

	Stage	N		LP/LU			LU/LT		N		LA/LT			LA/LP	
Calanoides acutus	CI	33	**3.66**	0.55	2.56 - 4.40	**0.22**	0.03	0,17-0,28	28	**0.83**	0.15	0,72-0,95	**1.09**	0.14	0.57 - 1.25
	CII	32	**3.81**	0.49	4,86-3,00	**0.21**	0.02	0,17-0,26	26	**0.95**	0.07	0,78-1,05	**1.21**	0.09	0.97 - 1.32
	CIII	30	**4.12**	0.42	3,33-5,00	**0.20**	0.02	0,17-0,23	30	**1.04**	0.10	0,83-1,13	**1.22**	0.12	0.99 - 1.40
	CIV	13	**3.77**	1.97	3,25-4,29	**0.21**	0.02	0,19-0,24	8	**0.97**	1.65	0,91-1,02	**1.23**	0.06	1.14 - 1.29
	CV	15	**3.33**	1.62	2,86-4,50	**0.23**	0.03	0,18-0,26	14	**1.00**	0.82	0,91-1,02	**1.29**	0.07	1.22 - 1.37
	CVI F	30	**3.59**	2.12	3,10-4,20	**0.22**	0.01	0,19-0,24	25	**1.02**	0.80	0,96-1,09	**1.30**	0.07	1.13 - 1.46
Calanus propinquus	CI	6	**4.12**	1.39	3,23-3,21	**0.21**	0.04	0,13-0,24	6	**1.18**	0.24	1,09-0,93	**1.48**	0.26	1.22 - 1.85
	CII	26	**3.71**	0.56	3,00-4,67	**0.22**	0.03	0,18-0,29	28	**1.31**	0.23	1,05-2,10	**1.67**	0.28	1.28 - 2.63
	CIII	42	**4.00**	0.49	2,67-5,44	**0.22**	0.02	0,16-0,27	38	**1.38**	0.12	1-1,61	**1.77**	0.17	1.25 - 2.10
	CIV	30	**3.24**	0.15	3,00-3,45	**0.24**	0.01	0,22-0,25	9	**1.31**	0.08	1,2-1,44	**1.72**	0.11	1.56 - 1.90
	CV	33	**3.21**	0.22	2,73-3,67	**0.24**	0.01	0,21-0,27	27	**1.02**	0.07	0,82-1,12	**1.34**	0.10	1.22 - 1.47
	CVI F	10	**3.17**	2.04	2,86-3,44	**0.24**	0.01	0,23-0,26	10	**0.97**	1.42	0,85-1,02	**1.28**	0.08	1.10 - 1.34

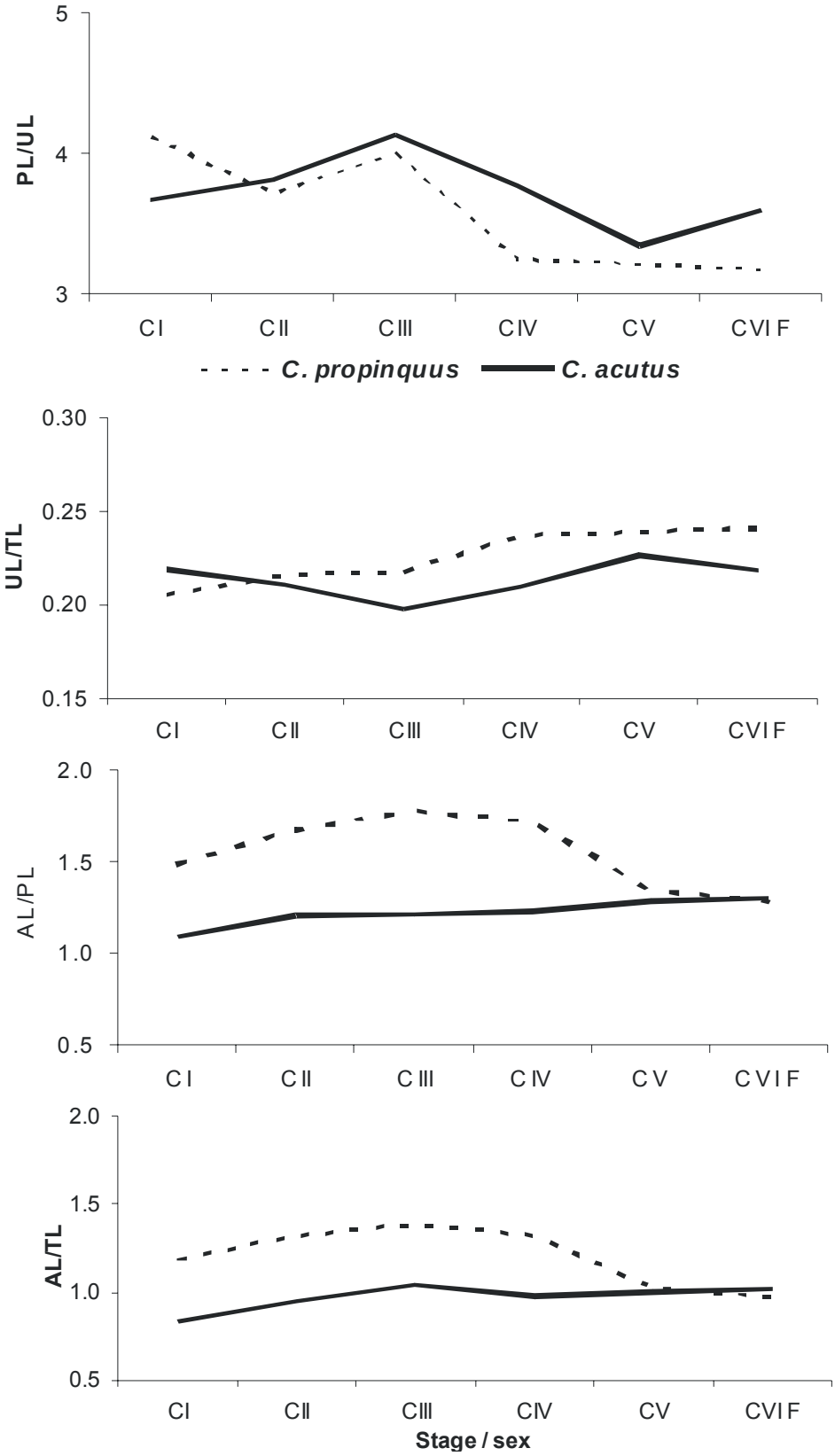

Fig. 2. Mean values of prosome length/urosome length (PL/UL), urosome length/total length (UL/TL), antennae 1 length/prosome length (AL/PL), and antennae 1 length/total length (AL/TL) ratios at different developmental stages of *Calanoides acutus* and *Calanus propinquus*. SD and range values as in Table 2.

The best fit in the regression analysis carried out on *C acutus* AL and PL data was achieved following a linear model. A highly significant regression coefficient ($b = 1.335$, $p < 0.001$) with a high adjusted determination coefficient (R^2 adj = 0.98) (Fig. 3) was obtained. When AL was regressed against TL, results were similar (Fig. 4). In the case of *C. propinquus*, the best fit was attained with an exponential regression model (Fig. 5). For AL vs. PL the regression coefficient was highly significant ($b = 2574.2$, $p < 0.001$) with a high adjusted determination coefficient (R^2 adj = 0.94). When AL was regressed against TL, results were similar (Fig. 6). PL proved to be the best predictor variable because the mean PL at the different developmental stages of both species evidenced lower and rather stable values of standard deviation than those corresponding to the other variables measured (such as, TL and UL).

However, the regression analyses carried out on both copepods using PL and TL data yielded highly significant regression coefficients. Therefore, and as in general, it is easier to measure TL than PL, the use of the former variable also is acceptable.

The above-mentioned results show significant differences in antennae 1 between *C. propinquus* and *C. acutus* at their different developmental stages. In our view, the regression lines as well as the respective equations developed in this study are useful tools to assign either to *C. propinquus* or to *C. acutus* those individuals whose identification is dubious as a result of their great morphological similarity particularly at their initial developmental stages. These tools can also be useful for those cases in which antennae 1 is broken and for the identification and sorting of live individuals. Body size in *C. acutus* from the West Antarctic Peninsula waters exhibits a high geographic variability (Zmijewska et al. 1999). Although the samples analyzed in the present study were collected from all along the west coast of the Antarctic Peninsula between 62 and 68º S, our observations on PL agreed with those reported by Zmijewska et al. (1999) particularly in relation with the data regarding individuals from Weddell Sea-related waters and Croker Passage. Also, and taking into account that the two copepod species studied in this research belong to the Calanidae fam., a spatial variability in size is also expected in *C. propinquus*. The data resulting from this morphological comparison of *C. acutus* and *C. propinquus* therefore, should be considered with special care on account of the fact that the sets of individuals corresponding to the two species studied were collected from different zones of the West Antarctic Peninsula coastal region. The size ranges registered for *C. acutus* and *C. propinquus* in the present research may not therefore be representative of the total population of both species in the study area.

The comparison between the average values of PL, UL, TL, and AL as well as the ratios between the variables measured at CI to CVI stages of both copepods, clearly show the principal similarities and the general differences among the different developmental stages (Table 3).

Furthermore, and taking into account particularly the measurements corresponding to copepodites I to III, it could be observed that, on average, antennae 1 in *C. propinquus* is notoriously longer than that of *C. acutus* (Table 1). In contrast, the overall average values and ranges corresponding to TL, PL, and UL indicate great similarity (Table 3). However, the data presented here show a certain variability among and within the developmental stages of *C. acutus* and *C. propinquus*. Ratio values were similar in general (Table 3) though with a slight variation among the different developmental stages (Table 2).

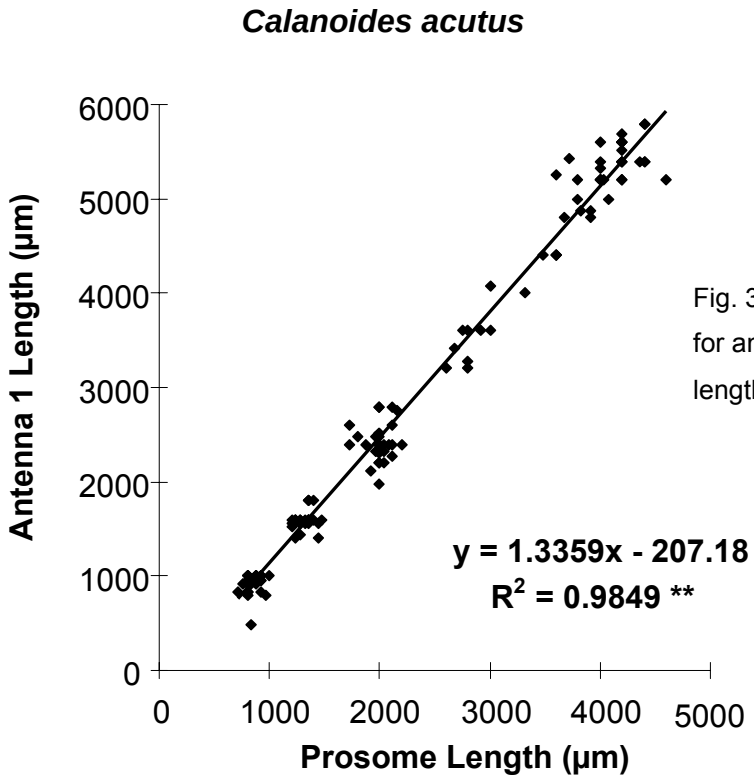

Fig. 3. Regression line and equation for antennae 1 length (y) and prosome length (x) of *Calanoides acutus.*

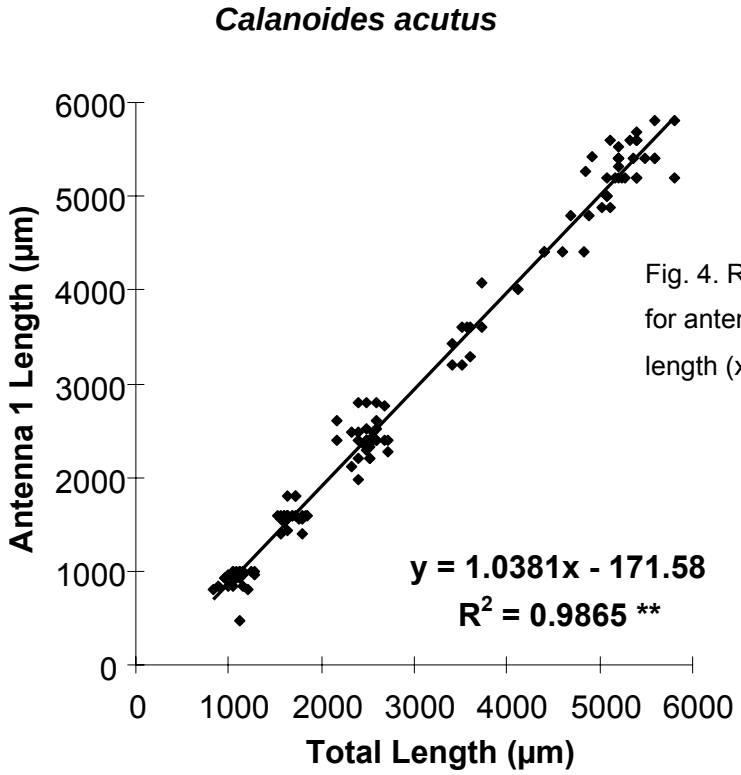

Fig. 4. Regression line and equation for antennae 1 length (y) and total length (x) of *Calanoides acutus.*

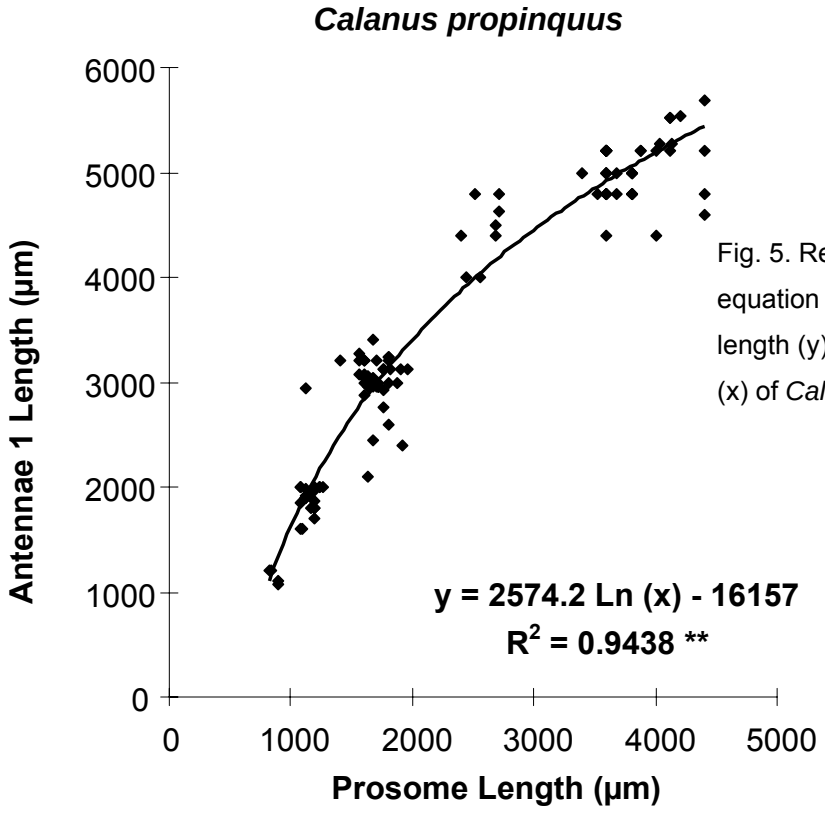

Fig. 5. Regression line and equation for antennae 1 length (y) and prosome length (x) of *Calanus propinquus.*

Table 3. Overall mean and SD (in µm) of prosome length, urosome length, total length, antennae 1 length values, and overall mean and SD of ratio values in *C. acutus* and *C. propinquus*.

	Calanoides acutus		***Calanus propinquus***	
Stage	CI -CVI females			
N	153		147	
PL	2458.09	159.73	2375.81	130.26
UL	678.08	86.23	716.28	49.59
TL	3136.17	229.21	3089.92	151.04
AL	3099.17	226.03	3510.92	327.17
PL/UL	3.71	1.20	3.57	0.81
AL/TL	0.97	0.63	1.19	0.36
UL/TL	0.21	0.02	0.23	0.02
AL/PL	1.22	0.09	1.54	0.17

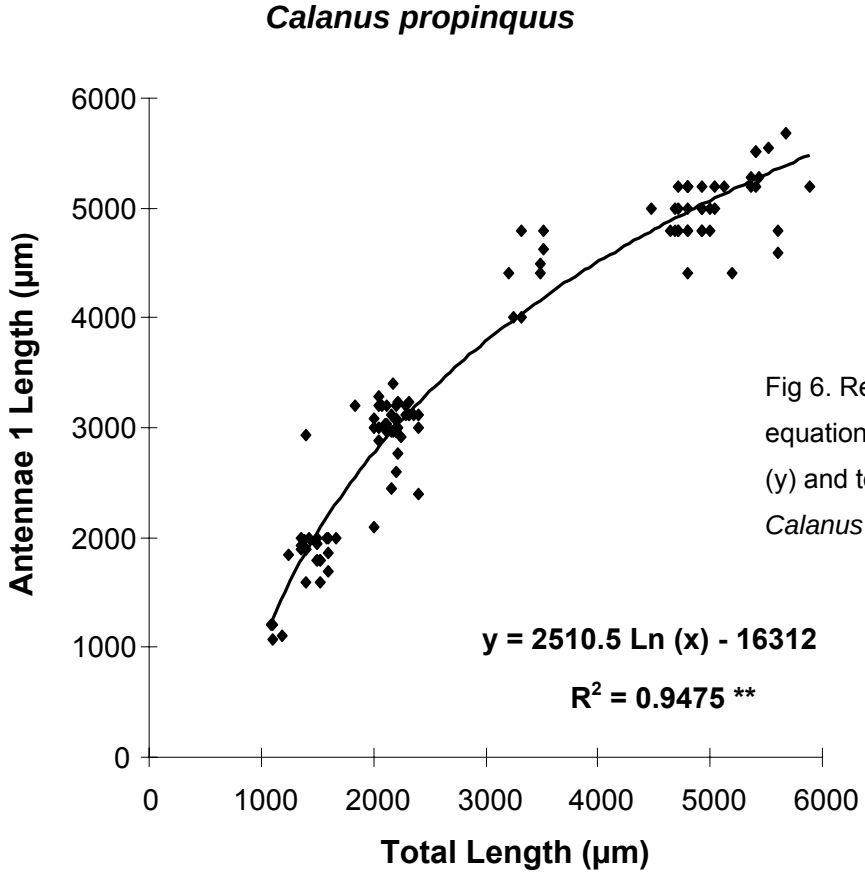

Fig 6. Regression line and equation for antennae 1 length (y) and total length (x) of *Calanus propinquus.*

Overall, our results demonstrate that, apart from the differences in the shape of the cephalic segment of *C. propinquus* and *C. acutus* prosome, antennae 1 length (AL) which is longer in the former than in the latter, is the most distinctive feature that greatly contributes to identifying individuals of the two species studied, particularly at their initial developmental stages. So, our findings corroborate empirical previous observations. They also lead to conclude that both prosome length (PL) and total length (TL) are good predictor variables for the estimation of AL in the two large copepods *C. acutus* and *C. propinquus*.

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General ecology of coastal fish from the South Shetland Islands and West Antarctic Peninsula areas

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General Introduction

The Antarctic fish fauna is unique in being dominated in terms of diversity (35%) and biomass by an endemic coastal demersal group, the suborder Notothenioidei, which includes six families and occurs as deep as 1200-1500 m. There is a lower diversity of Antarctic fish species on the continental shelves (139 spp.) compared with other cold-water seas (> 350 spp. in the North Atlantic). However, although the diversity of the notothenioids is limited compared with the large size of the ecosystem, there is no other fish group in the world with such a diversification and dominance in a continental shelf habitat (Eastman 1995).

The role of fish as predators and prey in the Antarctic marine ecosystem is important for understanding energy flow from benthos and pelagic nekton, such as Antarctic krill, to predatory seals and penguins, and for understanding the potential for fisheries. This matter has been the object of several studies during the last three decades (reviewed in Kock 1992; Hureau 1994; Barrera-Oro 2002). Accordingly, this paper summarises published information from the long-term research program carried out by the Ichthyology Project of the Instituto Antártico Argentino in the period 1999-2006, focused on ecological aspects of fish in inshore sites of the South Shetland Islands and west Antarctic Peninsula. This research program allowed the development of studies about the ecology of fish (e.g. role in the food web, populational aspects, trophic position, age and growth, predator-prey interactions), as well as about the impact of the offshore commercial fishery/scientific programs on inshore fish of the area (see Barrera-Oro et al., this issue).

Dietary overlap as an indicator of food competition

The Notothenioidei have developed varied feeding strategies which allow them to utilise food resources in all regions of the Antarctic Ocean (Gröhsler 1994). These strategies may help to reduce dietary overlap and therefore to mitigate competition among coexistent fishes in specific ecological zones of the marine ecosystem.

An analysis of dietary overlaps between notothenioid species was carried out among three fish assemblages in the South Shetland Island archipelago

(Barrera-Oro 2003). The study areas were Potter Cove and Admiralty Bay at King George Island and the Elephant Island shelf (mainly the NW) (Fig. 1). The fishes were demersal shallow water fish which spend most of their life cycle inshore, with the exception of the pelagic Antarctic silverfish, *Pleuragramma antarcticum*.

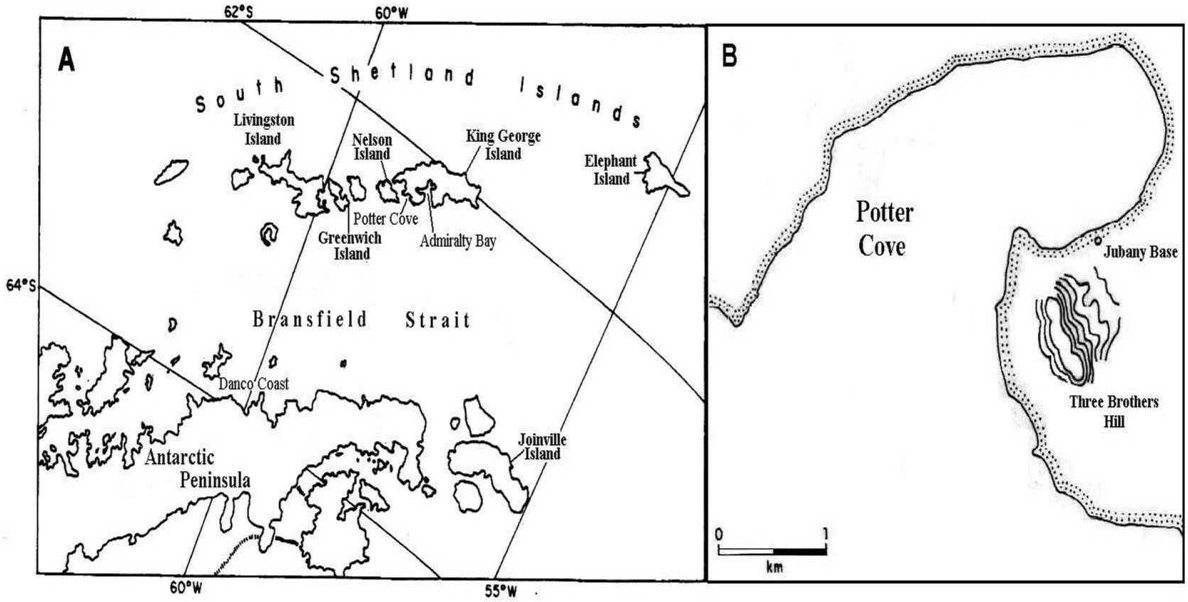


Fig. 1. The study area in the South Shetland Islands and west Antarctic Peninsula (A) and an enlargement of Potter Cove at King George Island (B).

The data of fish from Potter Cove (n=992) were taken from Casaux et al. (1990) and Casaux (1998) and correspond to a sampling period of August 1985-May 1986 using trammel nets at 8-48 m depth, except those on *Harpagifer antarcticus*, which specimens were caught in January-February 1992 by hand in tide pools. The data on fish from Admiralty Bay (n=683) was from Linkowski et al. (1983) for the winter of 1977 and the summer of 1979/80. Hooks, gill nets, traps, beam trawls and pelagic trawls were used to catch the fish between 10 and 150 m depth. Data on fish at Elephant Island (n=1930) between 89 and 493 m depth caught by bottom trawl are from Gröhsler (1994) for autumn/winter 1986. Stomach contents were assessed using mainly the dietary coefficient "Q" (Hureau 1970), which is the product of the percentage by number and by weight of each prey type, where a Q > 200 represents main prey and 200 > Q > 20 indicates econdary prey.

Dietary overlap between species was analysed following Tyler (1972). In this method, the reoccurrence of prey or percentage overlap among predator species is the number of reoccurrences of prey among predators divided by the number of possible reoccurrences (Table 1). Thus, one reoccurrence means that a prey taxon occurs in two predator species. The total number of reoccurrences possible is the number of predators minus one, multiplied by the number of prey.

In Potter Cove, the reoccurrence of main and secondary prey was 33% in summer and 37% over the year. In Admiralty Bay prey reoccurrence was 25% and 7% during summer and winter respectively. Around Elephant Island, 20% of the possible reoccurrences of prey among fish predators was detected. Like-

wise, using the "S" index of Linton et al. (1981) the diet similarity between 75% of species pairs was under 50%. High S values of 87% and 88% were obtained between *Trematomus newnesi* and *H. antarcticus* in Potter Cove and between *T. newnesi* and *P. antarcticum* in Admiralty Bay, respectively (Barrera-Oro 2003, table 2).The observations in Potter Cove are confirmed by those of Linkowski et al. (1983) and Gröhsler (1994), because the three fish assemblages of the South Shetland Islands had relatively low dietary overlaps. This may be explained by the about equally divided occurrence of generalised feeders and specialised feeders. The proportional prey overlap among fishes may be even less if the fish are separated in size classes and if the prey are listed specifically rather than grouped in broad categories such as gammarideans. Such is the case between *T. newnesi* and *H. antarcticus* in Potter Cove. Likewise, the high food overlap between *T. newnesi* and *P. antarcticum* in Admiralty Bay is due to a high availability of krill (mainly *Euphausia superba*) in the area in summer.

Cases of trophic specialisation are described for most of the species treated here (Barrera-Oro 2003). In this summary, we give only some examples. The most generalist species, *Gobionotothen gibberifrons, Notothenia coriiceps* and juvenile *Notothenia rossii*, have a wide trophic spectrum. However, among generalist benthophagous fishes interspecific competition is reduced by resource partitioning. For example, competition would seem to be high in Potter Cove, due to the importance of gammarideans as prey of several fishes. However, different fish species may feed on different gammaridean species, for example amphipods among macroalgae may be prey for *N. coriiceps* whereas epibenthic gammarideans may be prey for *G. gibberifrons* (Casaux et al. 1990). Although interspecific food overlap between *N. coriiceps* and *N. rossii* was detected at Chile Bay and Fildes Bay (South Shetland Islands), both species coexist. During summer *N. coriiceps* feeds on a greater diversity of prey than *N. rossii* (Moreno & Bahamonde 1975). Furthermore, *N. coriiceps* may prey actively by searching for prey among macoalgae or it may adopt an ambush feeding strategy (Barrera-Oro & Casaux 1990; North 1996). *Harpagifer antarcticus* is a specialist feeder that hunts mobile epibenthic amphipods (mainly *Gondogeneia antarctica*) in rubble bottoms in cobble substrate coves, but occasionally may prey chiefly on krill when it is abundant close to the bottom (Casaux 1998).

Information on diet overlap among the majority of the fish species included in this study for nearby areas is in agreement with our results. For fish from the South Orkney Islands area Targett (1981) found that prey overlap was generally low, usually much less than 0.50 by weight, but was high when krill was the main prey. For fish off the Antarctic Peninsula Daniels (1982) indicated that when a high degree of diet similarity occurs, then the overlap in the spatial distribution of the predators tends to be low. In both studies it was concluded that prey resources were clearly partitioned among the fish community, and that this diminished competition.

In fish communities of the Weddell Sea, the Ross Sea, and other high Antarctic regions the dietary overlap between most of the notothenioid species

Table 1. Fish prey overlap in three fish assemblages at the South Shetland Islands (Tyler's method). Dots indicate annual data at Potter Cove and autumn/winter data at Admiralty Bay and Elephant Island; crosses indicate summer data. The predator species are numbered as follows: **1**, *G. gibberifrons*; **2**, *N. coriiceps*; **3**, *N. rossii*; **4**, *T. newnesi*; **5**, *H. antarcticus*; **6**, *L. nudifrons*; **7**, *P. antarcticum*; **8**, *L. larseni*; **9**, *C. aceratus*; **10**, *P. charcoti*.

PREY	PREDATOR	POTTER COVE							ADMIRALTY BAY							ELEPHANT ISLAND								
		89 123	671	46	63		No. of Reocc.		220	263	42	51	10	No. of Reocc.		626 12	318	263	341	70	76	224		No. of Reoc c
		1	**2**	**3**	**4**	**5**	SUMMER	ANNUAL	**2**	**3**	**4**	**6**	**7**	WINTER	SUMMER	**1**	**2**	**3**	**8**	**6**	**7**	**9**	**10**	AUTUMN
Algae		+•	+•	+•			2	2																
Polychaetes		+•					0	0		•		+		0	0									
Bivalves									+						0									
E. superba			+	+•	•		1	1			+•		+	0	1	•	•	•	•		•		•	5
Gammarideans		+•	+•	+•	+•	+•	4	4	+•	+•		+		1	2	•				•				1
Hyperiids			+•	•	+•		1	2			+•		+	0	1									
Copepods											•			0							•			0
Pycnogonids												+			0									
Salps									+•	+•		+	+	1	3									
Ascidians																•			•	•				2
Fish eggs																				•				0
Fish indet.			+•				0	0		•				0					•			•	•	2
G. gibberifrons																						•		0
Total Reoccurrences							8	9						2	7									10
Maximum possible							24	24						28	28									49
% of Reoccurrences							33	37						7	25									20

pairs was less than 0.50, because food resources are partitioned, mainly with depth (Schwarzbach 1988; Vacchi et al. 1994). In cases of higher overlap, interspecific competition is thought to be mitigated by taking different prey or different amounts of the same prey (La Mesa et al. 1997).

Information from demersal fish assemblages in similar non-Antarctic cold marine ecosystems like the Boreal and Arctic, also agree on the existence of a reduced interspecific competition due to resource partitioning (Tyler 1972; Arntz 1980; Atkinson & Percy 1992).

In general, it has been indicated that in polar marine ecosystems the predominant demersal fish exhibit a low degree of trophic specialisation (Andriashev 1987). However, this premise is only valid in comparison to, for example, analogous ichthyofaunas from temperate or tropical zones, in which high diversity fish faunas must share its food, this resulting in the development of a great variety of specialised feeding types. In conclusion, there was no evidence of food competition among the shallow cold-water fish communities reviewed in this work.

Ecological aspects of fish from the Danco Coast, Antarctic Peninsula

Most of the ecological studies on coastal demersal fish from the South Shetland Islands and west Antarctic Peninsula waters (FAO Statistical Subarea 48.1) were done in the first area (reviewed in Barrera-Oro 2002) and a fewer number in the second area (Moreno et al.1977; Daniels 1982). In 1997/1998, the scope of our research project was extended to the Danco Coast, a less investigated area of the Antarctic Peninsula, which has remained outside the influence of the commercial fishery (Fig. 1). New data on occurrence**,** morphometry, reproduction and diet of demersal fish from this region, was given by the analysis of 1103 specimens obtained by trammel-nets at four sites surrounding Cierva Point (Moss Island 1; Moss Island 2; Sterneck Island; Leopardo Island) between 2 February and 31 March 2000 at depths of 20-70 m (Casaux et al. 2003) (Fig. 2).

Nototheniid species (471 *N. coriiceps*; 265 *T. newnesi*; 215 *G. gibberifrons*; 45 *Trematomus bernacchii*; 28 *Lepidonotothen nudifrons*; 3 *N. rossii* and 3 *Trematomus hansoni*) dominated in the samples whereas the families Bathydraconidae (12 *Parachaenichthys charcoti*) and Channichthyidae (8 *Chaenocephalus aceratus*) were scarcely represented (Table 2).

The fish species caught at the Danco Coast in the present study have been previously reported for neritic waters of the Antarctic Peninsula, and agree in terms of composition with those sampled also with trammel-nets in the South Shetland Islands area (Casaux et al. 2003). *Notothenia coriiceps* was the dominant fish in number and mass in the sampling sites, except at Sterneck Island where *G. gibberifrons* was the most abundant. The total length ranges observed in *N. coriiceps*, *T. newnesi* and *G. gibberifrons*, coincide with those reported by Casaux et al. (1990) for fish caught with similar nets at Potter Cove.

The analysis of the *N. coriiceps*, *T. newnesi*, *L. nudifrons*, *T. hansoni*, *C. aceratus* and *P. charcoti* specimens at gonad stages III and IV indicates that the sampling time and the size of these fish agree with the spawning time and the length at first spawning reported for these species at other localities (summarised in Casaux et al. 2003). A high proportion of the *L. nudifrons* (91.7%)

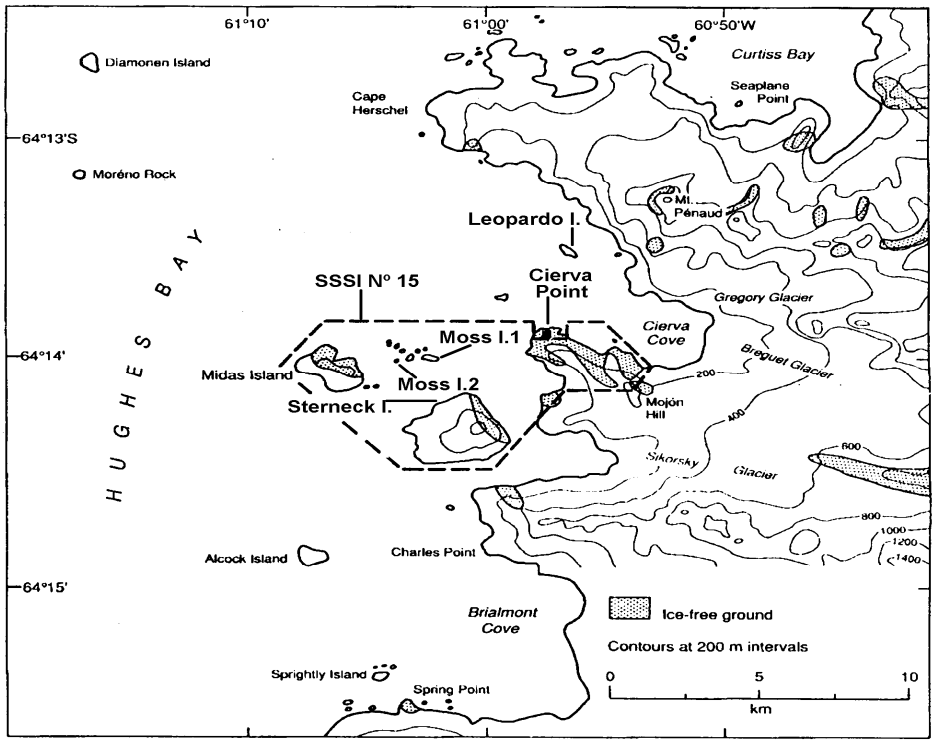


Fig. 2. Location of the sampling sites in the proximity of Cierva Point, Danco Coast, Antarctic Peninsula.

and *T. bernacchii* (73.5%) specimens were females. Except one stage III, all *L. nudifrons* ovaries were at stage IV, which suggests a pre-spawning female aggregation in the sampling area at that time.

Full stomachs predominated in the two most important fish by mass, *N. coriiceps* and *G. gibberifrons*; only empty stomachs was found in *C. aceratus* and *T. hansoni* and this stage predominated in *T. bernacchii* and *T. newnesi* (Casaux et al. 2003, table 9). The main preys found in the stomachs have been previously indicated in the diet of the same fish species in other areas. Although they seem to be primarily benthos (*N. coriiceps*, *G. gibberifrons*, *L. nudifrons* and *T. bernacchii*) or plankton/water-column feeders (*T. newnesi*, *N. rossii* and *P. charcoti*), all of them preyed on both benthic-demersal and pelagic organisms. Most of these fish species are specialised feeders (see coefficient *Q* in Table 3). Gammarid amphipods were largely the main food of *G. gibberifrons*, *L. nudifrons* and *T. bernacchii*; krill was the main prey of *N. rossii* and *T. newnesi* whereas *P. charcoti* foraged mainly on fish. The diet composition of *N. coriiceps* was the most diverse and changed in the different sampling sites. This fish is an opportunistic feeder, and therefore its diet reflects the food availability of benthos at different sites and depths. Algae constituted a main food item for *N. coriiceps* and *N. rossii*, in line with the theory of deliberate selection of this item by fish (Barrera-Oro & Casaux 1990; Iken et al. 1997).

Comparison of relative abundance of *G. gibberifrons* in inshore trammel-net catches between present results from the Danco Coast and those from the South Shetland Islands area indicates high and very low values, respectively (this study; Barrera-Oro et al.2000). The effect of the commercial

fishery around the South Shetland Islands at the end of the 1970s is the reason for the decline in the inshore population of *G. gibberifrons* in that area during the last 22 years (Barrera-Oro et al. 2000). This study supports this explanation because the data were obtained in an area that has remained outside the influence of the fishery.

Table 2. Fish caught in trammel-nets at the Danco Coast. The frequency of occurrence (F) and the importance by number (N) and mass (M) are expressed in percentage.

	Total **F%** **N%** **M%**	**Moss I. 1** **F%** **N%** **M%**	**Moss I. 2** **F%** **N%** **M%**	**Sterneck I.** **F%** **N%** **M%**	**Leopardo I.** **F%** **N%** **M%**
G. gibberifrons	74.3 19.5 11.6	25.0 0.8 0.9	100 27.0 13.9	87.5 38.5 24.7	37.5 1.0 1.0
L. nudifrons	42.9 2.5 0.4	--- - -- ---	60.0 3.2 0.5	50.0 5.0 0.9	25.0 0.7 ***
N. coriiceps	100 42.7 74.2	100 97.5 97.7	100 32.3 67.6	100 28.1 61.0	100 47.9 85.9
N. rossii	5.7 0.3 0.5	25.0 0.8 1.1	--- - -- ---	12.5 0.8 1.5	--- --- ---
T. bernacchii	48.6 4.1 1.5	--- - -- ---	53.3 3.9 1.3	62.5 2.9 2.1	50.0 6.9 2.3
T. hansoni	8.6 0.3 0.2	--- - -- ---	6.7 0.2 0.3	12.5 0.4 0.3	12.5 0.3 0.3
T. newnesi	80.0 24.1 7.7	25.0 0.8 0.3	100 27.5 7.9	87.5 24.0 9.5	75.0 28.2 10.1
C. aceratus	20.0 0.7 2.1	--- - -- ---	40.0 1.6 4.7	--- - -- ---	12.5 0.3 ***
P. charcoti	20.0 1.1 1.8	--- - -- ---	40.0 2.5 3.8	--- - -- ---	12.5 0.3 0.4
Unidentified	22.9 4.7 -- -	--- - -- ---	13.3 1.8 -- -	12.5 0.4 -- -	62.5 14.1 ---

The distinct ecological role of fish in inshore and offshore waters in the Antarctic marine food web.

The ecological role that fish play in the Antarctic ecosystem has been well described by Kock (1992) and Hureau (1994), without distinction between the inshore and the offshore species. Some questions remain: is the role of demersal fish in the Antarctic food web similar in inshore shallow waters and offshore waters? What is the role of fish in comparison to that of krill and other key organisms in both of these zones? The review by Barrera-Oro (2002) aimed to provide some insight into these questions, taking as an example the coastal marine communities of the southern Scotia Arc (South Orkney Islands and South Shetland Islands) and the west Antarctic Peninsula (Fig. 1). The main fish species considered are the most abundant notothenioids distributed in the two contiguous portions of the continental shelf: inshore, shallow waters, at depths down to 110 (littoral, coves, shallow fjords)-200 (deep fjords and bays) m and offshore waters up to the shelf break, between 110 m and c. 450 m.

The inshore zone

Harpagifer antarcticus (from tide-pools), *N. coriiceps* (from 5 m depth) and *N. rossii* (juveniles, from 10 m depth) are the more neritic species, *G. gibberifrons*, *L. nudifrons* and *T. newnesi* occur more frequently from 30-45 m depth, whereas *T. bernacchii*, *P. charcoti* and *C. aceratus* are caught mainly from a depth range of 70-90 m downwards. The occurrence of pelagic fish (myctophids, *P. antarcticum*) inshore is rarely reported (Daniels 1982, Linkowski et al. 1983). Their presence in shallow water zones could be sporadic and has been related to temporal introduction of nekton from adjacent offshore areas (e.g. from the Bransfield Strait to Admiralty Bay, King George Island, Skora 1993). Recent observations of flying birds feeding nearshore close to the surface suggest that the occurrence of pelagic fish in inshore areas might be higher than previously believed (see Barrera-Oro 2002).

In this zone benthos feeders and benthophagous, non generalist species, which also feed sporadically in the water column (benthos-plankton feeders) are predominant. Plankton feeders, which prey almost exclusively in the water column, are early juvenile stages of many notothenioids (e.g. *C. aceratus*). All the feeding behaviours are represented in this zone: ambush, slurp, grazing and water column. Some species use these strategies alternatively or combine more than one strategy to feed on a wide range of organisms. Water column feeding is typical of the pelagic Antarctic silverfish *P. antarcticum* and the cryopelagic *Pagothenia borchgrevinki* and the semipelagic *T. newnesi* in the High Antarctic Zone (Eastman & DeVries 1985, Gutt 2002). Inshore, water column feeding fish prey on krill when it is present and on other pelagic prey, but also feed on benthos when pelagic prey is not available. The energetic value of benthos is much lower than that of fish and krill (Brey 2001 and references therein). Furthermore, some of the benthic organisms preyed on throughout the year by generalist demersal fish species such as *G. gibberifrons*, *N. coriiceps* and juvenile *N. rossii* are of very low energetic value (e.g. algae, sponges, corals, asteroids, ophiuroids, ascidians). It is likely that this apparent “energetic disadvantage” be balanced by seasonal variation in the demand for food (Coggan 1997, see below). Observations of seasonal variations in fish diets showed that benthic amphipods are the main prey during most of the year, but during

Table 3. Diet composition of the fish sampled at the Danco Coast, Antarctic Peninsula. F%= frequency of occurrence percent; Q= dietary coefficient.

	G. gibberifrons		*L. nudifrons*		*N. coriiceps*		*N. rossii*		*T. bernacchii*		*T. newnesi*		*P. charcoti*	
	F%	**Q**	**F%**	**Q**	**F%**	**Q**	**F%**	**Q**	**F%**	**Q**	**F%**	**Q**	**F%**	**Q**
Algae	24.1	2.3	12.5	2.3	66.8	299.6	66.7	590.2	---	---	---	---	---	---
Errant polychaetes	26.5	18.4	25.0	74.8	5.8	0.3	---	---	---	---	---	---	---	---
Gastropods														
Nacella concinna	11.8	10.5	---	---	12.2	9.8	---	---	19.1	15.2	---	---	---	---
unidentified	10.0	1.0	20.8	34.8	14.6	12.6	---	---	---	---	---	---	---	---
Bivalves														
Clams	26.5	84.1	---	---	0.8	0.0	---	---	---	---	---	---	---	---
Laternula elliptica	3.5	0.4	---	---	---	---	---	---	---	---	---	---	---	---
Quitons	5.9	1.1	4.2	0.2	0.8	0.0	---	---	---	---	---	---	---	---
Squids	---	---	---	---	0.3	0.0	---	---	---	---	---	---	---	---
Euphausiids														
Euphausia superba	0.6	0.0	8.3	9.2	12.7	94.8	66.7	2557.4	4.8	0.5	94.4	9707.0	11.1	4.3
Decapods	---	---	---	---	0.3	0.0	---	---	---	---	---	---	11.1	16.8
Amphipods														
Gammarids	52.4	1970.2	70.8	4382.0	45.4	263.8	---	---	52.4	3502.9	2.5	0.3	---	---
Hyperiids	0.6	0.0	---	---	1.3	0.0	---	---			1.9	0.0	---	---
Isopods														
Glyptonotus antarcticus	0.6	0.1	---	---	1.6	0.2	---	---	---	---	---	---	---	---
Serolis sp.	22.9	34.5	---	---	11.1	9.5	---	---	---	---	---	---	---	---
unidentified	1.2	0.0	---	---	0.3	0.0	---	---	---	---	---	---	---	---
Ophiuroids	8.2	1.2	---	---	---	---	---	---	---	---	---	---	---	---
Echinoids														
Sterechinus neumayeri	---	---	---	---	0.6	0.0	---	---	---	---	---	---	---	---
Nemerteans	0.6	0.0	---	---	0.6	0.0	---	---	---	---	---	---	---	---
Priapulids	6.5	3.9	---	---	0.3	0.0	---	---	---	---	---	---	11.1	5.2
Asciids	2.9	0.3	---	---	3.2	1.1	---	---	9.5	8.7	0.6	0.0	---	---
Salps	15.3	14.6	---	---	17.0	48.4	33.3	16.4	9.5	53.7	0.6	0.0	---	---
Fish	1.2	0.0	---	---	15.7	56.4	---	---	---	---	---	---	77.8	6473.3
Unidentified	47.1	---	20.8	---	14.3	---	66.7	---	47.6	---	1.9	---	---	---

the summer months, fish take advantage of the occurrence of krill and other pelagic organisms (e.g. hyperiid amphipods) inshore, to feed on them intensively (Casaux et al. 1990).

The offshore zone

The low-Antarctic species *G. gibberifrons*, *Champsocephalus gunnari*, *C. aceratus* and *Lepidonotothen squamifrons* are predominant in the whole southern Scotia Arc region; the high-Antarctic species *Chionodraco rastrospinosus, Cryodraco antarcticus* and some *Trematomus* species also occur in the whole region, but their abundance is scarce (Kock et al. 2000). The stocks of the former exploited species *N. rossii* remains low, after the offshore commercial fishery in the area in the late 1970s.

Pleuragramma antarcticum is circum-Antarctic and abundant in coastal waters (DeWitt & Hopkins 1977), including the area off the Antarctic Peninsula (Kellermann 1987). Species of the family Myctophidae, mainly *Electrona antarctica* and *Gymnoscopelus nicholsi*, occur on the outer shelf, but the entire family reaches maximum abundance in the open ocean pelagic ecosystem.

The feeding types and feeding behaviours described for fish inshore are also represented in offshore waters, but fish depend less on benthic organisms and feed more intensively on krill and other pelagic forms such as salps, hyperiid amphipods and pelagic fish (i.e. myctophids, *P. antarcticum*, juvenile stages). Water column feeding is a common feeding behaviour of fish in the offshore zone. For example, *N. rossii* occurs inshore in the juvenile stage, where they are mainly benthos feeders but also feed on epi-benthos, plankton and nekton (Casaux et al. 1990)). After the juvenile phase, this species migrates offshore to join the adult population, that feed mainly on krill and fish (Burchett 1982, Gröhsler 1994). The benthos feeder *G. gibberifrons* in offshore waters north of the South Shetland Islands showed a main diet of krill, supplemented with benthic organisms (Takahashi 1983). *Lepidonotothen larseni* and *Trematomus eulepidotus*, which occur inshore but have a wider offshore distribution, feed mainly on krill and secondarily on gammarideans, isopods and polychaetes (Barrera-Oro & Tomo 1987, Gröhsler 1994, Takahashi & Iwami 1997).

Krill as fish prey in inshore and offshore waters

The importance of krill in the diet of Antarctic fish has been widely recognised (Barrera-Oro 2002 and references therein). Independent of habitat and feeding style, krill is the main prey for most of the offshore demersal notothenioids. Moreover, it has been indicated that mesopelagic fish such as myctophids might have a greater impact on krill than coastal species (Sabourenkov 1991, Hureau 1994). The role of krill in inshore waters is less important for fish and the benthic community than in the offshore portion of the shelf. One explanation is that the occurrence of krill in inshore waters is restricted mainly to the summer season. On the other hand, there is evidence for inshore demersal fish, of seasonal variation in the demand for food (Coggan 1997).

Fish and krill predators

In their turn, the demersal fish are common prey of birds and seals (Fig. 3).
In Antarctica, the only flying birds that feeds chiefly on demersal coastal fish are the Antarctic shag *Phalacrocorax bransfieldensis* and the South Georgia shag *P. georgianus* (reviewed in Casaux & Barrera-Oro 2006). The fish taken by Antarctic penguins are mostly pelagic, only the gentoo (*Pygoscelis papua*) feeds inshore on pelagic and demersal species (Coria et al. 2000).

The fish eaten by Antarctic pinnipeds are also mostly pelagic (mainly myctophids) but the Southern Elephant seal (*Mirounga leonina*), the Leopard seal (*Hydrurga leptonyx*), the Weddell seal (*Leptonychotes weddellii*) and the Antarctic fur seal (*Arctocephalus gazella*) prey on demersal fish at least partially (summarised in Barrera-Oro 2002) (Fig.3).
Predation on demersal fish by birds and seals in the offshore fraction of the continental shelf is conditioned by their diving ability. Among the higher predators that also feed in nearshore waters, only Weddell, elephant and presumably leopard seals have the diving capacity to feed on demersal fish offshore, close to the seabed at depths deeper than 200 m (summarised in Barrera-Oro 2002). The Weddell seal is the most piscivorous one.

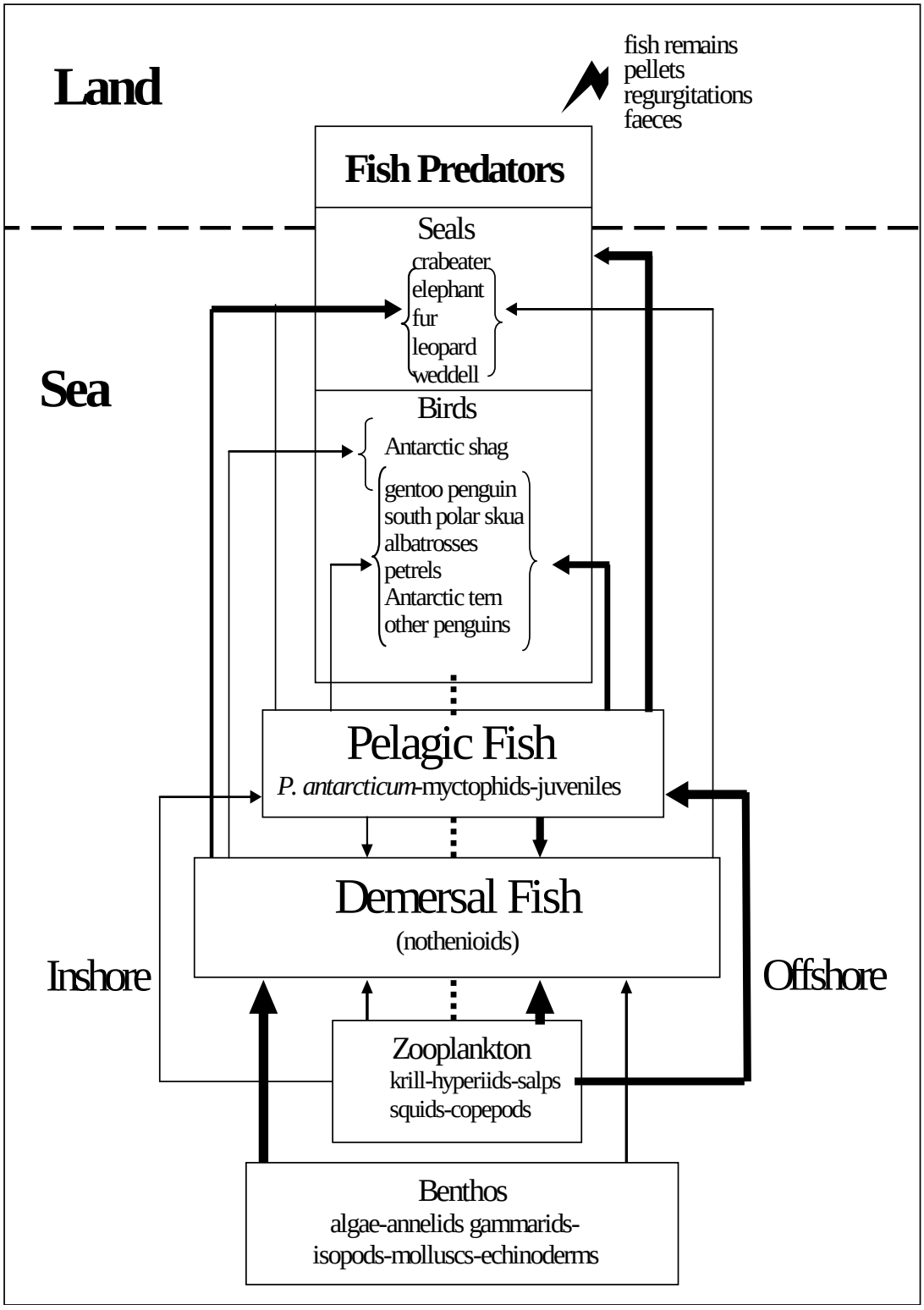
In offshore waters, predation by birds and seals on pelagic fish, instead, is more important quantitatively. Myctophids (lanternfish), are key components of the oceanic pelagic ecosystem in terms of fish biomass and as dominant krill predators (Lancraft et al. 1989). The other circum-Antarctic pelagic fish, *P. antarcticum,* is also an important prey of seabirds and seals; it plays a key role in the marine food web in the high Antarctic zone (Kellermann & North 1994, Williams & Duhamel 1994).

In the outer shelf and the open ocean ecosystem krill is the main prey for most of vertebrates. The accessibility of krill to fish could be explained in terms of occasional availability due to the highly aggregated distribution of krill (Miller & Hampton 1989). However, to higher predators the accessibility to krill depends also on its foraging behaviour, allowing them to search for krill over larger areas.

Although the calculations have not been refined lately, annual krill intake is about 30-50 Mt (million tonnes) by squids; 40-50 Mt by fish; 115 Mt by birds; 130 Mt by seals; 36-140 Mt by minke whales and 43 Mt by the remaining whales. On the other hand, annual fish consumption by birds and mammals has been estimated to be up to 15 Mt, which is lower than consumption of krill. The annual predator consumption of cephalopods (mostly *Martialia hyadesi*) is also comparatively low, estimated at 330.000 tonnes in the Scotia Sea. (summarised in Barrera-Oro 2002).

The reliance of offshore fish and most of the higher predators on krill as a food supply is probably because of the general availability of krill offshore throughout much of the year in most years (Kock 1992). The high energy content of krill in comparison with other organisms (see Brey 2001) may also constitute a significant benefit for its predators. This is valid also for the myctophids in oceanic waters (elsewhere in the Antarctic Polar Frontal Zone) and for *P. antarcticum* in the high Antarctic zone, explaining its high consumption by higher predators. The difference in energetic value is explained by the high lipid content of krill and pelagic fish such as Myctophidae and *P. antarcticum* in comparison to demersal fish (summarized in Barrera-Oro 2002).

Fig. 3. Diagram indicating the position of fish in the food web of the inshore-shallow water and the offshore communities.



Conclusions

The main pathway of energy flow through fish in the food web in the study area is shown in Fig.3. Inshore, the ecological role of demersal fish is more important than that of krill. There, demersal fish are major consumers of benthos and also feed on zooplankton (mainly krill in summer). They are links between lower and upper levels of the food web; they are common prey of other fish, birds and seals. Offshore, demersal fish depend less on benthos and feed more on zooplankton (mainly krill) and nekton, and are less accessible as prey of birds and seals. There, pelagic fish are more abundant than inshore and play an important role in the energy flow from macrozooplankton to higher trophic levels (seabirds and seals). Through the higher fish predators, energy is transferred to land in the form of fish remains, pellets (birds), regurgitations and faeces (birds and seals). However, in the general context of the Antarctic marine ecosystem, krill plays the central role in the food web because it is the main food source in terms of biomass for most of the high level predators from demersal fish up to whales. This has no obvious equivalent in other marine ecosystems. In Antarctic offshore coastal and oceanic waters the greatest proportion of energy from the ecosystem is transferred to land directly through krill consumers, such as flying birds, penguins, and seals. Beside krill, the populations of fish in the Antarctic Ocean are the second most important element for higher predators, in particular the energy-rich pelagic Myctophidae in open waters and the pelagic Antarctic silver fish *P. antarcticum* in the high Antarctic zone.

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Massive input of terrigenous sediment into Potter Cove during austral summer and the effects on the bivalve *Laternula elliptica*: a laboratory experiment

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Introduction

Although extensive studies on the coastal ecosystem of Potter Cove have been performed, our knowledge concerning pelagic-benthic coupling is still scarce. Benthic filter feeders play an important role in linking the pelagial with the benthic systems by filtering and biodeposition (Ahn, 1993), enhancing particulate organic carbon and downward fluxes of inorganic material that increase the food supply to the associated fauna. At Potter Cove filter feeders contribute to the regulation of the energy budget and particle flux to the benthic realm. However, at this location seawaters are phytoplankton-impoverished and during the austral summer large quantities of inorganic seston are deposited on the seafloor. This is due to the run-off through coastal meltwater streams, with a high input of terrigenous sediments. How does this affect the feeding ecology of filter feeders and their possible nourishing role? In order to answer this question, we performed an *in vitro* experiment with the bivalve *Laternula elliptica* (King & Broderip, 1831), which dominates the Antarctic infauna. We evaluated the transformation of the organic matter from three artificial diets to the sediment, analysing the chemical composition of the biodeposits. In our study, the first diet supply may simulate a normal feeding situation, the second one a highly stressful condition due to the addition of inorganic material and the last diet supply may simulate a possible benthic diatom resuspension event, during a stressful period. Preliminary results are presented. In a subsequent study, the analyses will be extrapolated to the population of *L. elliptica* inhabiting Potter Cove.

Materials and methods

L. elliptica was collected by scuba diving and separated into three size classes, corresponding to wet mass: < 50 g: small; 50-100 g: medium; > 100 g: large. Three different diets were tested: diet 1 (d1)**:** natural seawater, chlorophyll a: ≈ 0.5-2.5 µg/L, suspended matter: ≈ 2-14 mg dry mass/L, diet 2 (d2)**:** d1 + natural

silt (40 mg dry mass/L) and diet 3 (d3)**:** d2 + cultured natural phytoplankton (chlorophyll a: 10 µg/L). Each diet was poured over 24 h into 50 lt. experimental tanks, to keep the diet level constant. This was accomplished with the aid of peristaltic pumps (for exact seston dosage) and a constant natural seawater flux, pumped directly from the cove. In order to maintain the homogeneity of the seston and to avoid sedimentation, submersible circulation pumps were used. Tanks were aerated and the filtering activity of the animals was permanently checked. The water temperature of the tanks was kept constant within normal sea temperature values (1±1 °C). Each diet was offered for three consecutive days (twenty four-hour experiments, with three replicates for each diet) in the experiments described below. In order to test the experimental conditions and to determine the amount and chemical composition of the seston in each experimental tank, water samples were taken at regular intervals (every 3 to 4 hours). Contents of chlorophyll a (spectrophotometric method, Strickland and Parsons, 1972), suspended particulate matter (SPM) and percentage of organic matter (OM) (gravimetric method), POC and PON (particulate organic carbon and nitrogen, Carlo Erba NA-1500 analyser) were determined.

Biodeposition rates: Biodeposits (faeces and pseudofaeces) produced by *L. elliptica* were collected from small plastic containers (with open tops), placed on the bottom of the experimental tanks. In some of these “collectors” test bivalves were placed, separated by size class and with similar total animal wet mass per container. Additional containers were equipped with pebble rocks as controls. After each twenty four-hour experiment, the sedimented material collected in the containers was analysed for total matter and organic content (gravimetric method). POC and PON were determined. The amount of biodeposits was calculated by subtracting the average mass of deposits in the control containers from the mass of deposits in each container with clams (Ahn, 1993). Data were analysed using two-way ANOVA and the Tukey Honestly Differences Test (multiple comparison tests of means).

Chemical composition of biodeposits: In parallel experiments, thirty specimens of the three size classes were randomly mixed and placed together in shallow tanks (70 x 50 x 15 cm). Test bivalves were fed in the same way with the same diets as described previously. During the water sampling described above, faeces and pseudofaeces (rejected non digested particles) were separately collected every 3 to 4 hours by use of Pasteur pipettes from the bottom of these shallow tanks. This material was also analysed for chlorophyll a, OM percentage, POC and PON contents. Data were analysed using non-parametric tests (Kruskal-Wallis ANOVA) and the Tukey Honestly Differences Test (multiple comparison tests of means).

Results and Discussion

The experiments with and without test bivalves showed that the deposited matter was significantly higher (at $p<0.001$) in the presence of *Laternula elliptica*. A two-factors ANOVA was performed, with diet type and clam size class as factors. Post-Hoc comparisons tests were performed as described above. The interaction effect between the diet type and size class treatments

was significant (at $p<0.01$) for deposited matter. This could be observed in the response of the smaller clams (size class 1) to the increasing seston load from diet 1 to diet 3. Although not outstanding in the case of d1, d2 and d3 (seston-rich diets) yielded in increased deposited matter with decreasing specimens' size class (Fig. 1). The deposited matter was significantly different among the three diets tested (independently of the size class). Moreover, the total amount of deposits produced by size class 1 (small clams) was significantly higher than the deposits produced by the other size classes (independently of diet treatment) (Table I).

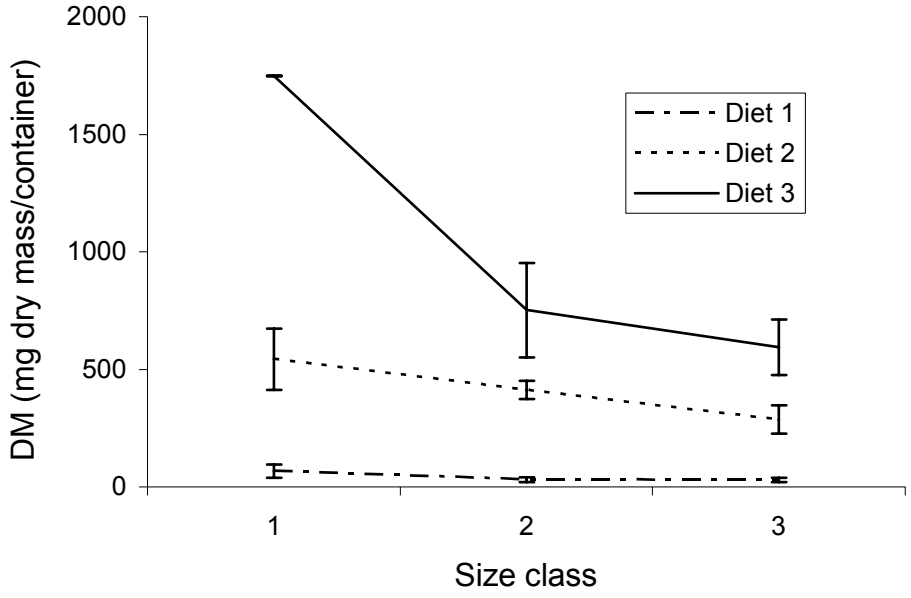

Fig. 1: DM: deposited matter (mg dry mass/container. Mean, ± SD); Size class of *L. elliptica*: 1: small, 2: medium, 3: large

Table I: Two-factors ANOVA, with diet type and clam size class as factors. Post-Hoc comparison tests results are not included.

Source	S.S.	DF	MS	F	P
Total	803125	48			
Diet	5125776	2	2562888	75.76	<0.001
Size	1584416	2	792208	23.42	<0.001
Interaction	1248412	4	312103	9.23	<0.001
Within cells	1353105	40	33827		

The interaction effect between the diet type and size class treatments was not significant for POC. However, the POC content of the deposited matter after d1 supply was significantly higher than the respective amount after d2 supply (independently of the size class). No significant differences were found between d2 and d3 nor between d3 and d1, on this respect. PON content estimations (data not shown) provide similar results (Fig. 2) (Table II).

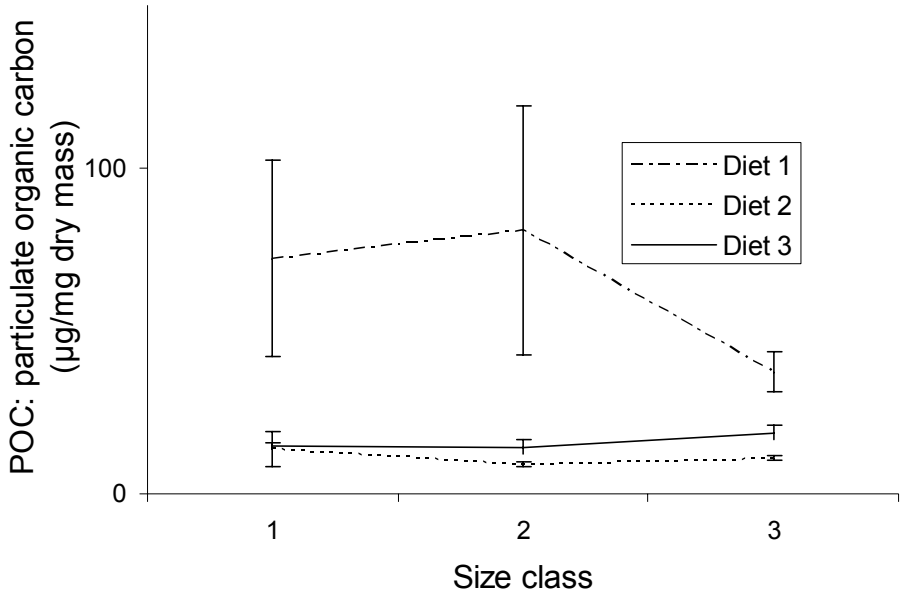

Fig. 2: POC: particulate organic carbon content (µg/mg dry mass of deposited matter. Mean, ± SD); Size class of *L. elliptica*: 1: small, 2: medium, 3: large

Table II: Two-factors ANOVA, with diet type and clam size class as factors. Post-Hoc comparison tests results are not included.

Source	S.S.	DF	MS	F	P
Total	110748.7	48			
Diet	30334.60	2	15167.30	8.03	<0.001
Size	1301.77	2	650.89	0.34	0.711
Interaction	4956.97	4	1239.24	0.66	0.626
Within cells	75536.10	40	1888.40		

One-factor ANOVA and multiple comparison tests of means were performed, to test for differences in C/N ratios of the seston, faeces and pseudofaeces among diets. The C/N ratio of the seston in the experimental tank during the supply of d1 was significantly lower than the other seston ratios studied (for d2 and d3). However, no significant differences were found between the respective values for d2 and d3 (Fig. 3-a, Table III). This is in line with the C/N ratios of faeces, but no significant differences were found among pseudofaeces. The higher C/N values of the faeces produced after d2 and d3 supply, may probably indicate a lower particle selection efficiency with increasing seston load, rather than a higher assimilation efficiency. Although the diets provided and faeces and pseudofaeces produced included different amounts of particulate organic carbon and nitrogen (per mg particulate dry mass), they have a potential high nutritional value. C/N ratios of seston, faeces and pseudofaeces ranged between 5 and 8, which is in the range of fresh phytoplankton (Schloss et al., 1999).

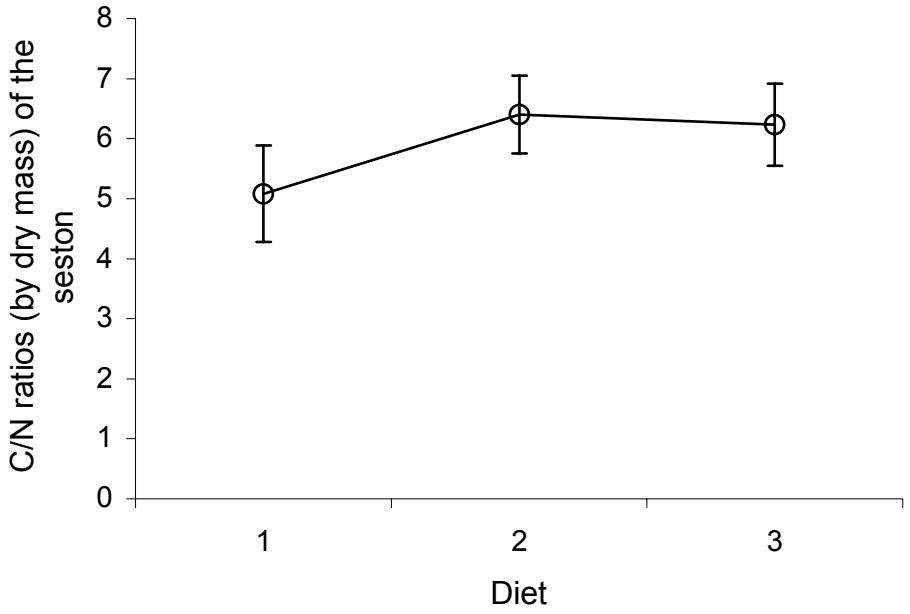

Fig. 3-a: C/N: organic carbon/nitrogen ratios of the seston in the 50 lt. experimental tanks (by dry mass. Mean, ± SD), during the supply of diets: 1, 2, 3

Table III: One-way ANOVA for the comparison of C/N ratios among diets. Post-Hoc comparison tests results are not included.

Source	S.S.	Df	MS	F	P
Total	35.92	39			
Diet	16.2	2	8.1	15.21	<0.001
Error	19.71	37	0.53		

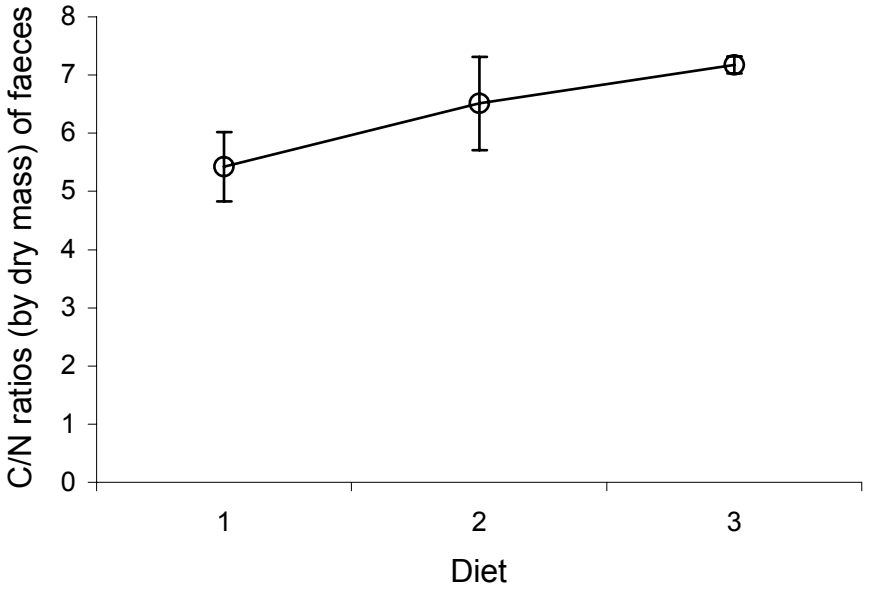

Fig. 3-b: C/N: organic carbon/nitrogen ratios of faeces of *L. elliptica* (by dry mass. Mean, ± SD); diets: 1, 2, 3

Table IV: refer to Table III for references.

Source	S.S.	Df	MS	F	P
Total	21.33	25			
Diet	10.99	2	5.5	12.22	<0.001
Error	10.34	23	0.45		

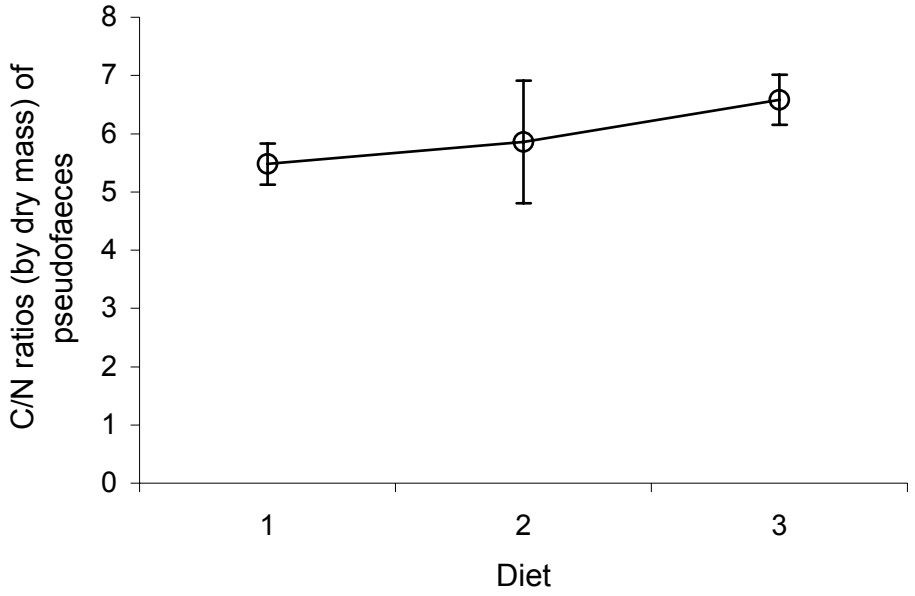

Fig. 3-c: C/N: organic carbon/nitrogen ratios of pseudofaeces of *L. elliptica* (by dry mass. Mean, ± SD); diets: 1, 2, 3

Table V: refer to Table III for references.

Source	S.S.	Df	MS	F	P
Total	174.39	25			
Diet	3.48	2	1.74	2.88	0.0768
Error	13.91	23	0.6		

Laternula elliptica may play an important role in the sedimentation process of both inorganic and organic material at Potter Cove (Ahn, 1993; Momo et al., 2002). Although the relative contents of POC and PON were low, the biodeposited material as well as the total amount of POC and PON deposited by the bivalve increased with seston load, where the activity of the smaller clams was outstanding (Fig. 1, Fig. 2). With a high nutritional/energetic value, the deposited matter in all the studied conditions may serve as a food source for the associated benthic fauna, like the deposit feeders at Potter Cove (Figs. 3-b, 3-c). However, the possible sources of the total organic matter transferred to the sea bed by the clam, especially during stressful conditions, may not be clear at present. Pseudofaeces production (higher with increased inorganic material load) involve the usage of mucus produced by the bivalve, to put together particles rejected from the mantle cavity. Moreover, in such conditions a high

amount of energy may be required to clean the filtering apparatus of the clogging material and to select food particles efficiently. Although the possible nourishing role of the bivalve may be assured and also unaffected by changes in the seston load, the impact of prolonged stressful conditions on the energy budget of the bivalve is uncertain. Is *L. elliptica* negatively influenced by high sediment load conditions? To what extent? Are the smaller clams prone to be especially affected?

To assess the ecological relevance of these concepts, they could be linked to the occurrence of natural events, especially like the massive input of terrigenous sediment through coastal meltwater streams, and the strong particle resuspension by the physical forcing factors of the water column. Both events take place during the austral summer.

Studies within the ClicOPEN Project (Impact of climate induced glacial melting on marine and terrestrial coastal communities on a gradient along the Western Peninsula) will provide further information to asses the effect of climate change-induced processes on Antarctic coastal benthic ecosystems.

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Role of benthic filter feeders in pelagic-benthic coupling: assimilation, biodeposition and particle flux

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Introduction

Potter Cove is characterized by two clearly distinct areas, the outer cove with high benthic primary production and the inner cove with low primary but high secondary benthic production. The substrate of the outer cove is comprised of solid rocks and big boulders which support a dense canopy of phaeophyte macroalgae down to 25 m depth (Klöser et al., 1994). The inner cove is dominated by sessile macrofaunal assemblages on a muddy substrate (Sahade et al., 1998). A rich filter-feeding community occupies this area, in spite of what appears to be unfavorable conditions for their their feeding activities. These conditions include high sedimentation and low pelagic primary production. In Phlebobranchiate ascidians, high suspended inorganic particles reduce the amount of food assimilated by limiting the intake of utilizable food by a process of dilution and/or reducing the efficiency of assimilation of such food (Robbins, 1985). In summer, creeks formed by melting ice transport large amounts of inorganic material from the surrounding land into the cove, resulting in high sedimentation rates of about 18-30 g m^{-2} day^{-1} in the inner cove, (Ferreyra et al., 2003). In Potter Cove, the sediment load in the water column is frequently enhanced in the summer by resuspension of the fine bottom material, when the sea surface is ice-free and winds result in turbulent mixing that affects the bottom to 30 m depth (Schloss et al., 1999). The suspended sediment then affects the penetration of light into the water column which together with the deep vertical mixing explains the low phytoplankton biomass within inner Potter Cove (Schloss and Ferreyra, 2002). Water-column analyses from Potter Cove suggested that some organic material in the deposited sediments is released from the water-bottom interface even during winter (Kowalke, 1999; Tatián et al., 2002). Although scarce, this material seems to be the food source for benthic organisms, especially long-lived species that undergo growth and reproduction during winter (Sahade et al., 2004). The origin and composition of this material is, nevertheless, not completely understood. One of the most reasonable hypotheses is that part of this material may be macroalgal debris that originated in the outer cove, which is then continuously carried into the inner cove by clockwise water currents (Ferreyra et al., 2003). This material is sometimes

easy to observe as little brown-greenish particles floating on the sea surface and a thick bed of brown material that is patchily present on the bottom.

Filter feeding animals can play a significant control on phytoplankton biomass, coupling the pelagic and benthic systems. The uptake of phytoplankton and particulate organic matter from the water column, followed by biodeposition by filter feeding animals is known to be one of the major trophic pathways in marine ecosystems (Kowalke, 1999; Ahn, 1993). In phytoplankton-impoverished coastal waters, as Potter Cove, the role of benthic suspension feeders is important, since their fecal depositions can nourish the benthic fauna.

The focus of this study was to measure assimilation efficiencies of natural and provided seston (powdered macroalgae) by a series of experiments performed with one bivalve and two ascidian species. The aim was to reveal insights into the possible response of filter feeders to a food source other than phytoplankton. The mass-specific deposition rate was measured, as well as the C:N composition of deposits (feces) should make it possible to determine the contribution of suspension feeders to the particle flux near muddy bottoms in Potter Cove.

Material and Methods

Experiments were carried out in aquaria disposed in a cold and wet room, at the Dallmann laboratory. Sea water was first pumped from the cove (1-3 m depth) into 2.500 l tanks were the temperature was maintained at 0º C. Seawater passed through filters to remove coarse suspended material before filling the experimental aquaria. Diets were natural seston (particles suspended in running seawater) and powders made from kelp fragments and inorganic material (precombusted diatomaceous earth) suspended in filtered sea water. The kelps *Desmarestia mensiezii* and *D. anceps* were collected prior to the treatments in the intertidal of the outer cove. The kelps were washed in freshwater to remove the salts, and a portion of the material was then lyophilized for 48 hs. The dried material was ground to powder and sieved through 50, 125 and 250 µm mesh. Diatomaceous earth was ashed (5 h 450º C) to eliminate residuals of organic matter and sieved as the kelp material. Different diets were composed by particle size and macroalgae/diatomaceous earth concentrations. These diets were stored at -20º C. Test filter feeders used were the clam *Laternula elliptica* (King and Broderip, 1831) and the ascidians *Cnemidocarpa verrucosa* (Lesson, 1830) and *Pyura setosa* (Sluiter, 1905).

Laternula elliptica: experiments were carried out during February and March 2002. Animals were collected at 10 m depth by SCUBA diving and immediately transported to the laboratory. Bivalves were cleaned of fouling organisms and debris and kept for several days in aquaria for acclimatization (open system with running sea water, at 1 $\pm$ 1ºC). Seven specimens were placed in individual 2 l - PVC aquaria and nourished near the oral siphon using a Pasteur pipette with a solution made by yeast and carmine-red in sea water. The time until the production of red feces (gut residence time, GRT) was recorded. Seven specimens were placed in individual 1 l - PVC flasks and two pebbles instead of bivalves (controls), putted randomly on the bottom of a 90 l aquarium, a close system filled with 0.45 µm filtered sea water. Animals were starved in the experimental aquarium for two days. Water was changed at the beginning of each experiment. During three days, animals were fed on different diets (Table I) with a suspension of 4 mg l^{-1} (initial concentration). Water in the

aquarium was stirred using airstones and resuspension pumps to keep particles in suspension. Every 24 h, triplicate water samples and deposits were filtered onto precombusted and preweighed GF/F filters. Suspended particulate matter, particulate organic matter (POM) and particulate inorganic matter were measured gravimetrically. POM was calculated after combustion of filters (5 hs at 450° C). Assimilation efficiency (AE) was calculated using the ratio of Conover (Navarro and Thompson, 1994) which assumes that an animal can digest and absorb the organic component of the food, but not the inorganic fraction. The organic matter is calculated as the percentage of mass loss after combustion of water samples, which is compared with the corresponding percentage of mass loss after combustion of feces. According to Ahn (1993), *L. elliptica* produces feces and pseudofeces. As it was not possible to continuously follow the deposition activity, a parallel experiment was used, collecting pseudofeces by a Pasteur pipette for correction purposes.

Cnemidocarpa verrucosa and *Pyura setosa*: experiments were performed from January to March 2004; the animals were collected by SCUBA diving at 20 m depth, cleaned of debris and fouling organisms and washed under flowing seawater. Acclimatization of the animals was allowed in an aquarium system similar to that used with *L. elliptica*. Gut residence time (GRT) experiments were performed accordingly. A total of six specimens of *C. verrucosa* and five specimens of *P. setosa* were placed in individual flasks on the bottom of two 90 l aquaria, as well as two controls. The first experiment was performed by feeding animals with natural seston (running sea water) in an open system. The following experiments were run in a closed system filled with filtered (0.2 µm) sea water. During five days, animals were fed on diets prepared with macroalgae and precombusted diatomaceous earth (aprox. 4 mg l^{-1} of seston). Dietary composition for each feeding condition is showed in Table I.

Table I: Diet composition of the different experiments performed to estimate assimilation efficiency (AE).

Species	Diet	Composition	Size particles
Laternula elliptica	1	Particulate macroalgae (60%), diatomaceous earth (40%)	< 50 µm
	2	Particulate macroalgae (30%), diatomaceous earth (70%)	< 50 µm
	3	Particulate macroalgae (60%), diatomaceous earth (40%)	< 125 µm
	4	Particulate macroalgae (60%), diatomaceous earth (40%)	< 250 µm
Cnemidocarpa verrucosa and *Pyura setosa*	5	running seawater, natural seston	
	6	Particulate macroalgae (25%), diatomaceous earth (75%)	< 50 µm
	7	Particulate macroalgae (13%), diatomaceous earth (87%)	< 50 µm
	8	Particulate macroalgae (25%), diatomaceous earth (75%)	< 125 µm

To estimate the assimilation efficiency (AE), determinations of total matter (organic and inorganic) were performed from triplicate filtered water and feces samples, dried (60º C, 24 hs), ashed (450º C, 5hs) and weighed filters and deposits. Feces were taken using a Pasteur pipette. Organic carbon and organic nitrogen were both determined from feces deposited after feeding Diet 5 using a Carlo-Erba NA-1500 analyzer, after removing calcium carbonate with ClH, to estimate the quality of the feces (C:N ratio). Once experiments were terminated, animals were sacrificed and the tunic dissected. The rest of the ascidian was dried (60º C, 24 h) before mass determinations (gDM). The biodeposition rate (BR) is calculated as mg DM of feces produced per individual per d^{-1}. Mass specific biodeposition rate (MSBR) as mg DM of feces produced per gDM^{-1} of animals per d^{-1}. Data on mean mass (gDM) and density (ind m^{-2}) of *C. verrucosa* and *P. setosa* at different sites of Potter Cove (E1: southern inner cove, muddy bottoms; E2: outer cove, hard substrates; E3: northern inner cove, moraine deposits) were used (Sahade, 1999; Kowalke, 1999, Fig. 1) to estimate biomass per square metter. This information made it possible to estimate total particle flux, and fluxes of particulate organic carbon (POC) and particulate organic nitrogen (PON) by deposits produced by both ascidians in these areas.

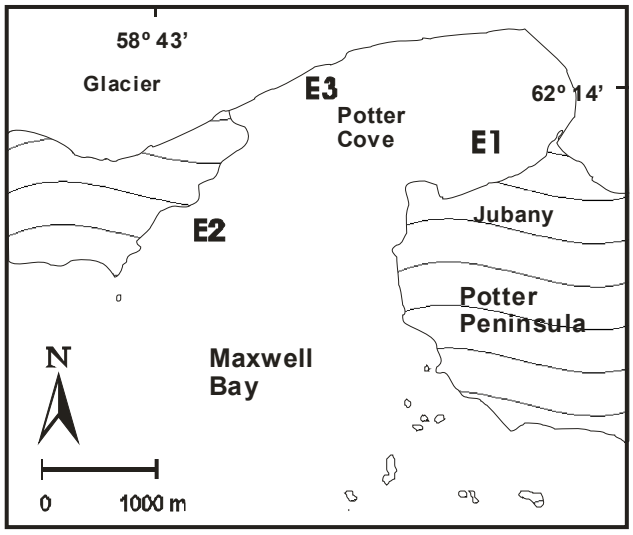

Fig. 1: Map of Potter Cove. E1: sampling site. E1, E2, E3: sites with available data on ascidian abundance.

Results

All *L. elliptica* specimens produced red feces 15-19 h after they were fed with the yeast-carmine solution. On the other hand GRT for the ascidians was difficult to estimate, because specimens commonly rejected the carmine solution or produced red feces through a very wide time range (between 20 to 90 h). Samples taken from the controls not revealed a significant sedimentation of particles during the experimental time. All diets were assimilated from the three test species: mean of the AE varied between 26-50 % in the case of *L. elliptica*, and 26-72% for ascidians, reaching higher AE when natural seston was sup-

plied (Fig 2 A, B). Differences between diets were not significant in *L. elliptica* (ANOVA F = 1,38, n = 28, *p* = 0.27), *C. verrucosa* (ANOVA F = 2.01, n = 23, *p* = 0.14) and *P. setosa* (ANOVA F = 2.65, n = 20, *p* = 0.08). The latter species was the less influenced by the sediment condition: highest AE was calculated under high amounts of inorganic material (Diet 7). This species did not decrease the assimilation efficiency in the presence of large particle sizes (Diet 8), but AE were lowered under these conditions in *L. elliptica* (Diet 4) and *C. verrucosa* (Diet 8).

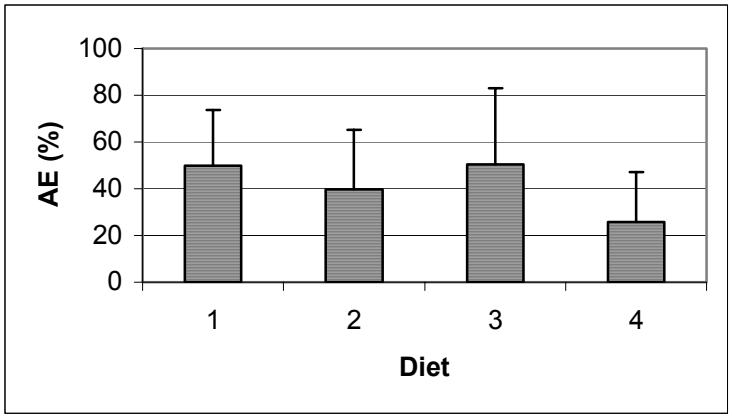

Fig. 2A: Assimilation efficiency (mean ± SD) with different diets: *Laternula elliptica.*

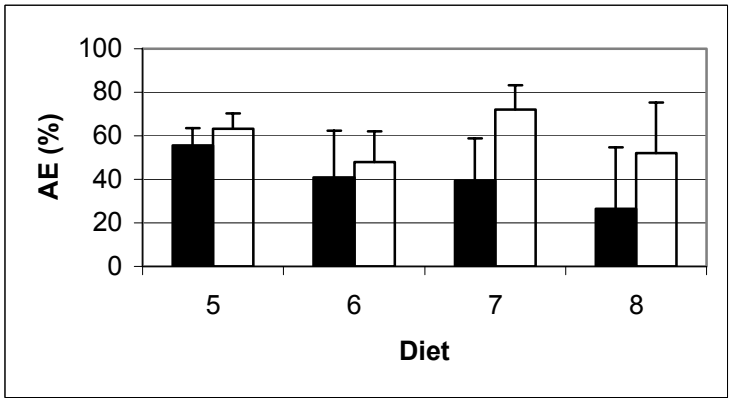

Fig. 2B: Assimilation efficiency (mean ± SD) with different diets: *Cnemidocarpa verrucosa* (black bars) and *Pyura setosa* (white bars)**.**

Estimated BR and MSBR while the organisms were feeding on natural seston was also higher in *C. verrucosa* than in *P. setosa* (Table II). Thus, the particle, POC and PON fluxes vary according to species, density and biomass (Table III).

Table II: *Cnemidocarpa verrucosa* and *Pyura setosa.* Deposition rate and mass specific deposition rate under running seawater (natural seston).

	Cnemidocarpa verrucosa n = 6	*Pyura setosa* n = 5
Biodeposition rate (BR) mg specimen^{-1} d^{-1}	10.9 ± 2.6	5.4 ± 0.3
Mass specific biodeposition rate (MSBR) mg gDM^{-1} d^{-1}	6.4 ± 2.7	3.8 ± 2.2

Table III: Estimated particle, PON and POC fluxes by *Cnemidocarpa verrucosa* and *Pyura setosa* at different areas in Potter Cove, 30 m.

	Cnemidocarpa verrucosa			*Pyura setosa*		
	E1	**E2**	**E3**	**E1**	**E2**	**E3**
Animal density (ind m^{-2})	1.8	1.9	3.6	0.9	-	0.1
Animal biomass (gDM m^{-2})	3.5	3.7	7	1	-	0.12
Total particle flux (mgDM m^{-2} d^{-1})	22.5	23.7	44.9	3.9	-	0.44
POC flux (mg m^{-2} d^{-1})	1.03	1.09	2.07	0.09	-	0.01
PON flux (mg m^{-2} d^{-1})	0.11	0.12	0.22	0.01	-	0.001

Discussion

Estimations of GRT depended on animal behavior and stress conditions. Clams fed, however, despite acclimatization time while ascidians did not. Ascidians are generally very sensitive to water movements, closing their siphons when disturbed. To estimate AE, GRT was assumed to be approximately 24 h as was previously stated for other ascidian species (Klumpp, 1984; Robbins, 1985). In the ascidian *Pyura stolonifera*, feeding depends on seston quality: squirting produces rejection of particles under high inorganic loads (25 mg l^{-1}, 3 % POM) and large particle size, > 65 µm (Klumpp 1984). According to Armsworthy et al. (2001), AE decreased significantly from 46% to 23 % when adding up to 46 mg l^{-1} sediment to the natural seston. High concentrations of particles can lower AE; however, such high concentration were not measured in Potter Cove, where particulate inorganic matter close to the bottom, at 30 m was an average 3 mg l^{-1} during a year-round period study (Tatián et al., 2002). Nevertheless, we need to emphasize that water samples analysed in this study were taken under calm weather conditions, higher values are possible during

storms. As an adaptation to inorganic sedimentation, in the case of the species *C. verrucosa*, *Molgula pedunculata*, *C. eumyota* and *Ascidia challengeri* from Potter Cove, less efficient retention and low pumping rates decrease the risk of filtering structures being clogged. Nevertheless, it remains unclear to which degree heavy particle loads lower production (Kowalke, 1999). The supplied inorganic particles did not decreased significantly the AE in the three species studied. Besides, abundance of these species suggests that impact of sedimentation is still low or present species can cope well with the actual sedimentation rates. Under low seston concentration (approximately 4 mg l^{-1} in all diets) our results show that differences in the AE depend on the diet, species and even individuals. In the case of *L. elliptica*, no significant differences in AE was observed owing to inorganic percentage nor in particle size up to 125 µm but, a decrease of the mean AE was measured when food particles in the provided diet were larger than 250 µm in size. *Cnemidocarpa verrucosa* appeared to be more affected by inorganic particles and particle size, showing a decrease in AE from natural seston (Diet 5) to diets enhanced with inorganic material (Diets 6, 7, 8) and from smaller to higher particle size. This was not the case in *P. setosa* that showed higher AE under powdered kelp suspensions and higher inorganic percentages in the diet. In the Arctic bivalve *Hiatella arctica,* AE decreased under increased food levels, from 90% to 32% (Sejr et al., 2004). Similarly *L. elliptica* produces fecal pellets with higher organic carbon and chlorophyll a/phaeopigment ratios when increased chlorophyll a concentration in the seawater (Ahn, 1997). This indicates an ineffective AE under higher water quality as this bivalve feeds in excess of its needs. AE varies according to diets: monoculture was reported to be efficiently (up to 92%) assimilated by ascidians (Fiala-Médioni, 1974). Other particles, i.e. natural seston were also efficiently assimilated: Tito de Morais and Fiala-Médioni (1985) estimated up to 95%. Although with less efficiency (up to 42%), kelp detritus was also reported to be assimilated by the ascidian *P. stolonifera* (Klumpp, 1984).

Antarctic sessile suspension-feeders consume a broad spectrum of the seston fraction in an opportunistic strategy, ingesting particles in proportion to their availability (Gili et al., 2001). Since filter-feeders are non selective, the use of macroalgal debris as food depends on their capacity for retention and assimilation of these particles. Minimum particle size for maximal retention efficiencies measured in four solitary ascidian species from Potter Cove, is 2 – 6.5 µm (Kowalke, 1999), but no data are available for maximal particle size. Gut content analyses carried out from the ascidians *C. verrucosa* and *Corella eumyota* immediately fixed after sampling revealed the presence of recognizable macroalgal debris of up to 500 µm in size (Tatián et al., 2004; this volume). Although with less food value than fresh material, decaying kelp detritus as part of the bacterial loop can be a potential food source (Tenore et al., 1982; Albertelli et al., 1998). Bacteria represent a nitrogen source of comparable importance to phytoplankton (Seiderer and Newell, 1985). Macroalgal diets were assimilated at least at 25% efficiency by the species studied: thus, suspension-feeding communities may use kelp detritus and the associated bacteria as alternative food source in this impoverished phytoplankton environment. Fatty acid studies performed in water column and sediment samples at Potter Cove (Graeve et al., this volume) revealed high amounts of detritus and low contributions of phytoplankton from the water column but in sediment samples, fatty acids were typical for bacteria and their degradation products.

Filter-feeding is successful within inner Potter Cove, as indicated by high abundance of clams and ascidians (Sahade et al., 1998). Abundance and capacity of these species to filter water samples seems to be important in pelagic-benthic coupling. The biodeposits (feces and pseudofeces) enhance particle flux nourishing the associated benthic fauna. BR estimated for *L elliptica* at the close Marian Cove, in King George Island was 0.26 - 2.17 mgDM gWM^{-1} d^{-1} (Ahn, 1993). BR was higher in *C. verrucosa* than in *P. setosa*. The higher abundance and biomass of the former species at different sites of Potter Cove (Sahade et al., 1998; Kowalke, 1999) suggests that *C. verrucosa* has an important role in the benthic community and in the particle flux. Particularly interesting are the POC and PON percentages measured in the feces of ascidian species: the C:N ratio is higher in comparison to filter-feeding bivalves (Table IV).

Table IV: POC, PON and C:N in feces in different suspensivores under running seawater (natural seston).

		POC (%)	PON (%)	C:N	Reference
Crassostrea virginica	Bivalve	18.1	3.4	5.3	Frankenberg & Smith, 1967 (cit. Ahn, 1993)
Geukensio demissa	Bivalve	2.4	0.35	6.9	Kraeuter, 1976 (cit. Ahn, 1993)
Laternula elliptica	Bivalve	5.68 ± 3.54	1.04 ± 0.72	6.0 ± 1.1	Ahn, 1993
				5 - 7	Mercuri et al., this volume
Adamussium colbecki	Bivalve	-	-	5.13	Chiantore et al., 1998
Halocynthia pyriformis	Ascidian	3.7 ± 0.7	0.3 ± 0,05	13.6 ± 1.1	Tatián et al., 2003
Styela rustica	Ascidian	9.1 ± 2.6	1.3 ± 0.4	8 ± 0.7	Tatián et al., 2003
Cnemidocarpa verrucosa	Ascidian	4.6 ± 1.3	0.5 ± 0.1	9.7 ± 1.97	This study
Pyura setosa	Ascidian	2.3 ± 1.2	0.3 ± 0.1	7.9 ± 2.1	This study

Taking into account that the bivalve *L elliptica* ceases feeding during the four winter months (Brockington, 2001), the continuous filtering activity of ascidians may be important, because implies the permanent feces production. This continuous particle flux from the animals to the system is especially important during low periods of primary production and when the water column is stable, lowering events that make particles available (i.e. advection, sedimentation and resuspension).

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Contribution of different seston components to ascidian food in Potter Cove

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Introduction

Benthic suspension-feeders typically feed on phytoplankton and consequently play an important role in the coupling of pelagic and benthic systems. In Potter Cove, suspension-feeders reach a high biomass and some taxa, particularly ascidians, demonstrate one of the highest species richness values reported in coastal Antarctic areas (Sahade et al., 1998). This is unexpected because of the scarcity of fresh phytoplankton (Schloss and Ferreyra, 2002) and the high input of inorganic material within inner Potter Cove (Schloss et al., 1999). Particles are also frequently resuspended from the fine muddy sediments that characterize the bottom. According to Robbins (1985) ascidians cannot survive in environments affected by heavy inorganic sedimentation since the particles increase the energy required to extract food items and also clog the filter system (branchial sac and associated mucous layer). Surprisingly, studies performed with sediment traps in the inner cove revealed a relatively high percentage of organic particles in the water column-bottom layer, at 25-50 cm from the bottom (Schloss et al., 1999). This suggests the presence of food and potential differences in the capability to retain particles from the water column in the area according to body length and distance in relation to the bottom. In order to obtain insights about the origin and composition of food items in the benthic filter-feeding community through the year, a number of ascidians were selected for our investigation. Monthly gut content analysis of the erect species *Cnemidocarpa verrucosa* (Lesson, 1830) in Potter Cove, revealed organic material decreasing along the gut. This suggested an intake and assimilation of organic particles throughout the year (Tatián et al., 2002). In contrast, microscopic analyses carried out in these gut contents revealed different degrees of stomach repletion (fullness expresed as percentage of the total stomach volume). A wide variety of particles mainly composed of detritus and particles less than 5 µm in size were found (Tatián et al., 2004). The ascidian *Corella eumyota* Traustedt, 1882 is one of the dominant macrobenthic species within inner Potter Cove, and can reach an abundance of 2.2 individuals m^{-2} (Kowalke, 1999). Despite its large size (up to 20 cm in length) it shows a depressed shape or "a body more or less compressed laterally" (Van Name, 1945) and it is attached to the bottom by its ventral side. It is commonly covered by mud with the siphons close to the bottom surface. This ascidian was selected for microscopical gut contents analysis to determine its diet throughout the year. Comparison between the erect *C. verrucosa* and the depressed *C. eumyota* will

help us to understand the structure of the diverse ascidian community that is affected by short-term changes characteristic of Antarctic-coastal ecosystems (Tatián et al., 2002).

Materials and Methods

Sampling

Five individuals of *Corella eumyota* were collected monthly between March 1996 and April 1997. Specimens were taken by SCUBA at 30 m within inner Potter Cove (E1, at the southern coast see Tatián et al., this volume) on muddy bottom. Immediately after sampling, the entire gut was removed, fixed and stored in 2.5% formaldehyde made up with filtered sea water. The stomach repletion was calculated as percentage by:

R = (volume of stomach contents/length of stomach)*100

Determination of volume was carried out by the displacement of a formaldehyde solution in a graduated test tube after the addition of the stomach contents.

Microscopic observations

Gut contents were stained with Bengal rose and stirred. Quantitative analyses were performed under a light microscope (up to 1600 magnification), through a Neubauer counting chamber (haematocytometer, 0.1 mm deep). Particles were recorded in the central grid of the chamber (1mm^{-2} area) and counted whenever they were present at one of the crosses of the grid (a total of 441 points). The particles comprising a high density and variety in terms of shape and size, represented the food items of an unknown volume of water filtered; therefore, the records only provide information on the relative abundance of the different particles in the stomach. Relating the monthly relative abundance of each component to the stomach repletion, we estimated the absolute amount of components per stomach throughout the year. This helped to determine if there is a real or only an apparent seasonality of particles in the gut. The origin of particles fed by ascidians (from the pelagic or benthic systems) during the year-round period was determined counting diatoms, discriminating between pelagic and benthic ones (Frenguelli and Orlando, 1958; Medlin and Priddle, 1990; Klöser et al., 1994; Klöser, 1998; Ahn, 1997). Sub samples were also placed in the Neubauer chamber and counted in ten random fields (total area, 6.75 mm^{-2}). All observations were performed using a light microscope equipped with an ocular micrometer to measure the size of the particles.

Results and Discussion

A marked seasonality in the repletion percentage of *Corella eumyota* was observed (Fig. 1). Highest values were calculated in January 1997. August was the month with the lowest stomach repletion. High values were also observed in April 1996 and 1997. This agrees with a second peak of primary production commonly registered in late March (Schloss et al., this volume), when ice-melting and sediment input into the cove diminish. Results for *C. ver-*

rucosa indicate that the ascidians find more food in summer (November) when phytoplankton growth starts (Fig. 1; Tatián et al., 2004). These stomach repletion were not only coupled with the seasonal cycles of primary production, but also with the year-round dynamics of the water column. Turbidity and mixing of the water layers by strong winds are only possible during ice-free months, while pack ice reduces the input and resuspension of particles during winter (Schloss et al., 1999). Comparing both species, a higher repletion was calculated for *C. eumyota* (Fig. 1). The gut contents were composed mainly by inorganic material especially during summer: this suggests that this species has a higher filtering effort to get food particles compared to the erect *C. verrucosa.* Inorganic particulate suspensions reduce the amount of food assimilated, limiting the intake of utilizable food by a process of dilution, having an adverse effect on growth and viability (Robbins, 1985). Nevertheless, mean repletion for *C. eumyota* was never higher than 60%, which reveals a higher potential for particle intake. Levels of sedimentation in Potter Cove are not to high to affect assimilation of food by ascidians (Tatián et al., 2002; this volume).

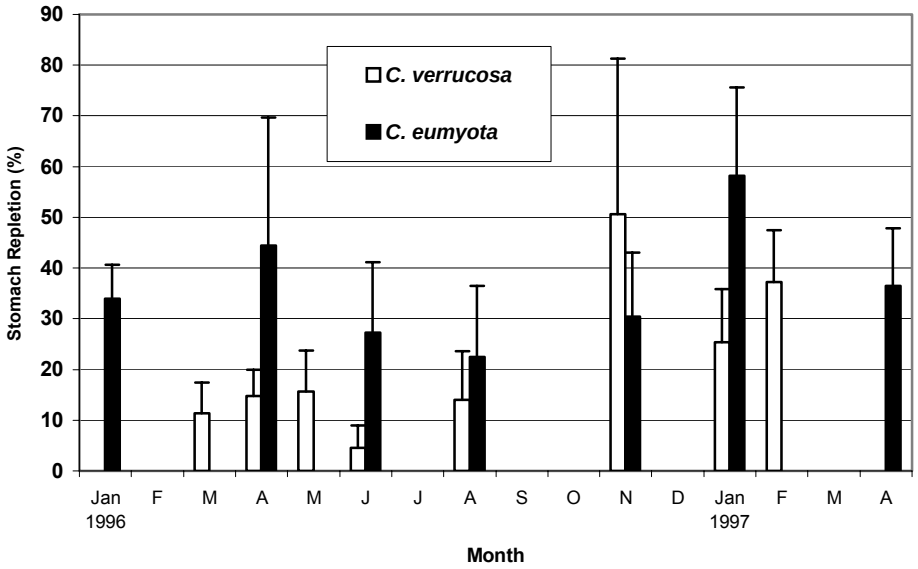

Fig. 1. *Cnemidocarpa verrucosa* and *Corella eumyota.* Stomach repletion percentage calculated during the studied period (mean ± SD). Lack of data in some months are due to bad weather conditions.

A wide range of particle-sizes was observed in the stomach contents. The largest particles were green and up to 0.5 mm in diameter (macroalgal debris) and dense aggregated brown measuring up to 1.3 mm (fecal pellets). Particles < 5 µm in size were difficult to recognize, but probably consisted of fine inorganic and minute organic material. Detrital material and fine particles constituted the main percentage of particles counted throughout the year (Fig 2). In both species microalgae (diatoms), reached highest densities in November (in *C. eumyota* up to 46% of gut contents). Scarce diatom frustules were observed during the winter months. Inorganic particles (sand) were relatively abundant in the stomachs of *C. eumyota* compared to *C. verrucosa* (Fig 2 A, B).

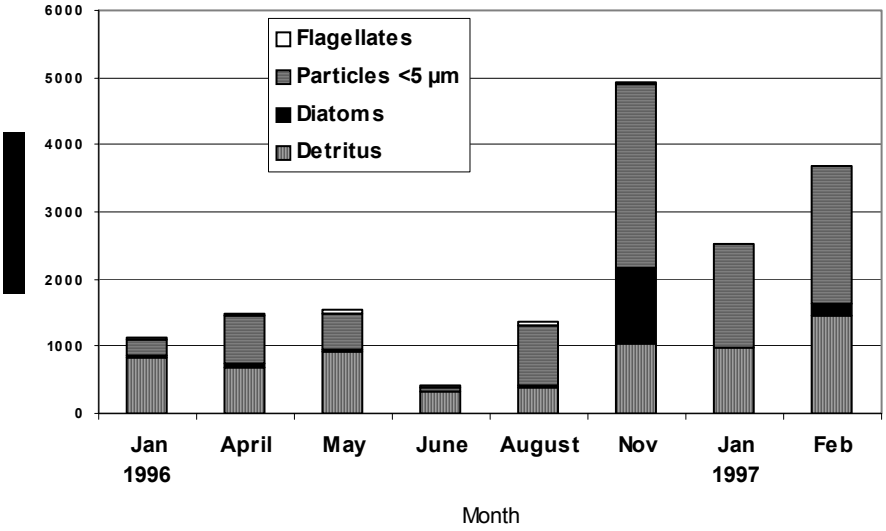

Fig. 2A. Estimated absolute numbers of components in the stomach content during the study period in *Cnemidocarpa verrucosa*

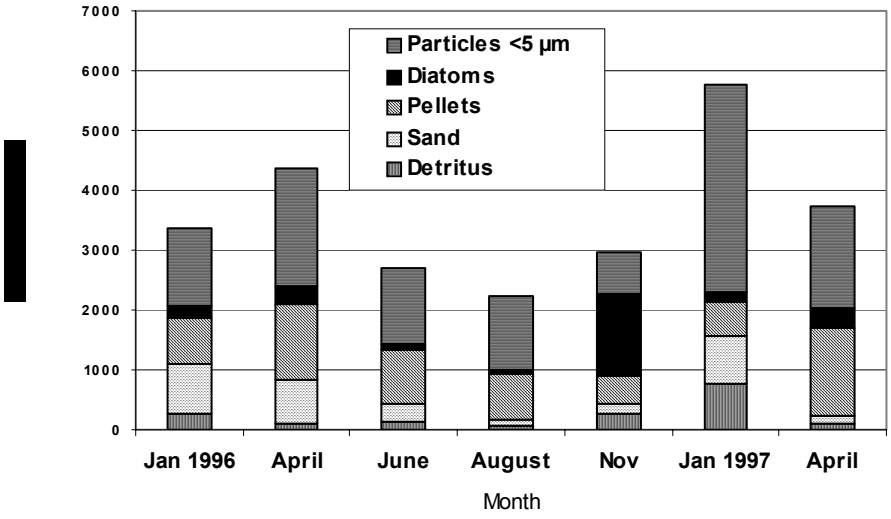

Fig. 2B. Estimated absolute numbers of components in the stomach content during the study period in *Corella eumyota*

Macroalgal debris was frequently observed floating on the water surface, and even dispersed on the bottom as patches (Tatián, personal observation). Although the south coast of Potter Cove, characterized by muddy sediments is not colonized by macroalgae, dense kelp beds dominate the rocky bottom of the outer cove and the north coast which is characterized by moraine deposits. Thus, the algal detritus in Potter Cove is apparently transported from these

areas by lateral advection by frequent clockwise currents, even during pack-ice months (Roese and Drabble, 1998). High benthic faunal stocks in empoverished primary production environments can survive owing to POC advected from highly productive areas (Feder et al., 2005). Zooplankton and even benthic organisms produce fecal pellets, that enhance the carbon flux to the bottom. Suspension feeders can use macrophytobenthic detritus (Charles et al., 1996) and may ingest fecal pellets (Ahn 1993). The detritus can be converted by microbial activity into material available to macroconsumers and the associated bacteria can also serve as food (Tenore et al., 1982). The specific mass of macroalgal detritus and fecal pellets should be different, since they were differently represented in both ascidians: while the erect shaped *C. verrucosa* exhibited higher amounts of macroalgal detritus, fecal pellets were rare in their stomach contents being abundant in the depressed shaped *C. eumyota*. Pelagic diatoms generally dominated the gut contents of both ascidians (Table I). The diatoms *Corethron criophilum and Thalassiossira* spp. were the most frequent species observed in the guts during the study period. Benthic diatoms (*Licmophora* spp., *Navicula* spp., *Gyrosigma* spp. and *Cocconeis* spp.) were present throughout the year but mostly during summer. Benthic diatoms are available by resuspension. Pelagic diatoms and fecal material seemed to be available to the ascidians mainly by sedimentation through the water column but also by resuspension. Again, resuspension seems to be more important during summer when winds strongly mix the water column, as supported by the higher amount of benthic diatoms in gut contents of *C. verrucosa* from January and February 1997 (Table I).

Table I. *Cnemidocarpa verrucosa and Corella eumyota.* Relative abundance of pelagic and benthic diatoms in the stomach contents through the studied period ("-" indicates lack of data because bad weather conditions).

	Cnemidocarpa verrucosa		*Corella eumyota*	
	Pelagic (%)	Benthic (%)	Pelagic (%)	Benthic (%)
January 1996	-	-	51	49
February	-	-	-	-
March	58	42	-	-
April	55	45	65	35
May	46	54	-	-
June	96	4	50	50
July	-	-	-	-
August	56	44	48	52
September	-	-	-	-
October	-	-	-	-
November	51	49	70	30
December	-	-	-	-
January 1997	34	66	64	36
February	41	59	-	-
March	-	-	-	-
April	-	-	78	22

Conclusions

The particle dynamics of the water-column bottom layer within Potter Cove are important to suspension feeders at different distances from the bottom. Organic particles (macroalgal detritus) with a lower specific mass than inorganic particles (sand and part of the fecal pellets), will be suspended for a longer period of time and be present at a greater distance from the bottom. Therefore, these particles are mainly available for erected fauna throughout the year. On the other hand, some depressed shape ascidians as *C. eumyota* appear to be adapted to withstand the inorganic particles from the intense sedimentation. This study revealed that both species retain a wide range of particle sizes and quality (< 5 µm to 1,3 mm in length and from fresh to partially decomposed material and inorganic particles) depending on their availability. These capabilities allow ascidians in Potter Cove to survive periods with low primary production and high sedimentation. Horizontal transport (macroalgal detritus), sedimentation (fecal pellets and phytoplankton) and bottom resuspension (benthic microalgae and other particles settled on the bottom) are important processes which supply these particles to benthic filter feeders.

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Metazoan meiofauna in Potter Cove, King George Island

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Introduction

Shallow water meiofauna has scarcely been studied for Potter Cove, so far. There are studies on metazoan meiofauna higher taxa and Foraminifera by Mayer (2000) and on benthic Harpacticoida by Veit-Köhler (2005). For King George Island only two more data sets are known from Martel Inlet (de Skowronski and Corbisier, 2002) and Admiralty Bay (de Skowronsky et al., 1998).
It was the aim of this study to assess small- and meso-scale heterogeneity of meiofauna densites on soft-bottom sediments in Potter Cove. A comparison with the data from other antarctic shallow water sites and a discussion whether usually applied sampling efforts induce reliable generalisations on meiofauna communities are presented.

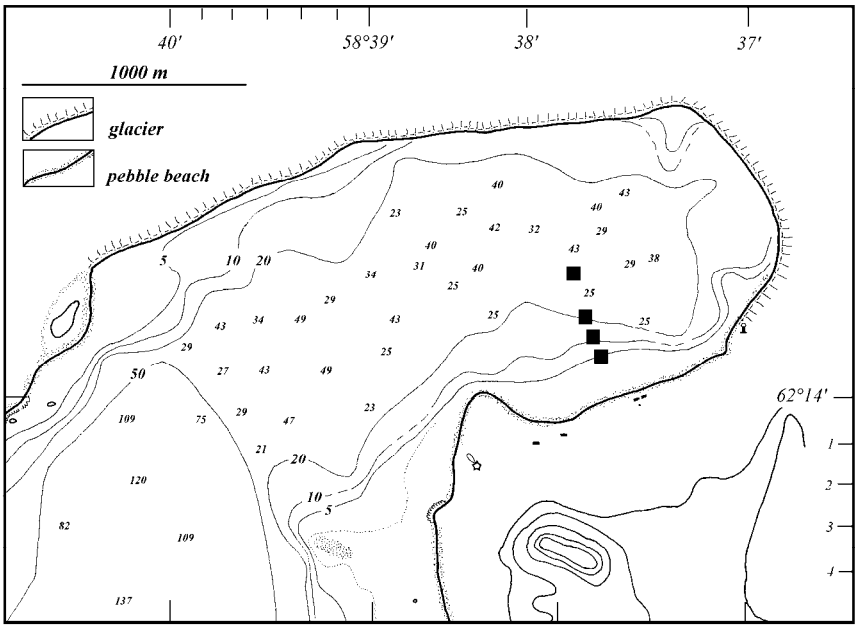

Fig. 1: Location of the depth transect (5, 10, 20, and 30 m) in the soft-bottom habitat of Potter Cove, King George Island

Material and Methods

Study site and sampling
Potter Cove (62°14'S, 58°40'W) is a small inlet connected to Maxwell Bay at King George Island, South Shetland Islands, Antarctica. Klöser and Arntz (1994) gave a detailed description of the site. A depth transect (5, 10, 20, and 30 m) was sampled during the antarctic summer (January – March 2004). The transect started rectangularly from the German Dallmann laboratory at the Argentinian base Jubany and represents the protected area of the inner cove sheltered by a shallow ridge at its entrance. Muddy sediments predominate.

Meiofauna samples were collected by scuba diving. At each station six sediment corers (6 cm inner diameter) were randomly pushed into the sediment and brought to the surface as undisturbed as possible. The upper 5 cm of oxidized sediment layer were preserved in 5% buffered formalin.

Meiofauna treatment
The fixed samples were washed with tap water through a 40 µm mesh sieve. Meiofauna and organic material were extracted from remaining particles by centrifugation with a colloidal silica polymer (H. C. Stark, Levasil 200/40%, ρ = 1.17) as flotation medium and kaolin to cover heavier particles (McIntyre and Warwick, 1984). Centrifugation was repeated three times at 4000 rpm for five minutes respectively. After each centrifugation the floating matter was decanted and rinsed with tap water. This supernatant contained all organic material which was thereafter stained with Bengal Rose before manually sorting to higher taxon level using a Leica MZ 12.5 stereo microscope. The individual numbers were counted and abundance values taken for further analyses and interpretation.

Statistical analysis
As the data were not distributed normally, no means and standard deviations could be calculated for the different treatments. Multivariate meiofauna community analyses were carried out on abundance data of the major taxa recorded using the PRIMER v6 package (Clarke and Gorley, 2006). Prior to analysis the data were square root transformed. Bray-Curtis similarity was calculated and samples were ordinated and classified (Non-metric multi-dimensional scaling: MDS) using group average linkage. Analysis of similarity (ANOSIM) was carried out for the factor “depth”.

Results

Altogether 18 higher metazoan taxa of the meiofauna size class (< 1 mm; > 40 µm) have been found in the samples from Potter Cove. Nematoda were numerically dominant followed by Copepoda and copepod nauplii, Annelida, and Cumacea. Priapulida and Gastrotricha reached appreciable individual numbers only at 20 m depth (Tab. 1). Several taxa were found only in very low abundances and only in some samples: Loricifera (5 m depth), Rotifera (every depth), Tardigrada (20 and 30 m), and Daphnia (5 m). Therefore they are not represented by the median values given in Table 1.

Table 1: Median single values of the meiofauna higher taxa, and median, minimum and maximum values of single cores (individual numbers per 10 cm^{-2}) found along a soft-bottom depth transect in Potter Cove, King George Island.

Ind. 10 cm^{-2}	5 m	10 m	20 m	30 m
Nematoda	2074.7	2328.3	8617.2	3004.7
Copepoda	28.3	18.9	98.7	50.8
Nauplii	20	18.7	341.1	101
Cumacea	5.3	10.6	1.8	2.7
Isopoda	1.1	0	0	1.4
Amphipoda	1.1	0.4	2.3	2.3
Tanaidacea	0	0	0.4	0
Ostracoda	4.4	1.2	2.1	7.4
Acari	0	0	0.2	0
Annelida	9.4	28.3	27.2	9.6
Priapulida	0.4	0.2	10.1	0.4
Kinorhyncha	0.2	0	1.2	3.4
Gastrotricha	0	0.4	14.3	0
Bivalvia	4.8	2	3.2	0.7
Gastropoda	1.1	0	0.9	0
Total per core				
Median	2183.4	2454.2	9467.6	3139.6
Minimum	1097.8	721.9	1585.9	1650.6
Maximum	2811	5910.7	16835.8	5952.8

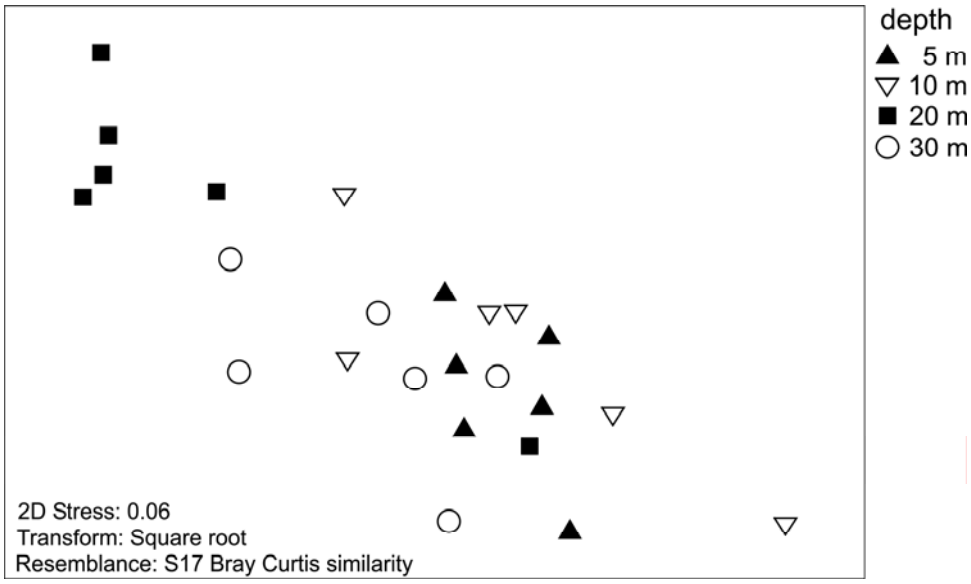

Fig. 2: Meiofauna along a soft-bottom depth transect (5, 10, 20, and 30 m) in Potter Cove. MDS of the Bray-Curtis similarity based on higher taxon level individual numbers (square root transformed data).

Median individual numbers at the 20 m station are three to four times higher than the densities found at 5, 10, and 30 m depth. This rise can be observed mainly in nematodes and copepods while copepod nauplii tend to be more abundant in both deeper stations. However, this is not consistent with all taxa and there are groups which are underrepresented at 20 m depth as compared to their relative abundances at other stations.
The MDS of the Bray-Curtis similarity (Fig. 2) shows the highly scattered pattern which is achieved for the 36 samples (six cores per station). An ANOSIM test for the factor "depth" was not significant (global R = 0.35). This indicates that samples from different depths were equally similar or distinct from each other as samples from the same depth. This is clearly visualised in Figure 2, where only five samples from 20 m depth can be regarded as somehow "grouped" which is due to the markedly higher individual numbers found there. The samples of all other stations and the sixth sample from 20 m depth are widely distributed over the array of the plot.

Discussion

The only study so far dealing with metazoan meiofauna communities in Potter Cove is by Mayer (2000). She reported a maximum individual density of 485.5 Ind. 10 cm^{-2} in a sample from 10 m depth (Mayer, 2000; p. 124, slice no. 73 – 77, 0-5 cm). Compared to those findings our study revealed much higher abundances in every of the 36 samples.

The individual numbers of our transect in Potter Cove are comparable to those of de Skowronski and Corbisier (2002) who studied meiofauna densities at various 15 m deep stations close to Potter Cove in Martel Inlet, Admiralty Bay, King George Island. They give ranges between 1953 and 6310 Ind. 10 cm^{-2}. Although de Skowronski and Corbisier integrated the upper 10 cm sediment layer and in this study only the upper 5 cm have been considered individual numbers are comparable. Following Mayer's findings (2000) from Potter Cove, it can be stated that the vast majority of the metazoan abundance is concentrated in the first 3 cm of the sediment, although she showed that animals can still be found in sediment depths of 10 to 16 cm.

A comparison to data from other shallow-water sites in the Antarctic is possible when sampling methods are comparable. Corers placed by scuba divers have been used by several authors working at Signy Island: Vanhove et al. (1998) showed two 10 m deep sites to be contrasting in meiofauna abundances. Although their five replicate cores (3.6 cm diameter) per station indicate a very patchy horizontal distribution of the meiofauna (high standard deviations) they find contrasting means of 13170 Ind. 10 cm^{-2} and 4950 Ind. 10 cm^{-2}, respectively. Vanhove et al. (2000) studied seasonal changes in Factory Cove (2 replicates per sampling date) and detected higher values in summer (> 10000 Ind. 10 cm^{-2}) and lower abundances in winter (< 5000 Ind. 10 cm^{-2}). Lee et al. (2001) investigated the recovery of meiofauna communities after ice-berg scouring (2 replicates per sampling date). The values found during recolonisation (maximum 4339.7 Ind. 10 cm^{-2}) and in the control samples (1431.6 Ind. 10 cm^{-2}) represent the patchy distribution of the meiofauna.

In our study minimum and maximum densities in single cores have revealed 721.9 Ind. 10 cm^{-2} (10 m) and 16835.8 Ind. 10 cm^{-2} (20 m), respectively, thus covering all values reported elsewhere from Antarctic shallow waters. Even at station level the six replicates can reveal highly differing abundances. Therefore a reliable discrimination between the stations cannot be made at the higher taxon level although mere median values seem to indicate a marked position of the 20 m station.

This study shows that the higher than usual sampling effort in our case rather leads to a more scattered than to a clearer picture. At the time being, generalisations cannot be made and further analyses on species level are necessary in order to investigate whether the soft-bottom sediments in Potter Cove are - although very variable on small scales - quite homogeneous in their patchiness at meso-scale level.

Acknowledgements

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Species composition and structure of the ciliate community in the benthos at King George Island, Antarctica.

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Introduction

The ciliates are a group of protozoan organisms widely distributed in both marine and fresh water. As consumers of bacteria and algae, and as predators and detritus-feeders, they are at the base of several special food chains in aquatic ecosystems, with crucial significance for the circulation of materials and the overall function of these systems. In the Antarctic benthic regions at King George Island, ciliates are the dominant protozoan group, in numbers of both species and individuals (Song & Wilbert 2002; Wilbert & Song 2005, 2007). An initial extensive investigation of the ciliate population in the benthos was done during the Antarctic summer in the years 2000, 2002, 2004 and 2006. This report presents results of these studies. Part of my programme was also to assess the ciliate population in the Antarctic benthos, with the aim of comparing it with that in the sea ice (Petz et al. 1995; Song & Wilbert 2000).

Materials and Methods

For studying the benthos a place was chosen where a rock in the surf, about 50 m wide, was protected from the ice movement. On the landward side of the rock, not directly exposed to the surf, were three pools about 15 cm deep, each with an area of about 0.5 m^2 . At low tide these were not flooded by the ocean for four hours. Pilot experiments showed that the abiotic conditions were not different from those in the open ocean, even during the four-hour isolation at ebb. Isolated ciliates were observed *in vivo* and used for morphological studies. Their infraciliatures and slverline systems were impregnated with protargol according to Wilbert 1975, by the method of Song and Wilbert 1995. For designating, evaluating and comparing the Antarctic ciliate coenoses, the following formula (as given by Schwerdtfeger 1979 and Bick 1998) can be used.

Species identity (according to Jaccard): I_A number representing the degree to which two compared samples are consistent with one another, regarding the species that are present:

$$I_A = \frac{g}{a + b - g} \cdot 100\,\%$$

where *a* is the number of species in Community 1, *b* is the number of species in Community 2, and *g* is the number of species present in both communities.

Results

In total 35 species were found in the benthal 58 % of these species have been found exclusively in Antarctica (Tab.1). The relative paucity of species (Tab.2) in the benthic ciliate coenosis reflects the extreme conditions for life at such sites. Here, in the tidal region, erosion by moving masses of ice has severe consequences, allowing the growth of organisms (periphyton) only in crevices. Another substantial factor is the occurrence of external temperatures below the freezing point; especially during storms, at ebb tide the wind-chill effect causes puddles and rock pools to freeze. The Antarctic benthal habitat is thus very unstable, and constant coenoses cannot become established here for long periods of time.

Table 1: List of ciliate species recorded in the benthos at King George Island (Species = 35, genera = 28, Species/Genus = 1.2).

Aeyriana paroliva SONG & WILBERT, 2002

Amphileptus sp. WILBERT & SONG, 2005

Amphisiella antarctica WILBERT & SONG, 2005

Aspidisca crenata FABRE-DOMERQUE, 1885

Aspidisca polypoda (DUJARDIN, 1841) KAHL, 1932

Aspidisca quadrilineata KAHL, 1932

Bickella antarctica nov.. gen., nov . spec. WILBERT & SONG, 2007

Chlamydonella sp.

Condylostoma remanei KAHL, 1932

Condylostoma cf. *magnum* SPIEGEL, 1926

Cyclidium varibonneti SONG, 2000

Diophrys oligothrix BORROR, 1965

Diophrys scutum DUJARDIN, 1841

Dysteria calkinsi KAHL, 1931

Dysteria parovalis WILBERT & SONG, 2005

Euplotes balteatus (DUJARDIN, 1841) KAHL , 1932

Euplotes rariseta CURDS et al., 1974

Hartmannula cf. angustipilosa DEROUX & DRAGESCO, 1968

Hemigastrostyla szaboi WILBERT & SONG, 2005

Heterostentor coeruleus WILBERT & SONG, 2005

Holosticha diademata (REES, 1884) SONG & WILBERT. 2002

Holosticha sp.

*Intranstyl*um antarcticum Wilbert & Song, 2005

Litonotus antarcticus SONG & WILBERT, 2002

Metanophrys antarctica SONG & WILBERT, 2002

Metaurostylopsis rubra SONG & WILBERT, 2002

Orthodonella shenae SONG & WILBERT, 2002

Philasterides sp.

Pithites pelagicus WILBERT & SONG, 2005

Pleuronema coronatum KENT, 1881

Strombidium apolatum WILBERT & SONG, 2005

Telotrochidium sp.

Thigmokeronopsis magna WILBERT & SONG, 2005

Uronychia transfuga (MUELLER, 1776) STEIN, 1859

Urotricha antartica WILBERT & SONG, 2007

The constancy of species in the benthal

The constancy of the benthic species can be calculated from the investigations in 2000, 2002, 2004 and 2006. Of the 35 species observed on these occasions, 14 are cosmopolites. The remaining species are new to science, including the new genera *Heterostentor* and *Bickella*. The species *Bickella antarctica* Wilbert & Song, 2007 (Fig. 1)*, Metaurostylopsis rubra* Song & Wilbert 2002, and *Orthodonella shenae* Song & Wilbert, 2002 exhibit a constancy of 100%, and can be regarded as characteristic species in the investigated benthal of King George Island. None of the other species exceeds a constancy of 50%.

Table 2: The ciliate community in marine and freshwater benthal (Song & Wilbert 1989) interstitial, North Atlantic (Dragesco 1960) and in sea ice of the Weddell Sea (Petz et al. 1995, Song & Wilbert 2000) categorized according to method of feeding.

Coenosis	Benthal (marine) Jubany Station	Benthal (freshwater, Germany)	Interstitial	Sea ice (Weddell Sea)
species in total	38	155	161	55
grazers	33	10	0	10
raptorial feeders	8	29	45	20
vagile filter feeders	45	17	17	23
hemi sessile filter feeders	6	13	38	47
sessile filter feeders	8	31	0	0*

* The percentages of the various feeding categories in the different biotopes

The ratio of species to genera in the coenoses of the benthal and sea ice

In general the coenoses are distinguished by the fact that each genus is represented by only one species. The coenosis in the benthal has a species-to-genus ratio of 1.2 (Tab. 1). This ratio also corresponds to the values found for other ciliate coenoses.

Conclusion

The benthic character of the sea ice ciliates (Tab. 2) initially suggested that they had colonized the ice from the benthal. However, now that populations of Antarctic benthic ciliates have been examined, enabling the two coenoses to be compared, this has now been refuted. The species identity shows that the ciliate fauna in the sea ice is autochthonous.

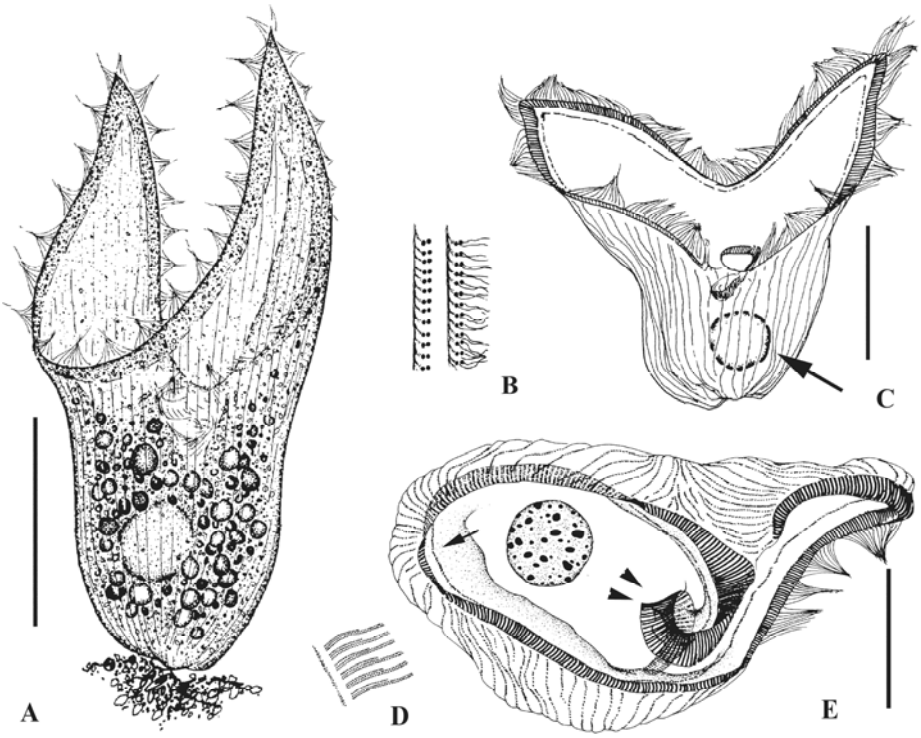

FIG. 1 A-E: *Bickella antarctica* n. gen., n. sp. Wilbert & Song, 2007, from the benthal of Potter Cove, Jubani Station. The drawings serve as an example of the way species newly discovered in the benthal and ice are described. They show the results of *in vivo* examination and the features of the infraciliature that are typical of the species.
(A) From life and after protargol impregnation. (**B)** Somatic kineties, to show the fibres associated with dikinetids. (**C)** Lateral view, arrow marks the macronucleus. (**D)** Details of membranelles and paroral membrane. (**E**) Apical view, arrow marks the paroral membrane, arrowheads indicate proximal end of adoral-zone of membranelles, which extends deeply into the buccal cavity. Scale bars 100 µm.

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Bentho-pelagic coupling at Potter Cove, Antarctica: A fatty acid approach

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Introduction

Antarctic benthic communities are diverse and rich in biomass. A striking feature is the dominance of benthic filter feeders in most of the studied areas (Arntz et al. 1994, Barnes 2005, Gili et al. 2006). The biology of these filter feeders is usually linked to the strong seasonality of the primary production, characteristic for polar ecosystems (Clarke 1996). However, this linkage could be much more important in shallow coastal ecosystems than in deeper continental shelf areas, because a clear and direct coupling between primary pelagic and secondary benthic production is an obvious feature in shallow waters (Dayton and Oliver 1977, Clarke 1996, Cattaneo-Vietti et al. 1999, Corbisier et al. 2004).

At Potter Cove planktonic primary production is low, and the phytoplankton blooms, which are characteristic for other Antarctic coastal areas, are almost absent (Schloss and Ferreyra 2002), while the benthic secondary production is characterised by high densities and biomass of benthic suspension feeders, especially bivalves, pennatulids, ascidians and sponges (Sahade et al. 1998, Sahade et al. this issue). Moreover, it was an unusual finding that two of the most successful ascidians *Molgula pedunculata* and *Cnemidocarpa verrucosa* do have their reproductive season during winter, totally uncoupled from summer primary production pulses (Sahade et al. 2004). This suggest that either ascidians are able to store energy for reproduction, or the energy demand of the benthic filter feeders during winter is not as high as usually assumed (Gili et al. 1996, Ahn et al. 2003). Therefore, the main energy source for this benthic community is probably not based on local primary production. Reinforcing this idea, particles analysed in ascidian gut during a year-round period revealed only little micro-algae contribution, even during summer (Tatián et al. 2004).

To study food web interactions and possible energy sources, fatty acid trophic markers are a useful tool. This is due to the fact that specific fatty acids are transferred almost unchanged from algae into the lipids of higher trophic organisms and top predators (Dalsgaard et al. 2003). For instance, typical fatty acid markers of diatoms are 16:1(n-7) and 20:5(n-3) and of dinoflagellates 18:4(n-3) and 22:6(n-3). In addition, long-chain fatty acids like 20:1(n-9) and 22:1(n-11) are major fatty acids of calanoid copepods, which could play an important role providing energy to the benthic system (Graeve et al. 1997, Lee et al. 2006). These fatty acids, which may be part of their ingested material, can

be egested with faecal pellets, which have high sedimentation rates, thus contributing to the carbon flux to the benthic system.

In this study, fatty acid compositions of particulate material of water column and sediments, and benthic suspension-feeders are used to trace the possible origin and pathway of the energy flux that fuels the benthic ecosystem in Antarctic shallow waters.

Materials & Methods

Monthly sampling of suspended particulate matter (SPM), sediments, and the benthic organisms, *Laternula elliptica*, an infaunal bivalve, and *Pareugyrioides arnbackae*, a stalked ascidian, was carried out in the Potter Cove (King George Island, Antarctica) from January 2003 to March 2004. The stalked ascidian species has not been previously reported for this area (Tatián et al. 1998) but was present at considerable abundance during the sampling year in shallow waters (10 to 12 m). Samples of some months are lacking due to unfavourable weather conditions that hindered scuba diving . Five individuals of the animal species and 5 sediment samples were taken in the inner cove per month and stored in chloroform:methanol (2:1) at -20ºC. From the sediment cores only the first centimetre was used for further analysis. For SPM two stations, one in the inner and one in the outer Potter Cove were sampled at depths of 0, 15 and 30 m. One litre of water or less, if the filter was clogged, was filtered through precombusted GF/F glass-fibre filters (47 mm Ø), and the filters were also stored in chloroform:methanol (2:1) at -20ºC.

The fatty acid compositions of the total lipid extracts were analysed by gas-liquid chromatography according to Kattner and Fricke (1986). Lipids were converted to fatty acid methyl esters (FAME) by transesterification in methanol containing 3% concentrated sulphuric acid at 80°C for 4 hours. After extraction with hexane, FAME were analysed with a Hewlett-Packard 6890 Series gas chromatograph equipped with a DB-FFAP fused silica capillary column (30 m x 0.25 mm inner diameter, 0.25 µm film thickness) using temperature programming (160-240°C at 4°C min^{-1}, hold 15 min). For recording and integration, Class-VP software (4.3) (Shimadzu, Germany) was used. FAME were identified with standard mixtures, and additional confirmation was carried out by gas chromatography-mass spectrometry (Kattner et al. 1998).

Results & Discussion

Fatty acid data were averaged independently of depth and seasons in order to get a direct overview of possible relationships between the benthic filter feeders and the food sources (Table 1). The fatty acid compositions of the individual samples of SPM were variable but showed no clear trend during the different sampling periods. SMP was characterised by the saturated fatty acids, 16:0 and 18:0, and the monounsaturated fatty acid 18:1(n-9), which reflect a considerable degradation of the material and thus a high amount of detritus within the SPM (Kattner et al. 1983). These fatty acids are more stable than polyunsaturated fatty acids. Small proportions of the polyunsaturated fatty acids 20:5(n-3) and 22:6(n-3) in combination with the fatty acids typical of diatoms

(16:1 and 18:1 both as n-7 isomers) and flagellates (18:4(n-3)) indicate a small contribution of phytoplankton to SPM.

Table 1: Fatty acid compositions of the different pelagic and benthic components (mean of mass % ± standard deviation (SD)). Data of stations and seasons are averaged, n = number of samples.

Fatty acids	SPM (n=22)		Sediment (n=7)		*P. arnbackae* (n=10)		*L. elliptica* (n=18)	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
i14:0	0.0	± 0.0	1.5	± 0.7	0.0	± 0.0	0.0	± 0.0
a14:0	0.0	± 0.0	1.0	± 2.4	0.0	± 0.0	0.0	± 0.0
14:0	5.0	± 1.8	7.7	± 3.2	1.1	± 1.1	1.2	± 0.4
i15:0	0.1	± 0.2	4.2	± 1.9	0.0	± 0.0	0.0	± 0.0
a15:0	0.0	± 0.0	4.2	± 2.1	0.0	± 0.0	0.0	± 0.0
15:0	1.8	± 0.6	3.4	± 0.8	0.3	± 0.2	1.3	± 0.3
15:1(n-6)	0.0	± 0.0	0.9	± 0.7	0.0	± 0.0	0.0	± 0.0
16:0	22.8	± 5.8	31.5	± 6.6	10.0	± 6.2	20.2	± 2.1
16:1(n-9)	1.9	± 1.8	1.5	± 1.2	0.0	± 0.0	0.0	± 0.0
16:1(n-7)	5.5	± 2.0	10.1	± 10.6	8.1	± 5.8	2.2	± 0.5
16:1(n-5)	0.2	± 0.3	0.8	± 1.0	0.5	± 0.2	0.2	± 0.1
i17:0	0.0	± 0.0	2.3	± 1.3	0.0	± 0.0	2.0	± 0.5
a17:0	0.0	± 0.0	1.4	± 1.0	0.0	± 0.0	0.7	± 0.2
16:2(n-4)	0.9	± 1.0	1.1	± 2.0	2.0	± 0.7	0.8	± 0.3
16:3(n-4)	1.0	± 1.2	0.0	± 0.0	1.4	± 1.7	0.3	± 0.1
16:4(n-1)	0.0	± 0.0	0.0	± 0.0	0.4	± 0.3	0.1	± 0.1
17:0	0.0	± 0.0	1.7	± 0.6	1.1	± 0.5	2.7	± 0.6
18:0	13.7	± 6.2	8.1	± 4.0	5.6	± 1.8	6.9	± 2.0
18:1(n-9)	20.1	± 9.6	4.1	± 4.0	1.8	± 1.1	1.6	± 0.3
18:1(n-7)	3.5	± 1.3	3.1	± 3.9	16.9	± 5.0	2.8	± 0.7
18:2(n-6)	5.2	± 2.4	1.1	± 0.9	0.2	± 0.2	1.3	± 0.2
18:3(n-3)	2.0	± 2.3	0.0	± 0.0	0.1	± 0.1	0.3	± 0.1
18:4(n-3)	2.5	± 2.1	0.0	± 0.0	0.7	± 0.6	1.3	± 0.8
20:0	0.0	± 0.0	1.1	± 1.1	0.1	± 0.2	3.2	± 1.5
20:1(n-11)	0.0	± 0.0	0.0	± 0.0	1.1	± 0.8	0.0	± 0.0
20:1(n-9)	3.9	± 3.6	0.4	± 0.8	4.2	± 1.6	13.1	± 2.7
20:1(n-7)	1.6	± 2.0	0.0	± 0.0	1.6	± 0.6	2.0	± 0.3
20:2 (n-6)	0.0	± 0.0	0.0	± 0.0	0.0	± 0.0	0.9	± 0.2
20:4 (n-6)	0.0	± 0.0	0.0	± 0.0	1.2	± 0.6	0.2	± 0.1
20:4(n-3)	0.0	± 0.0	0.0	± 0.0	0.9	± 1.0	1.3	± 0.4
20:5(n-3)	3.6	± 3.4	0.0	± 0.0	21.4	± 7.6	15.5	± 4.6
22:0	0.0	± 0.0	3.3	± 4.1	0.0	± 0.0	0.0	± 0.0
22:1(n-11)	0.0	± 0.0	0.0	± 0.0	0.8	± 0.5	0.0	± 0.0
22:5(n-3)	0.7	± 0.9	0.0	± 0.0	6.8	± 3.5	1.7	± 0.3
22:6(n-3)	2.6	± 2.5	0.0	± 0.0	5.9	± 1.8	13.9	± 2.5

In addition, the presence of the 20:1(n-9) fatty acid shows that also material originating from herbivorous copepods contributed to SPM. However, the absence of fatty alcohols as moiety of copepod wax esters clearly shows that live copepods were not part of SPM, but copepods can provide an important energy source via faecal pellets. The only copepods in Antarctica producing the 20:1(n-9) fatty acid are *Calanoides acutus* and *Calanus propinquus* (Kattner et al. 1994). However, we may exclude contribution of *C. propinquus* to SPM because of the lack of 22:1(n-11), the predominating fatty acid of this species. These results are consistent with a long-term study on the dynamics of zooplankton communities carried out at Potter Cove (Fuentes unpubl. data).

The fatty acid composition of surface sediments was clearly different from that of SPM being highly variable in percentage. The 16:0 fatty acid was predominant accounting for almost one third of all fatty acids. The other major fatty acids 18:0 and 18:1(n-9) of SPM were less abundant in the sediment, whereas 16:1(n-7) occurred in higher amounts but also in highly variable proportions. This might be due to the presence of benthic diatoms, which can have a very patchy distribution (Gili et al. 1996). The origin from planktonic diatoms is unlikely, because phytoplankton blooms are almost absent at Potter Cove (Schloss and Ferreyra 2002). The total lack of polyunsaturated fatty acids suggests that only remnants of these diatoms were sampled. The fatty acids characterising organic material in the sediments were odd-chain and branched components, which contributed about 20% to the total fatty acids. These fatty acids are typical for bacteria and their degradation products (Dalsgaard et al. 2003) and thus indicating a considerable microbial turnover of organic matter in the sediment.

The fatty acid compositions of the two possible food sources should present a signature in the benthic filter feeders because of the fatty acid transfer within the food web. The fatty acid trophic marker signal is clearest in storage lipids since membrane lipids are less influenced by the diet (reviewed by Dalsgaard et al. 2003). The benthic species studied have an opportunistic feeding behaviour and store only small amounts of lipids (Ahn et al. 2000, 2003). Therefore, it is difficult to identify feeding preferences via fatty acid compositions. *L. elliptica* had a very constant fatty acid composition without seasonal variations. It was characterised by high proportions of fatty acids typical for membrane lipids, which are 16:0, 20:5(n-3) and 22:6(n-3) (Albers et al. 1996), contributing together on average about 50% to total fatty acids. Fatty acids, which are indicative of phytoplankton feeding, occurred only in small proportions. Small amounts of odd-chain fatty acids reflect ingestion of the highly degraded organic matter from the sediment, which might be taken up probably after resuspension into the water column. The high proportion of the 20:1(n-9) fatty acid together with small proportions of its n-7 isomer indicates feeding on copepods. This fatty acid is a major component of *C. acutus* and might be accumulated by *L. elliptica* through SPM or by filtering directly small copepodite stages from the water column. It is also possible that *L. elliptica* incorporates high amounts of the 18:1(n-9) fatty acid from the SPM and elongates this fatty acid to 20:1(n-9) to increase its calorific value. A third possibility is the total de novo biosynthesis of this fatty acid, which is so far only known from herbivorous calanoid copepods (Sargent and Henderson 1986). Elevated amounts of the 20:0 and 20:1(n-9) fatty acids in *L. elliptica* gills may also suggest de novo synthesis (Ahn et al. 2000). The overall fatty acid composition reflects an opportunistic omnivorous feeding behaviour, and *L. elliptica* seems to be independent on lipid storage in times of food shortage (Ahn et al. 2003).

The fatty acid composition of *P. arnbackae* was clearly different from that of *L. elliptica* and more variable. The dominant fatty acid was 20:5(n-3), whereas 16:0 was less abundant and the membrane fatty acid 22:6(n-3) occurred only in small amounts. In contrast, the 22:5(n-3) fatty acid exhibited higher proportions than in *L. elliptica* and the organic matter. The proportion of the marker fatty acid of diatoms 16:1(n-7) accounted for 8.1% on average. 18:1(n-7) was the second abundant fatty acid of *P. arnbackae* (16.9%). This

high proportion is very unusual for marine organisms, especially in combination with very low percentages of the 18:1(n-9) fatty acid. The resulting 18:1(n-9) to (n-7) ratio is the lowest so far reported for benthic species. Some brittle star species from the Arctic, that are generally known to be opportunistic in their feeding mode and food source (Warner 1982), have also low ratios (Graeve et al. 1997), but not as low as *P. arnbackae*. The origin of the 18:1(n-7) fatty acid is difficult to explain. It might originate from elongation of the 16:1(n-7) fatty acid accumulated from the diet, or it is totally synthesised de novo. The low proportions of odd-chain and branched fatty acids support that sediment derived organic matter is probably not the major food source of *P. arnbackae*.

Multivariate analyses (MDS) (Fig. 1) using all fatty acid compositions revealed a closer relationship of the animals with SPM than with the sediments indicating that SPM is the preferred food source. *L. elliptica* showed a slightly higher proximity to sediments than the *P. arnbackae*. This is probably due to the infaunal living condition of *L. elliptica*, which feeds closer to the sediment-water interface, and is thus more susceptible to resuspension events than ascidians, which are feeding higher above the bottom (ca. 15 cm). It should also be considered that the interactions between sediments and water column could be strong due to resuspension caused by winds and storms. The fatty acid composition of *L. elliptica* was less variable than that of the *P. arnbackae*, SPM and sediments. This indicates that the fatty acid composition and probably the life strategy of *L. elliptica* in general is less dependent on seasonal changes in food availability, which is influenced by sedimentation events, sediment resuspension, and patchy distribution of benthic algae.

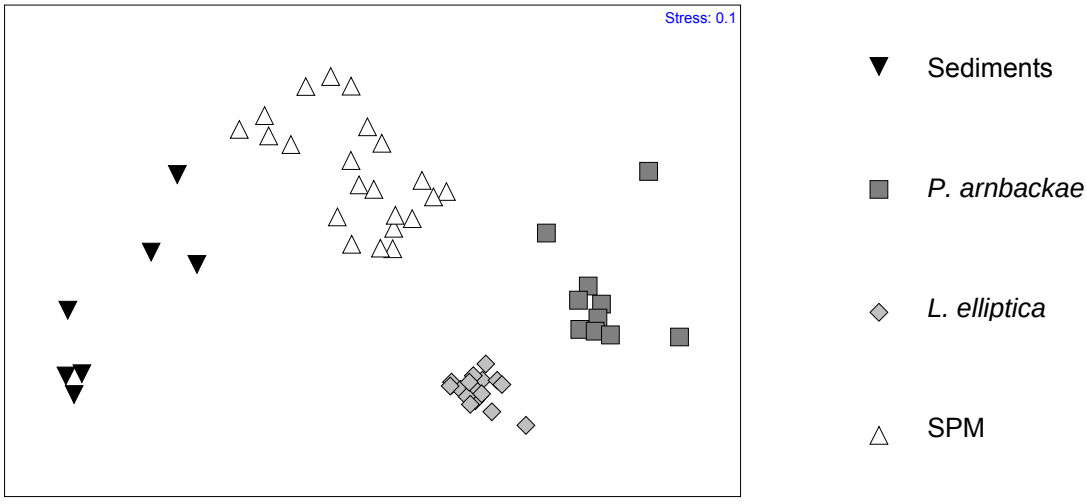

Fig. 1: MDS ordination graph based on fatty acid compositions of the different items analysed. Data were square root transformed to slightly even the weight of dominant and less abundant fatty acids; Euclidean distance was used to calculate the similarity matrix.

In conclusion, fatty acids are useful to reveal trophic relationships between the various components of the Potter Cove ecosystem. We hypothesise that the shallow benthic ecosystem in Potter Cove is not only

fuelled by local pelagic primary production but also by benthic primary production in form of micro- and macro-algae. The absence of bacterial tracers in the particulate matter was striking suggesting only small contributions of bacterial biomass, whereas bacterial activities are important in sediments. This study is the first approach to reveal food web structure and energy pathways in the Potter Cove and showed the necessity to extend studies on other ecosystems compartments as well as to intensify investigation of seasonal and inter-annual dynamics of this ecosystem.

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Spatial and temporal variability of chlorophyll-a and particulate organic matter in the sediments and the water column of Potter Cove (Antarctica)

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Introduction

Sunlight fuels marine primary production. Light reaching the seafloor sustains benthic primary production (Ackleson, 2003) that contributes to the total primary production of coastal ecosystems and consequently to the organic matter availability for the consumers in the system (e.g., Delesalle *et al*., 1993). Benthic diatoms can retain photosynthetic capacity in the dark and even inside sediments, thus potentially forming an important pool of primary producers, which can resume photosynthesis if exposed to light again (Fielding *et al*., 1988).

Several authors showed that total phytoplankton biomass in the South Shetlands is very low (Brandini and Rebello, 1994). This is also the case in Potter Cove, where the combination of mixing processes (Schloss and Ferreyra, 2002) and low water transparency due to the input of suspended particulate matter from freshwater runoff (Klöser *et al*., 1993, 1994) could be responsible for the low phytoplankton biomass (Schloss *et al*., 1997).

The pulses of organic matter derived from phytoplankton production in the water column have long been considered to be one of the main sources of particulate organic matter (POM) for zoobenthos nourishment (Barnes and Clarke, 1994; Fabiano et al., 1997). Benthic consumers are usually dependent on productivity in the water column. Phytoplankton, and especially diatoms, constitute an important food source for benthic organisms such as suspension and deposit feeders (Goddard and Hoggett, 1982). However, the low phytoplankton production found in Potter Cove would not be sufficient to fulfil the nutritional requirements of the high densities of benthic filter feeders (Tatián, 1999). We therefore hypothesize that microphytobenthos biomass represents a significant fraction of the POM pool in this environment, contributing to satisfy benthic suspension and deposit feeders energy needs.

The aim of this paper was to investigate the temporal and spatial variability of chlorophyll-a (Chl-a) and organic matter in surface sediments of Potter Cove (Antarctica) in a year-round study. To address the importance of water column-derived material for the sedimentary organic matter pool, we also analysed the seasonal variation of pelagic total particulate matter (TPM), suspended POM, Chl-a, as well as light penetration in the water column. Fluctuations of wind

speed and direction were registered and analysed throughout the year in order to understand their influence on the dynamics of these parameters.

Materials and methods

Study area

Potter Cove is a small tributary embayment inside the Maxwell-Guardia Nacional Bay system, and located in the vicinities of the Argentinean Station Jubany and the Argentinean-German Dallmann laboratory (King George-25 de Mayo Island, South Shetland Islands, 62º 14`S, 58º40`W). Within the cove, two zones can be distinguished: an inner cove, where maximal depths can reach 50 m (average = 30 m), and an outer deeper cove (>100 m). Muddy soft bottom characterize the inner cove. The outer cove is bordered by hard substrate down to 30 m (Klöser *et al.,* 1994). Microphytobenthic algae dominate the inner cove while high densities of macroalgae are found in the outer cove (Klöser *et al*., 1994). This study was performed in the inner cove, at the site of a fixed long-term monitoring station (S1, Fig.1), from December 1997 to December 1998.

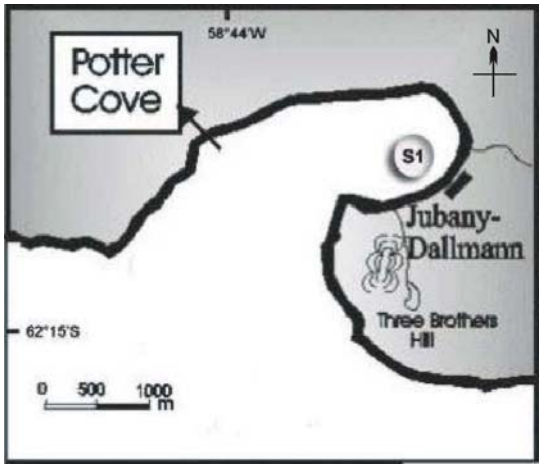

Figure 1: Map showing the location of Potter Cove and the sampling site studied (S1).

Sample collection and analysis

Three replicate sediment samples were collected in monthly intervals at 5, 10, 20 and 30 m depth by SCUBA diving using a hand-corer (inner diameter: 5 cm). Only the upper 3 cm were analysed. Each core was divided into 3 sections of 1 cm intervals (0-1, 1-2 and 2-3 cm). Variable volumes of a suspension of a sub-sample of the sediment core were collected for the determination of Chl-a and TPM. Chl-a was extracted during 24 h in cold and dark conditions with 90 % acetone and read on a Pharmacia Biotech Ultrospec 3000 UV/Visible spectrophotometer. Correction for phaeopigments was done according to Strickland and Parsons (1972). Total particulate matter (TPM) as well as its organic and inorganic fractions were measured gravimetrically. Samples were dried 24 h at 60 ºC, weighed, then combusted for 5 h (500º C) and weighed again. The particulate organic matter fraction (POM) was estimated as the weight difference between TPM and the ash weight after combustion. The percent of organic matter (%POM) was then calculated from POM and TPM.

Simultaneously, water column samples were also collected by means of a 5 l Niskin bottle at 0, 5, 10, 20 and 30 m depth for the analysis of Chl-a and TPM. A volume of 0.5-1 l seawater was filtered through Whatman GF/F filters and analysed as described for sediments.

Wind speed and direction were measured at Jubany`s meteorological station every 3 h (Servicio Meteorológico Nacional, Argentina). Light (PAR, photosynthetic available radiation, 400-700 nm, µE m^{-2} s^{-1}) penetration in the water column was measured with a LI-193 sensor (Li-Cor, USA).

Data Analysis

Values are presented as mean ± standard error unless indicated otherwise. Analysis of variance (ANOVA) was applied to detect differences in the vertical distribution of the studied parameters (Chl-a, POM, %POM).

Results and discussion

Wind Conditions

Figure 2 shows the average wind speed (10 ms^{-1}) between December 1997 and December 1998. The dominant winds came from the West, although a pronounced SW - NE bi-directionality was also observed, consistent with the longitudinal axis of Potter Cove. Wind speeds of this force have been shown to be strong enough to intensely mix the water column in Potter Cove (Schloss and Ferreyra, 2002).

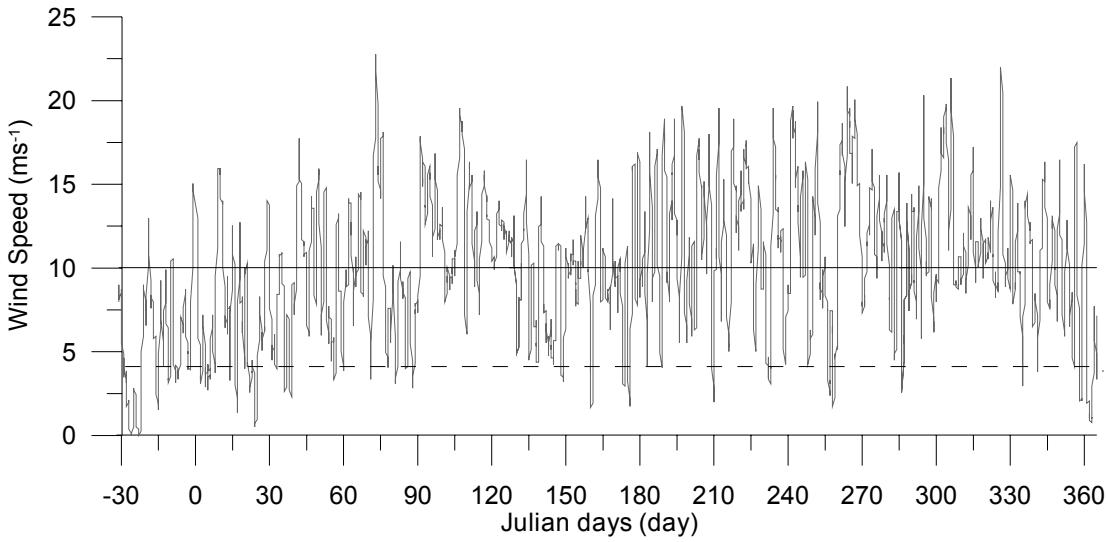

Figure 2: Daily average wind speed between December 1997 and December 1998. The annual mean of 10 ms^{-1} (continuous horizontal line) is also shown along with the point at which the wind speed intensity begins to play a significant role in vertical mixing of the water column (dotted line).

Underwater light conditions

Typical examples for the contrasting underwater light conditions in the inner and outer part of the cove are shown in Fig. 3. The depth of the euphotic zone (Z_{eu}, the 1 % of incident PAR immediately below the sea surface) at the inner station reached only ~5 m depth, while it was around 21 m in the outer part of the cove on the same date. The low depth of the euphotic zone at our sampling station can be explained by the glacier meltwater input draining through loose moraine material. This land-derived freshwater contributes high amounts of particulate matter to the water column in Potter Cove.

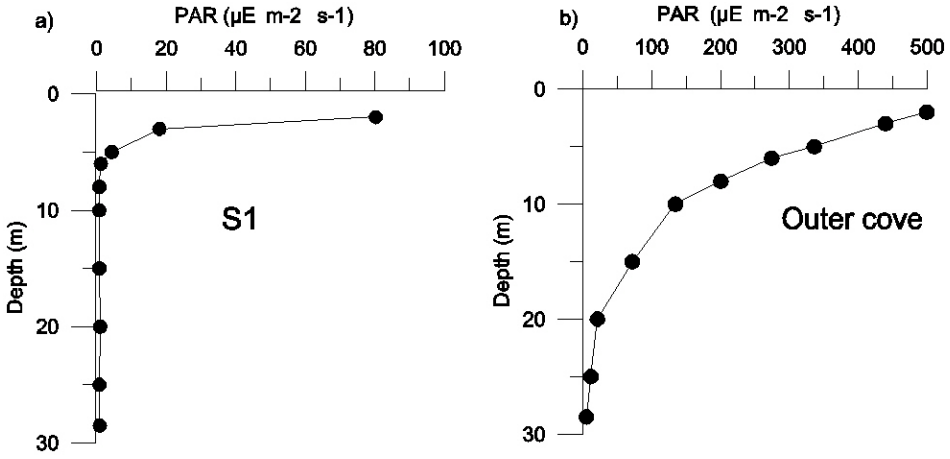

Figure 3: Photosynthetically active radiation (PAR; µE m^{-2} s^{-1}) in the water column at S1 (a) and the outer cove (b) in February 13 1998. Note the different scales in x-axis (PAR)

Water Column POM and Chlorophyll-a

The concentrations of pelagic POM (measured in mg l^{-1}) showed a variable seasonal pattern with somewhat higher values during summer and winter (Fig. 4a). The yearly average of POM at S1 was 3.28 (± 0.01) mg l^{-1}, with maximum values recorded on February 20 and July 11 (7.66 and 6.8 mg l^{-1}, respectively). The average percentage of particulate organic matter during winter was 51% of Total Particulate Matter. Despite POM concentration in the samples during winter was high, low values of Chl-a were recorded (winter average=0.11 µg l^{-1}). These high winter POM values were not linearly correlated to Chl-a ($p=0.8$). This means that during winter, when the Chl-a was low, another source of POM could be present in the water column.

Significant differences of Chl-a were found between seasons ($F_{22.90}=19.84$, $p<0.01$) with highest values during summer, reaching 4.72 µg l^{-1} on February 13 (Fig. 4b). In contrast, Chl-a concentrations between different sampling depths did not differ significantly ($F_{4.13}=19$ $p=0.94$).

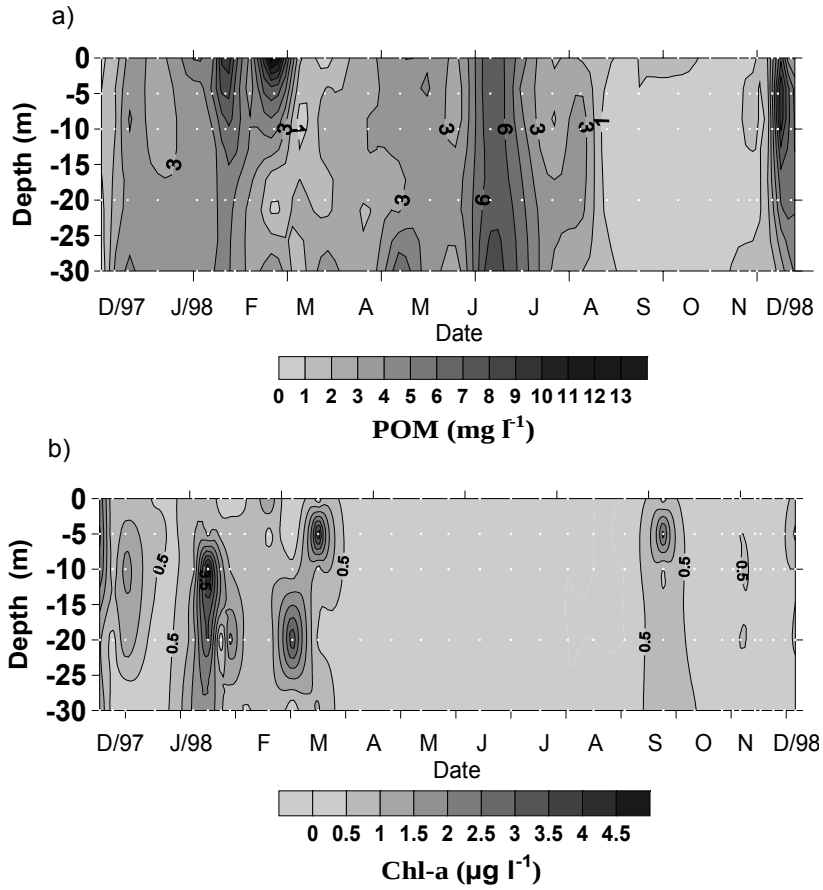

Figure 4: Temporal variability of POM a) and Chl-a b) in the water column of Potter Cove.

POM and Chlorophyll-a in the sediments

The annual mean POM estimated in the upper 3 cm of the sediments was 108.95 (± 0.21) g m^{-2}. Average sediment POM concentrations for the different seasons were: 100.00 (± 0.42) g m^{-2} for the first summer (December 1997 to March 1998), 118.25 (± 1.07) g m^{-2} during autumn, 117.70 (± 1.31) g m^{-2} during winter, 107.96 (± 4.39) g m^{-2} during spring and 119.83 (± 1.64) g m^{-2} during summer 1998 (see Fig 5a).

The ANOVA analysis evidenced significant seasonal differences ($F_{4.36}$=2.89, p<0.01). POM showed high values during late winter, with maximum values detected in September, (169.85 g m^{-2}). This highest value was observed at the same time as a decrease of POM levels in the water column (2.62 ± 0.37 mg l^{-1} in the same month). Figure 5a shows a similar pattern of the seasonal distribution of POM in the water column and in the sediments. However, a significant correlation between both variables was not found (r=0.04). Moreover, no correlation was found between between Chl-a in the water column and in the sediments (p=0.352), which suggests that processes in these two different environments may not be uncoupled.
The mean Chl-a concentrations in the sediments were 5.04 (± 0.05) mg m^{-2} in summer 1997-1998, 6.17 (± 0.05) mg m^{-2} in autumn, 3.07 (± 0.14) mg m^{-2} in winter, 1.05 (± 0.05) mg m^{-2} in spring, and 3.6 (± 0.5) mg m^{-2} in summer 1998.

No significant differences between seasons were found for Chl-a ($F_{15.12}$=1.61, p=0.08). However, differences in Chl-a in the sediments were significant between depths ($F_{3.55}$=5.83, p<0.01). There was no correlation among the Chl-a and the POM measured in the sediments (p=0.382) suggesting that POM present in the sediments are not generated from the active microphytobenthic biomass.

In this study we have shown that the POM values both in the water column and in the sediments exhibit a strong seasonality. However, we have found no significant differences between the different depths. The lack of significant differences in pelagic POM between different depths could be explained by relocation of particles due to the strong wind induced vertical mixing in the water column in the site, as shown in previous studies (Schloss et al., 1999).The annual wind speed average was ~10 ms-1, which had a strong impact on water column stability (Fig. 2). As previously shown, the kinetic energy produced by winds > 3 ms^{-1} blowing over 3-5 hour periods is higher than the potential energy in the water column of Potter Cove. Under such scenarios stratification is eroded, with a significant resuspension and re-location of particles (Schloss et al., 1997).

Wind direction and intensity are thought to have an effect on the origin of the POM in Potter Cove. Roese and Drabble (1998) described a cyclonic gyre in the cove, advecting water and particles from the outer to the inner cove. In this regard, West winds could favour the inflow of macroalgae detritus from the outer Cove to the inner Cove, which accumulate in the sediments. Furthermore, water movements are also associated with processes of resuspension and deposition of sediments, which may affect the composition and distribution of the microphytobenthic community.

Despite that Z_{eu} was very low (~5m depth) during summer, when the TPM was maximum, the microphytobentic algae biomass was abundant suggesting the presence of dark-adapted benthic microalgae. The diatom population in Potter Cove's sediments is usually composed by epipelic and epiphytic diatoms genera, principally *Gyrosigma spp*., *Cocconeis spp*. *Licmophora spp.* and *Achnantes spp.* (Atencio, 2006, unpublished data). Resuspension of these organisms may represent an important process in providing food to benthic suspension feeders all year-round. On the other hand, POM in the sediments was not correlated with Chl-*a*. This could be explained by the existence of other carbon sources in the sediments such as zooplankton faecal pellets, macroalgae detritus and bacteria among others, which have been observed by Schloss *et al.* (1999) and Atencio (unpublished data) inside sediment trap samples, and may represent an additional energy source for the benthic fauna. This will be subject of further studies.

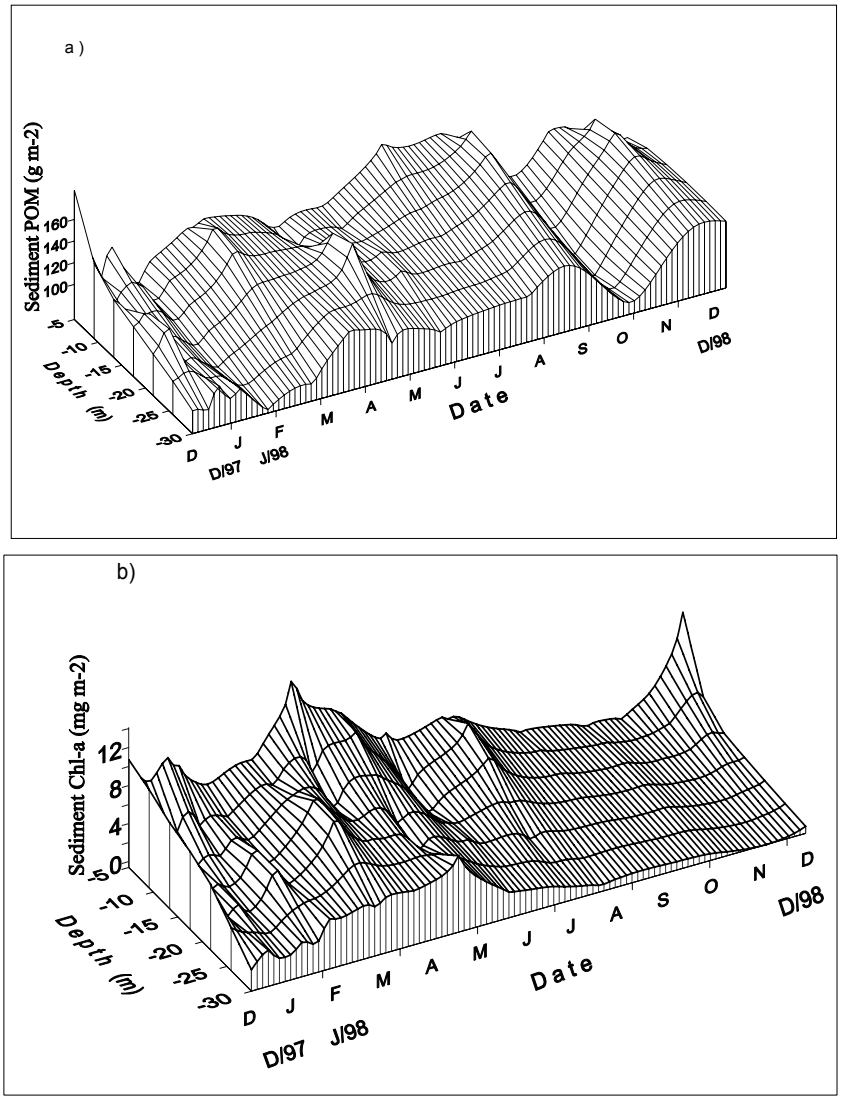

Figure 5: Annual distribution of a) POM (in g m^{-2}), and b) Chl-a (in mg m^{-2}) in the sediments of Potter Cove as a function of depth (m) and time.

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Benthic animal communities of Potter Cove (King George Island, Antarctica): Observed patterns and explanatory models

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Introduction

A variety of factors have been proposed to determine the structure of Antarctic benthic communities. For instance, physical disturbance caused by ice action (Sahade et al. 1998a) and biological interactions such as grazing, competition or predation (Iken et al. 1998). It is known that the shallow sublittoral zone of Antarctica is unfavourable for the establishment of sessile animals owing to ice effects (Dayton et al. 1970, Gili et al. 2006). Recently, more attention has been focused on the effects of ice melting, especially the increase in the concentration of inorganic matter at the water column (Tatián et al, 1998).

A complex spatial pattern in the vertical structure has been described for the benthic communities at Potter Cove (Sahade et al. 1998b) determining different responses to the presence of detritus in water column and to ice perturbation. Depending on the type of substratum, the hydrological characteristics and the perturbation regime, the dominant species occur at different zones of the cove. The inner cove is less stressed than the outer cove, and exhibits less ice disturbance and ample supply of organic food such as microalgae, bacteria and particulate organic matter (Sahade et al. 1998a). However, this zone can be the most affected by an increment in ice melting correlated with an enhanced input of inorganic particles in the water column.

The relatively high density of the bivalve *Laternula elliptica* at depths shallower than 15 m may be explained by their capacity to bury themselves, enabling them to avoid the ice impact. The pennatulids (sea pens), opportunistic species with a r-strategy (Sahade et al. 1998b), are also very abundant in these shallow areas. They grow very fast and complete their life cycle within one year.

In order to explain the observed patterns of species distribution, it is important to develop dynamic models based on the main biological and ecological relationships allowing to predict future changes in the community structure. The objective of this work is to present a simple mathematical model that includes biological interactions and physical perturbations in an explanatory framework of the benthic community structure at Potter Cove.

The model

According to the different life traits of benthic organisms we defined four biological compartments. The first compartment (N_1) is formed by pennatulids. These organisms are inferior competitors but exhibit high growth rates. Consequently,

pennatulids are the “pioneer” species that colonize denuded space after physical disturbances. The second compartment (N_2) is the population of *Laternula elliptica,* an infaunal species and able to resist the ice perturbation. This species is dominant in soft bottom down to 15 m together with pennatulids. *Laternula elliptica* has different life traits than pennatulids: It is an infaunal species with periodic recruitment and low growth (Momo et al. 2002); whereas pennatulids are epifaunal animals with quick and non periodical recruitment (Sahade et al 1998a,b). The third compartment (N_3) is formed by depressed shaped ascidians, mainly *Ascidia challengeri* and *Corella eumyota*. These tunicates appear to be less sensitive to sediment disturbance (Sahade et al. 1998a,b). Finally, the fourth compartment (N_4) includes erect ascidians (*Cnemidocarpa verrucosa* and *Molgula pedunculata*) that are capable to exclude other ascidians by competition but are the most sensitive species to physical disturbances. Moreover, inside each compartment (N_3 and N_4), ascidians have different food niche specialization in relation to the filtered particles size (Kowalke, 1999); in consequence, we may assume that intra-compartmental competition is negligible compared to inter-compartment competition.

Our model is a first approximation and had to be simplified in order to allow a mathematical treatment. That is why we did not include all the components of benthic community.The generic form of the model is a Lotka-Volterra competition equation (Hastings 1997) that is given by:

$$\frac{dN_i}{dt} = (r_i - q_i G - s_i H) N_i \left(1 - \frac{\left(N_i + \sum_{j \neq i} \alpha_{ij} N_j \right)}{K_i} \right)$$

where r_i is the intrinsic growth rate for the i^{th} compartment; q_i represents the sensitivity of this compartment to the enhancement of inorganic sediments in the water column; G is the inorganic sediment concentration in the water column; s_i represents the sensitivity to ice disturbances; H is some measurement of the total ice disturbance. For each compartment, there is a carrying capacity K_i. Finally, the terms α_{ij} represent the competitive coefficients of each j^{th} compartment on the i^{th} compartment.

The sediment and ice disturbance (G and H respectively) were represented as relative variables in an arbitrary scale from 0 to 1. G and H influence population dynamics similar as decrements in the effective growth rates. Taking into account the biological traits explained above for each biological compartment, we assumed the following relationships:

$$r_2 \leq r_3 \leq r_4 << r_1; q_2 < q_1 << q_3 < q_4; s_2 << s_1 \leq s_3 \leq s_4$$

Table 1 shows the parameter values used for the model simulations:

Table 1. Symbol, value and units for each parameter included in the model.

Symbol [units]	Value	Symbol [units]	Value
r_1 [day^{-1}]	1.6	s_1 [day^{-1}]	0.06
r_2 [day^{-1}]	0.6	s_2 [day^{-1}]	0.02
r_3 [day^{-1}]	0.55	s_3 [day^{-1}]	0.1
r_4 [day^{-1}]	0.45	s_4 [day^{-1}]	0.55
q_1 [day^{-1}]	0.03	K_1 [Ind m^{-2}]	95
q_2 [day^{-1}]	0.01	K_2 [Ind m^{-2}]	55
q_3 [day^{-1}]	0.1	K_3 [Ind m^{-2}]	50
q_4 [day^{-1}]	0.4	K_4 [Ind m^{-2}]	45
α_{12}	1.3	α_{31}	0.4
α_{13}	1.8	α_{32}	0.9
α_{14}	1.9	α_{34}	1.1
α_{21}	0.6	α_{41}	0.3
α_{23}	0.9	α_{42}	0.85
α_{24}	1.1	α_{43}	0.8

According to our field observations and experience, we consider that the sediment disturbance is highest in the littoral zone and then decreases linearly with depth according to the following equations:

$$G = G_{Max} \Leftrightarrow z \leq z_c$$
$$G = G_{Max} - b_1\left(z - z_c\right) \Leftrightarrow z > z_c$$

Although this assumption is unrealistic it is a useful simplification in order to analyze the model. The stability properties equilibrium points are not strongly affected by the exact form of the function; therefore, for this study is sufficient consider a decreasing function. Ice disturbance can be expressed by a Hill's function, which provides a general form for threshold phenomena (Scheffer 1989), as the following:

$$H = \frac{b_2}{b_2 + z^p}$$

Figure 1 shows the shape of these functions.

Simulations of the model were run under four different scenarios. The first simulation assumed that both kinds of perturbation, ice and sedimentation, are low. The variables *G* and *H* were fixed at 0.1. The second and third simulations represent situations in which one of the two perturbations is important and the

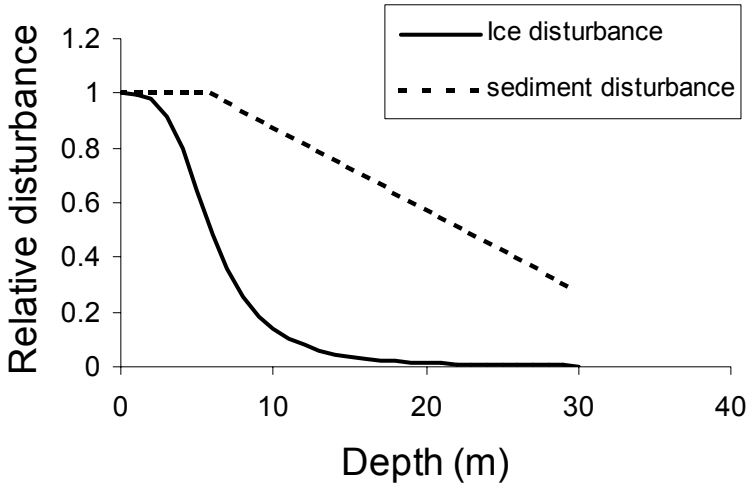

Figure 1: Sediments disturbance and ice disturbance versus depth

other is negligible. So, in the second (ice disturbed), *G = 0.1* and *H = 0.9*; whereas in the third (sediment disturbed), *G = 0.9* and *H = 0.1*. Finally, the fourth simulation considered both perturbations as equally important, and used values of *G = 0.9* and *H = 0.9* too.

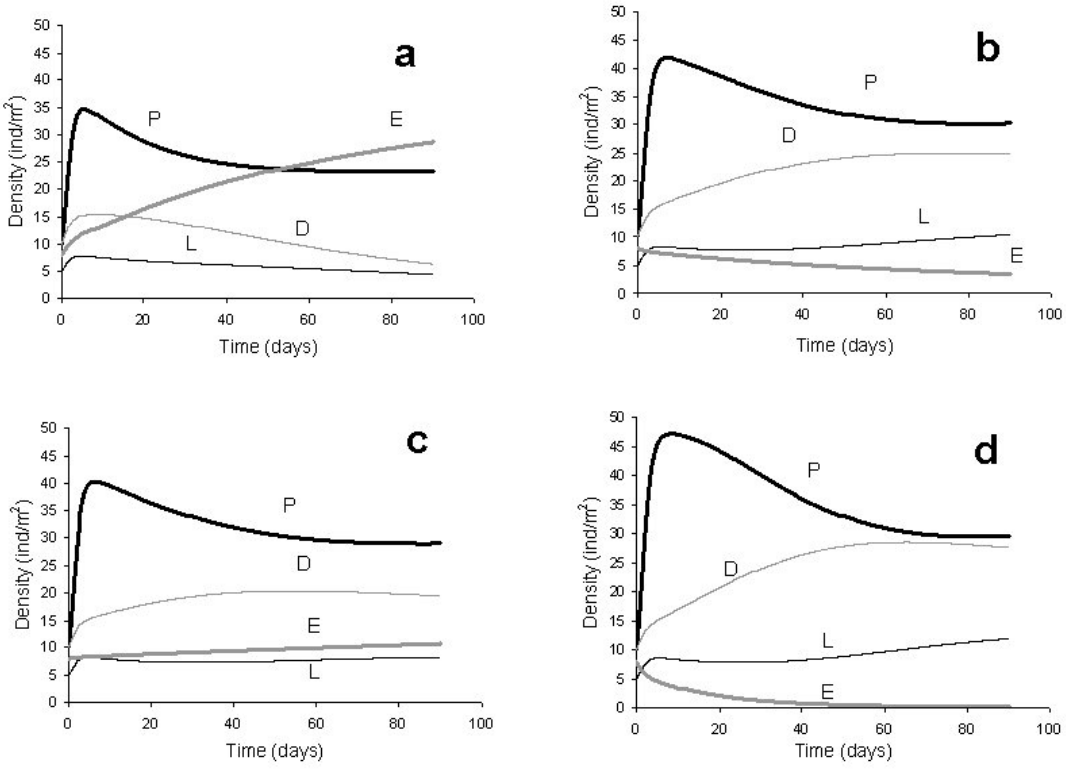

Figure 2: Results of the model under four different scenarios: 2a. Lacking perturbations. 2b. Ice disturbed only . 2c. Sediment disturbed only. 2d. Sediment and ice disturbed (P = pennatulids; E = erect ascidians; D = depressed shape ascidians; L = *Laternula elliptica*).

Results and Discussion

The results of the simulations are shown in Figure 2. In a non-disturbed scenario (Fig. 2a) pennatulids (*P*) and erect ascidians (*E*) are the dominant taxa, whereas depressed ascidians (*D*) and bivalves (*L. elliptica*) are subordinate species. Erect ascidians are dominant because off their competitive capability; on the other hand, pennatulids are favoured by their high reproduction and colonization rates.

In the case of ice disturbance only (Fig. 2b), the density of erect ascidians is diminishing, and depressed ascidians become dominant together with pennatulids. *L. elliptica* is also favoured by the perturbation. When we simulate the sediment disturbance only (Fig. 2c), the situation is similar to 2b, but erect ascidians are less affected. In this scenario depressed ascidians like *Corella eumyota* have a high capacity for particle intake and reduce the negative effects of the high sedimentation (Tatián et al, 1998). Finally, for the concurrence of both perturbations (Fig. 2d), depressed ascidians and pennatulids become highly dominant, *L. elliptica* is benefited and erect ascidians are practically extinct.

It is clear that in areas highly affected by ice action and inorganic sediments two different strategies can be successful: one infaunal (*L.* elliptica) and the other opportunistic with fast colonization of disturbed areas (pennatulids). A gradient of perturbations like that shown in Figure 1 probably will produce a mosaic of situations given a spatial pattern across the cove.

These results coincide with community shifts described by Sahade et al. (this volume). They showed an unexpected change in community structure during only 3 years (94/95 to 97/98) in which dominant organisms at 15 m, bivalves and pennatulids, extended their dominance down to to 20-25 m displacing the previous dominant organisms, mainly ascidians and sponges to deeper waters. It has been suggested that this process could be linked to the Fourcade glacier retreat, which causes the increment of inorganic particulate matter observed in the Cove (Sahade et al., this volume; Schloss et al., this volume). Bivalves and pennatulids are more resistant to sediment load than ascidians and sponges. And among ascidians the species *Ascidia challengueri* and *Corella eumyota* are more tolerant than *Molgula pedunculata*. The density of the latter species is extremely reduced (see Sahade et al. in this volume for more data). Our actual knowledge about the population dynamics of Antarctic benthic filter feeders is still scarce, and the changes could also be related to local extinctions, recruitment events or interannual or interdecadal population variations.

In this context the main challenge is to identify the proxies to assess and predict the effects of the Global Change on the ecosystems, and mathematical models provide a powerful tool for that. Moreover, the Antarctic Peninsula region is probably the best place to do this since changes take place very rapidly.

Acknowledgements. We wish to thank the Hortensia's Foundation for support.

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A decade of fundamental ecological research on storm-petrels at the Tres Hermanos colony, Potter Peninsula, King George Island

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Introduction

Most life history studies on birds are conducted in species where the findings might be masked by several factors like extra-pair paternity, predation effects or sibling competition. Therefore it is of great advantage to focus on species where some of these influences can be excluded. Due to their special life-style the Tubenoses (Aves, Procellariiformes) are most suitable model organisms for studying life history traits. The long-lived seabirds forage exclusively at sea, where they can forager over long distances and only return to their breeding sites for reproduction. Among the Tubenoses open ground-nesting species (e.g. albatrosses) as well as burrow breeders (e.g. prions, storm-petrels) can be found. They lay single-egg clutches and most of them were found to be socially monogamous (Warham, 1990).

The occurrence of breeding sites/colonies for breeding storm-petrels on Potter Peninsula (King George Island, South Shetland Islands, 62°14'S, 58°40'W) had been previously documented (Araya and Arieta, 1971; Aguirre, 1995), but only an extensive survey in 1997/98 revealed a high breeding pair density in the colony around the degraded volcano stump Tres Hermanos (Hahn, 1998a, 1998b). The mixed colony of Wilson's storm-petrels (*Oceanites oceanicus*) and Black-bellied storm-petrels (*Fregetta tropica*) on Potter Peninsula turned out to be a unique study site. The close proximity to Jubany Station/Dallmann Laboratory and the high number of breeding birds in a small area provided a great opportunity for studies of avian ecology. These advantages allowed many research projects to take place in conjunction with a long-term monitoring program carried out by scientists of the University of Jena since the austral summer of 1994/95. Previously, Black-bellied storm-petrels and Wilson's storm-petrels had only been the subject of a few studies and just basic aspects of their life history were known. As the Wilson's storm-petrel is considered to be one of the most abundant seabirds worldwide (Warham, 1990), and play a significant role in the food web of the Southern Ocean. This underlines the importance of fundamental knowledge of their ecology gained at the Tres Hermanos colony and its use as model organism for questions regarding the adaptation to environmental conditions of the Maritime Antarctic.

All studies revealed important insights into population development, e.g. colony size, number and ratio of breeding and non-breeding birds and biometric analyses as well as data of the breeding and behavioural ecology of both species.

Selected results

One of the first studies in the Tres Hermanos colony dealt with Black-bellied storm-petrel's foraging habits. By sampling regurgitates through stomach flushing, we could show that fish and crustaceans are the main components in the chick's diet and this composition was consistent throughout the season. Additionally, feeding frequencies and meal size for Black-bellied storm-petrel chicks were determined for the first time (Hahn, 1998b).
Hahn (2000) quantified the high synchronization between activity time and the solar cycle which was qualitatively described by Beck and Brown (1971). Flight and vocal activities varied with both the diurnal and the seasonal period. Black-bellied storm-petrels were never observed flying over land during daytime. These patterns could be explained as a predation avoidance strategy as the hunting success of their main predators (skuas *Catharacta* spp.; Mougeot et al., 1998) mainly depends on light levels. The seasonal changes in activity pattern correlated with the length of the nights in the proceeding summer resulting in an extension of the daily flight period in the advancing season.
Population estimates in the colony using mist-netting yielded in 1400 – 2280 breeding pairs of Wilson's storm-petrels and 639 – 852 pairs of Black-bellied storm-petrels in the season of 1995/96 (Copestake et al., 1988; Hahn, 1998a). Despite their lower numbers Black-bellied storm-petrels were much more affected by the predation of Skuas (Hahn and Quillfeldt, 1998). They show a more straight-line flight style and have higher wing loading which makes them easier to catch by a predator, an obvious reason for their reduced and highly synchronized nocturnal activity pattern in comparison to Wilson's storm-petrels.
This initial work led to more extensive population studies in Wilson's storm-petrels. For instance Quillfeldt et al. (2000) showed that the extense of black markings in the inner yellow part of the foot web is significantly more developed in breeding birds. Therefore the yellow-black foot web coloration pattern can be used to estimate the proportion of breeders (mature birds) and prebreeders (immature birds) in mist-net catches. As the determination of the reproductive state of seabirds in a breeding colony can be very difficult, this finding is of high relevance.
Wilson's storm-petrels prey primarily in the pelagic marine environment mainly on Antarctic krill (*Euphausia superba*), myctophid fish and amphipods (e.g. Obst, 1985; Wasilewski, 1986; Croxall and North, 1988) and their diet composition was known to be highly affected by temporal and spatial prey variability in the Antarctic (Sarhage, 1988). Quillfeldt (2002b) studied the diet of *O. oceanicus* in the breeding colony on the Potter Peninsula analysing regurgitated food samples in order to test for changes in composition within and between seasons. Breeding birds showed a decrease of krill and an increase of alternative prey from the incubation to the chick-rearing period. However, there was no change in the diet composition of non-breeders which indicates that the seasonal pattern is not simply a response to the decreased krill availability at

the end of the Antarctic summer, but that the increase in the fish component of the diet is caused by selective prey choice of breeding birds to meet the nutrient demand of their chicks.

More recently, analyses of stable isotope composition of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) in feathers and eggs were used to extend former findings (Quillfeldt et al., 2005; Gladbach et al., 2007). The differences in isotope ratios are caused by fractionation during chemical processes, a selective stronger binding of the heavy isotope in comparison to the lighter isotope (Michener and Shell, 1994). Therefore, organisms showed higher δ values than their diet. Because fractionation occurs tissue specifically, isotope composition can be used as archives for diet compositions over different periods (Bearhop et al., 2002). Hence, isotope compositions can be used to determine the trophic level and the foraging area of organisms. In Wilson's storm-petrels we verified a dietary shift between incubation period and chick-provisioning period could be verified with this method (Quillfeldt et al., 2005; Gladbach et al., 2007). Using reference samples from a variety of seabird species, we additionally showed that Wilson's storm-petrels feathers and albumen had a similar $\delta^{15}N$ signature more like that of skuas, who forage mainly on fish and vertebrates, rather than pure krill-feeding penguins. Moreover, $\delta^{13}C$ analyses of feathers indicated four different foraging areas of Wilson's storm-petrels depending on their breeding stage. During egg formation females foraged at the sea ice edge, the area of main krill distribution in the Antarctic spring. Breeding Wilson's storm-petrels fed in the area around the colony, while they migrate to the subtropical front and beyond during the interbreeding period.

Regarding the annual variation in diet composition derived from regurgitation, the food composition differed between the years (Quillfeldt, 2002b). Indices for krill biomass density indices revealed years of low food availability (Siegel et al., 2002). This was in line with results obtained from feeding rates calculated by daily weighing of the chicks (Quillfeldt, 2001). Very low food availability resulted in low feeding rates and high chick mortality up to 49 % (Quillfeldt, 2002b). In years with poor food supply amphipods were taken in a much larger proportion by both breeding and non-breeding birds. This suggests that amphipods are a suboptimal alternative prey only used in situations where there is a shortage of krill and fish, thus leaving breeding and non-breeding birds without possibilities for prey choice.

Studying the chick provisioning of Wilson's storm-petrels, detailed information on chick feeding and chick growth were presented by Quillfeldt & Peter (2000). Variation in offspring growth rates could be explained by different factors with feeding frequency by parents, hatching date and egg size in order of decreasing importance. Additionally, in accordance with previous studies (Wasilewski, 1986) offspring mass development was highly synchronistic with season's date but not with age of the chicks. However, this synchronization could not be explained alone by adverse weather conditions.

Generally, the body size of Wilson's storm-petrels increases from northern to southern populations (Copestake and Croxall, 1985). Thus, the observed increase in meal size from north to south might be partly be explained by a larger carrying capacity of larger birds in the southern colonies (Quillfeldt and Peter, 2000).

The long-term data set of chick provisioning and breeding success in the Wilson's storm-petrel at the Tres Hermanos colony allowed for the preliminary

conclusions about the influence of environmental factors on reproduction of this species (Quillfeldt, 2001; Büßer et al., 2004). The environmental conditions and the resulting food availability varied greatly between the studied seasons. This was reflected in the variation in feeding rates which were correlated with data of the Antarctic krill abundance in the area in the Elephant Island region. Consequently, the overall breeding success (measured as chicks per pair) was highly variable, but was low overall, ranging from 0 to 59% of clutches (Fig. 1).

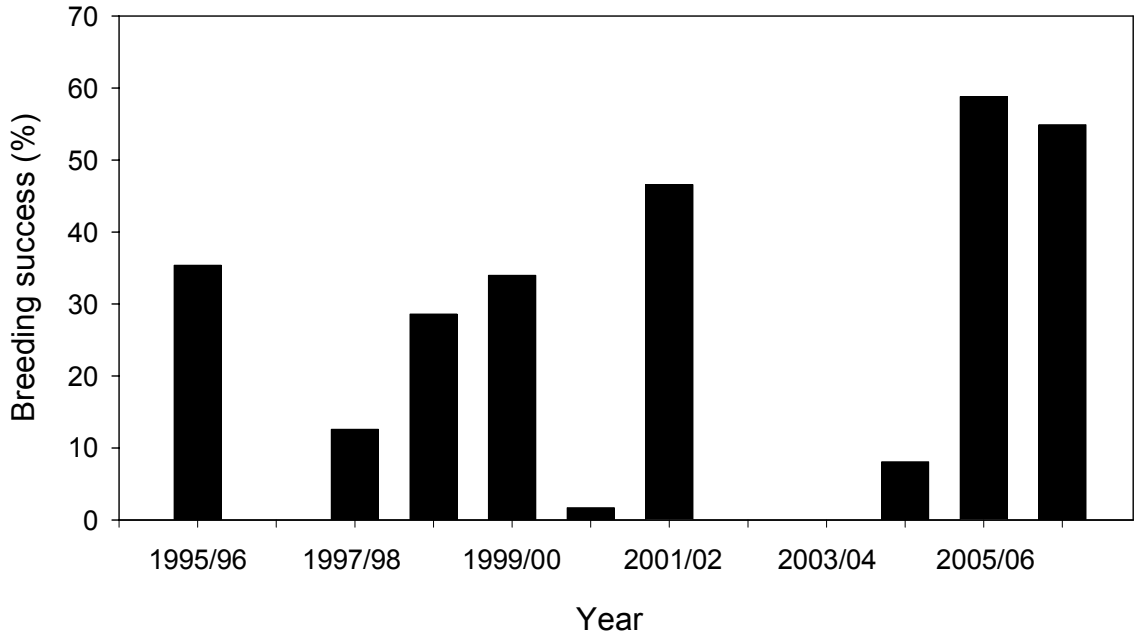

Fig. 1: Breeding success over 11 seasons (no data from 1996/97, total breeding failure in 2002/03 and 2003/04)

Factors that dramatically reduced breeding successes even in years with excellent food availability were long-lasting snow storms and unfavourable wind conditions (i.e. in periods of prevailing easterly winds). The wind directions were found to have a strong influence on the krill availability to storm-petrels (Quillfeldt, 2001; Büßer et al., 2004) as the area around the South Shetland Islands does not have a standing stock of krill (Siegel, 1986), and the transport from the Bellingshausen Sea to the South Shetland Islands of krill may be disturbed by the absence of westerly winds.

Our data provide evidence for a strong correlation between declining breeding success of Wilson's storm-petrels and the less frequent strong winters with extensive sea ice cover caused by the rapid regional warming (Büßer et al., 2004). As Wilson's storm-petrels prey mainly on krill and are highly sensitive to local changes in krill abundance; their feeding rates can be used to assess krill availability in "real-time" during the chick-rearing season between February and April within the foraging zone of the species.

When resources are limited, life-history theory predicts that animals with a long life-span should invest available resources in body maintenance rather than in current reproduction in order to maximise their lifetime reproductive success. To understand how the resources are partitioned between provisioning parents and chicks of the long-lived Wilson's storm-petrel the glucocorticoid excretion was

measured (Quillfeldt and Möstl, 2003). Faecal glucocorticoid concentration was negatively correlated with chick body condition and thus can be used to estimate the physiological stress in the focal chick. In a breeding season with low food availability the faecal and urine glucocorticoid levels were elevated during a period of chronic starvation. In contrast, adults did not show such an elevated glucocorticoid levels suggesting that Wilson's storm-petrels responded to adverse conditions by maintaining their own body condition and reducing provisioning rates to their chicks.

In conjunction with these studies, analyses of the variation in body mass of breeding birds during incubation showed a mass decrease in good seasons, but a mass increase in poor seasons of both males and females (Quillfeldt et al., 2006). This suggests that mass loss during chick rearing is in fact an adaptation to environmental conditions and not primarily a result of stress. Thus adults in poor condition due to diminished food supply may buffer against unpredictable food availability by gaining body mass, even at the expense of their chick.

Furthermore haematology and plasma biochemistry values were used to measure stress factors and provide information on the physiological state and adaptation of individuals to their habitat, changes in nutritional state, body condition and the level of parasite infestation (Quillfeldt et al., 2004). A positive correlation was found between plasma triglycerides and body mass in adult birds. Compared to adults, chicks showed a higher plasma triglyceride level indicating a resorptive nutritional state, during which dietary fat is deposited in adipose tissues. In contrast to adults, well-fed chicks did not show a stress response to handling, while corticosterone levels increased in adults. However, starvation through entombment, caused by snow storms, which blocked the nest entrances for a period of several days, was reflected in a stress response in excess of that of adults. Additional effects were found in terms of ectoparasite load with the feather louse *Philoceanus robertsi*, the abundance of which was positively correlated with corticosterone peak levels. This suggests that the stress response is increased when more stressors act simultaneously.

To determine sexual differences in parental investment the genetic mating system of Wilson's storm-petrels was analysed using Multilocus-DNA-Fingerprinting. No extra-pair paternity and no intraspecific brood parasitism were detected, revealing that Wilson's storm-petrels are socially and genetically monogamous (Quillfeldt et al., 2001).

Our long-term data showed clearly that parental investment related to nest attendance was identical between the sexes (Büßer, unpubl. data). Both sexes provided their single chick equally with food, no differences in feeding frequency and feeding mass were evident, but the variation between pairs as well as between study years was large.

The begging of avian nestlings has often been used to study the parent-offspring conflict and the evolution of signalling. As a species with single-chick brood, storm-petrels are ideal models to study the significance of chick begging during parent-offspring conflict periods, because the influence of nestling competition is eliminated. Analysing the begging calls of Wilson's storm-petrel chicks it was shown that chicks conveyed information about their current nutritional needs by vocalization. Parents responded directly by the size of the delivered meals (Quillfeldt, 2002a).

Outlook

Currently, the focus of ongoing studies lies on the evolutionary significance of sex-specific provisioning within breeding pairs and begging behaviour as well as the continuation of the long-term monitoring of the influence of environmental conditions on the colony. As marine ecosystems are very complex and hard to study, the knowledge of the influence of environmental variability on the Antarctic marine ecosystem is still very limited. The growing concern about the human influence in terms of global warming on the Antarctic ecosystem (Croxall et al., 2002) requires a better understanding of the influence of climatic conditions on the various parts of the food web. In this context storm-petrels, as an abundant marine top-predator in the Maritime Antarctic, could play an important role for a better understanding of the environmental variability in the Southern Ocean. As the Antarctic Treaty Members urge long-term monitoring and sustained observations of the Antarctic environment and the associated data management, to enable the detection, and underpin the understanding and forecasting of the impacts of environmental and climate change (ATCM 2007), the storm-petrel studies should be continued.

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Recent studies on the Antarctic shag *Phalacrocorax bransfieldensis*

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Introduction

In a previous synopsis of research performed in relation to the Potter Cove coastal ecosystem we reported on our studies on the Antarctic Shag *Phalacrocorax bransfieldensis* carried out in the South Shetland Islands between 1991 and 1997 (see Casaux et al. 1998a). There, we provided information on different aspects of the trophic ecology of this bird such as diet composition, variations in the diet among and within seasons and among colonies, variation of the foraging strategy throughout the breeding cycle, colony food requirements during the breeding period and on the feasibility of using the analysis of the diet of the Antarctic Shag to monitor trends in coastal fish populations. Since 1998, our project related to the Antarctic Shag has continued in the South Shetland Islands and has been also extended to the Antarctic Peninsula region.

In this paper we summarise the advances of our project on the Antarctic Shag during the period 1998-2006. Most of this information has been provided in Casaux & Barrera-Oro (2006).

Diving behaviour

The diving depths of the Antarctic shag at Harmony Point, South Shetland Islands, was analysed by the use of capillary-tube depth gauges (Casaux et al. 2001) and Time and Depth Recorders (Casaux & Coria 2004). The maximum diving depth (MDD) and duration recorded for the Antarctic shag is 112.6 m (Casaux et al. 2001) and 5.35 minutes (Casaux & Coria 2004) respectively; these are the deepest and longest duration record for any flying bird in Antarctica. This MDD value is similar to that reported for *Phalacrocorax georgianus* at South Georgia (Croxall et al. 1991, Kato et al. 1992, Wanless & Harris 1993), but deeper than the reported for other sub-Antarctic shags such as *Phalacrocorax nivalis* at Heard Island (Green & Williams 1997) and *Phalacrocorax purpurascens* at Macquarie Island (Kato et al. 1998). On the

contrary, the mean MDD registered for the Antarctic shag is similar or slightly shallower to that reported for *P. purpurascens* but markedly shallower than the registered for the South Georgia shag at South Georgia. The mean MDDs registered for the different shags may reflect the food availability and/or the characteristics of the foraging areas rather than the diving abilities of the species.

The diving behaviour was studied by direct observation on individuals foraging at Harmony Cove (maximum depth = 40 m) (Casaux 2004). The individuals observed foraged in shallow waters (mean = 7.9 m) and presumably dived predominantly aerobically. They displayed relatively short diving bouts (mean = 8.9 min) composed of few dives (mean = 7.1). In some seasons the duration of the dives decrease with the number of dives per bout and increase with the diving depth, whereas the duration of the bouts increase with the number of dives per bout. The mean diving efficiencies of the bouts were not significantly affected by the mean duration of the dives, the number of dives per bout or the mean diving depth. These birds display anticipatory or reactive dives possibly according to the foraging conditions (Casaux 2004). In this sense, the diving strategy seems to be an indicator of the foraging conditions rather than being a feature of the species.

We investigated gender differences in MDD and in diet composition of breeding Antarctic shags at Harmony Point (Casaux et al. 2001). The mean MDD estimated by the capillary-tube depth gauge technique was 37.8 m. Females dived significantly deeper than males and reached the MDD registered (112.6 m). The analysis of the stomach contents recovered when the individuals with capillary-tubes returned to the nest from foraging trips indicated that the stomach contents of males were heavier and contained a lower number of fish, but of larger sizes than those of females. Although fish constituted the bulk of the diet in both sexes, females foraged more intensively on invertebrates than males. *Notothenia coriiceps* was the most important fish prey for both sexes, but whereas males preyed almost exclusively on this species females did mainly on smaller specimens and on smaller species (such as *Harpagifer antarcticus* and *Lepidonotothen nudifrons*). Shallower dives and the capture of larger fish by males may be related to the fact that males deliver more food to the chicks and visit the nest more often than females (see Casaux 1998, Favero et al. 1998). Given that these birds can deplete fish stocks in waters close to their colonies (Casaux et al. 2001, Casaux 2003), the differences observed suggest that individuals of both sexes partitioned foraging depths and food resources, which may diminish the intra-specific competition during the breeding season and probably allows males to undertake a higher breeding effort and/or the population prevent the depletion of the fish stocks around the colony (Casaux et al. 2001).

Foraging strategy

By direct observations on breeding individuals we studied the foraging strategy in the Antarctic Shag at Harmony Cove. Antarctic shags forage once a day (Favero et al. 1998, Casaux & Baroni 2004), usually females early in the morning and males when their partners return to the nest. As chicks grow older

and the energy requirements at the nest increase, the parents increase the number of foraging trips, usually alternating the time at sea.
Antarctic shags forage during daylight hours (Casaux & Baroni 2004) and the extension of this activity varies throughout the season, mainly in relation to the energy requirements at the nest. The starting time of foraging activities in females is positively correlated with the sunrise (Bernstein & Maxson 1984, Casaux & Baroni 2004).

Whereas in the Antarctic Peninsula *P. bransfieldensis* frequently forage in groups of up to 200 individuals (Bernstein & Maxson 1985), breeding individuals at Harmony Cove usually forage solitarily but occasionally in aggregations of 2-8 individuals (Casaux 2004). Foraging in group is advantageous when shags forage in the water column and/or in turbid waters (Van Eerden & Voslamber 1995) or when the prey is patchy distributed (Orians & Pearson 1979). *Notothenia coriiceps*, the main prey of *P. bransfieldensis* around the South Shetlands (Casaux et al. 1998a), is a demersal-benthic fish with a strong site fidelity (Barrera-Oro & Casaux 1996) and is uniformly distributed in rocky bottoms with algal beds. This would explain why solitary foraging is the most common strategy at Harmony Cove, an area with clear waters. However, foraging in large groups might be more frequent when fish availability is scarce (see Casaux 2004) or during the post-breeding period.

Antarctic shags were seen swallowing fish at the surface in only 12 % and 4.3 % of the dives observed by Casaux (2004) in the 1995 (n = 225) and 1996 (n = 211) breeding seasons at Harmony Cove. The fish swallowed at the surface were larger than 15 cm; smaller fish as well as invertebrates may have been ingested underwater. Shags were seen swallowing at the surface up to three fish within a diving bout. At the surface, fish were manipulated in order to be swallowed head-first. It was occasionally observed that during such manipulations the fish caught by shags were kleptoparasited by kelp gulls *Larus dominicanus*, southern giant petrels *Macronectes giganteus* and brown skuas *Catharacta antarctica*.

Agreement between diet and conventional gear

In the previous synopsis we reported for the South Shetland Islands on the good agreement both qualitatively and in relative numbers between the fish species sampled by means of conventional fishing equipment and those represented in pellets of the Antarctic shag in the same site (see Casaux et al. 1998a). Such agreement was also evidenced through recent studies carried out at the Danco Coast, Antarctic Peninsula (Casaux et al. 2002, 2003). Interestingly, among the species caught with nets inshore the South Shetland Islands (reviewed in Barrera-Oro 2002), only *Notothenia rossii* and *Gobionotothen gibberifrons* were absent or scarcely represented in the pellets. For this area this is not surprising, since these species have decreased markedly in trammel-net catches over the last 21 years, due to the offshore commercial exploitation at the end of the 1970s (see Barrera-Oro et al., this issue). This contrasts with the high incidence of *G. gibberifrons* in the diet of shags and in trammel-net catches both at the Danco Coast, reflecting higher availability of this fish in an area remote from the main historical fishing grounds (Elephant Island and north of Livingston/King George Islands) and the Antarctic

Peninsula (Joinville Island) (Kock 1992) The geographical distribution of *N. rossii* barely reaches the Danco Coast area (DeWitt et al. 1990) supporting the low frequency in the diet.

All this information evidence the potential of using the analysis of the diet of the Antarctic Shag to monitor trends in coastal fish populations (Casaux & Barrera-Oro 2006).

Shag populations: status and trends

The estimated population size for the Antarctic Shag is 10900 breeding pairs (Orta 1992). Although this figure might be underestimated, a steady declining trend in the number of breeding pairs of this species has been reported for the last fifteen years at several colonies in the South Shetland Islands and the Antarctic Peninsula (see Casaux & Barrera-Oro 2006).

Among the causes proposed as major perturbations in seabird populations in the Southern Ocean (human disturbance, introduced predators, climatic changes, and changes in availability of preys), only the changes in prey availability could explain the decline observed in the Antarctic Shag breeding populations at the South Shetland Islands. Casaux & Barrera-Oro (1996) suggested that the decline in the inshore populations of *G gibberifrons* and *N. rossii* over the last two decades in shallow waters of the South Shetland Islands may be one factor influencing that trend. Given that the monitoring of the status of the Antarctic shag population in the South Shetland Islands started after the declining process of the fish populations in question, more conclusive data cannot be provided.

At the Danco Coast, Casaux et al. (2002) observed that the fish prey consumed by shags at Py Point differed markedly from those consumed in other close colonies. Among colonies there were marked differences in the size of the fish consumed, the smaller specimens being eaten by shags from Py Point. This was mainly influenced by the number of specimens of the smallest fish prey species, *H. antarcticus*, consumed at that colony. The relative high consumption of *H. antarcticus* at Py Point was related to a scarce availability of *G. gibberifrons* and *N. rossii* around the colony, a scenario that, although related to natural causes, resemble that at the South Shetland Islands. Interestingly, the diet of shags from Py Point was broadly similar to that of shags breeding at the South Shetland Islands (see Casaux et al. 1998a for review). Compared to other two colonies located at the Danco Coast, Midas Island and Primavera Island, the shags from Py Point displayed longer foraging trips and invested more time in foraging activities (Casaux & Baroni 2002). Although at the beginning of the study the number of chicks per nest observed at the three colonies was similar, the breeding output at Py Point was markedly lower, possibly due to the differences in fish prey consumption between these shags and those from Midas Island and Primavera Island (Casaux & Baroni 2002). The facts that *H. antarcticus* lives sheltered under rocks and that larger fish provide proportionately more energy than smaller ones (Hislop et al. 1991) support this view.

In the Antarctic Shag low breeding output and high foraging effort might result in low recruitment and high adult mortality respectively, both factors

adversely affecting the population trend of this species. Considering the information from the Danco Coast reported above, the present low availability of the former abundant prey *G. gibberifrons* and *N. rossii* in inshore waters of the South Shetland Islands (see Barrera-Oro et al. 2000) may be at least partially responsible for the decrease in the number of breeding Antarctic shags in that archipelago.

Advantages of the potential use of antarctic shags as biomonitors

The good agreement between the fish species represented in the diet of the Antarctic Shag and those regularly sampled by means of conventional fishing gears (see above) suggest that this bird could be used as proxy monitors of inshore demersal fish populations. Points supporting this suggestion are:

- demersal fish contribute roughly 99 % of the diet of the Antarctic Shag by mass (Casaux et al. 2001).
- the Antarctic Shag is able to dive up to 113 m deep (Casaux et al. 2001), thus covering the depth distribution range of inshore demersal fish.
- the Antarctic Shag show a strong breeding site fidelity over years (Bernstein & Maxson 1982, Casaux 1998) and forage relatively close to the colonies (usually up to 10 km from the colony; Bernstein & Maxson 1985, Casaux et al. 2004), thus reflecting local conditions.
- the analysis of pellets is an adequate method to estimate qualitatively and, after the development of feeding trials and the estimation of correction factors to compensate for the differential lost and digestion of the otoliths of the different fish preys (see Casaux et al. 1995 and 1998b), quantitatively the diet of the Antarctic Shag (Casaux 2003) and can reflect differences in fish availability between seasons and areas (Casaux et al. 2002).

After six years of testing of a Standard Method implemented in CCAMLR, relative to the analysis of the diet of the Antarctic Shag, it was recognised that the method had the potential to provide information on ecological relationships and changes in populations of certain fish species (CCAMLR, 2003, paragraph 3.57). These species are adult (e.g. *Trematomus* spp., *Lepidonotothen* spp.) and juvenile/early adult stages (e.g. *N. rossii*, *G. gibberifrons*, *N. coriiceps*) of demersal inshore fish populations, including commercially important species (CCAMLR, 2003). Considering the similarities in foraging strategies and reproductive behaviour between the Antarctic Shag and other shags distributed in the Southern Ocean, the methodology proposed may well be readily used with other Antarctic and sub-Antarctic shag species.

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Micromycetes isolated from King George Island, South Shetland Islands, Antarctica

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Introduction

The severe climatic conditions (extremely cold and dry, high UV irradiance) have a significant influence on the components of the Antarctic terrestrial ecosystem. The indigenous Antarctic microorganisms must be closely adapted to survive in these extreme conditions. In a number of previous works the presence of indigenous fungi, either psychrophiles or psychrotolerants, have been described from some Antarctic areas (e.g., Gray & Lewis Smith 1984, Fletcher et al. 1985, Mercantini et al. 1989, Kerry 1990, Baublis et al. 1991, Abyzov 1993, Williams et al. 1994, Vishniac 1996, Zucconi et al. 1996, Azmi and Seppelt 1997, 1998, Chambers et al. 1998, McRae & Seppelt 1999, McRae et al. 1999, Hughes et al. 2003, Onofri et al. 2004, Tosi et al. 2004).

There have been relatively few studies of microorganisms from either maritime or continental Antarctic regions. We have initiated a preliminary screening of the microbiota of Potter Peninsula, the coastal areas of Potter Cove and other marine areas near Jubany Station, King George Island (25 de Mayo Island). An exhaustive sampling was designed to cover soil and sediments under different physical, chemical and biological conditions (pristine, hydrocarbon contaminated, exposed to solar radiation, etc.), as well as microfungi associated with bryophytes, lichens, vascular plants, feathers and wood debris of diverse origin). From the results of this work, carried out during the Antarctic expeditions 1999-1995), we discuss the microfungi isolated and identified in the proximity of Jubany scientific Station during the past eight years of collaboration between the Insituto Antartico Argentino (I.A.A.) and the Rovira & Virgili University (U.R.V.) scientific staff. A number of new reports for King George Island (25 de Mayo Island) and for Antarctica are mentioned.

Materials and Methods

Samples used in this study

Soil samples were collected around Jubany scientific Station (62°14'S, 58°40'W) on King George (25 de Mayo) Island located between 61° 49' 54''S and 62° 17' 13''S, and 57º 26' 11''W and 59º 60' 55''W). All samples were preserved at –20 ºC until processed at the Rovira & Virgili University.

Isolation techniques

In order to recover the fungi in pure culture two different techniques were employed: the direct soil plating proposed by Warcup (1950), for general fungal isolation, and dormant ascospore activation by acetic acid solution described by Stchigel (1999). The latter technique is specifically focused on the selective isolation of ascomycetes (phylum *Ascomycota*; the most numerous of the kingdom *Eumycota*). The employed culture medium was the potato-carrot infusion agar (PCA) to which was added 50 mg l^{-1} of L–Cloramphenicol (to inhibit bacterial growth). Petri dishes were incubated at 10±1 ªC for 12 h in the dark, alternating with 12 h under a cool white fluorescent light.

Results

Using both isolation methods described above, we found that *Thelebolus microsporus* Kimbrough was the most frequently recovered fungus from soil and plant samples. Two strains corresponding to a non-described ascomycete were also detected when the acetic acid activation technique was employed. Their uncoloured ascomata (sexual fruiting bodies) consisted of a cluster of a few naked asci, without the protection of a peridial wall, and without paraphyses. The ascospores were ellipsoidal to fusiform, and with a spinose cell wall. In order to confirm its taxonomical position, we sequenced the ITS region of the nuclear rDNA. The obtained nucleotidic sequence was compared with others from different taxa in the families *Ascodesmidaceae*, *Eurotiaceae*, *Onygenaceae*, *Pezizaceae*, *Sordariaceae* and *Thelebolaceae*. Based on the morphological and molecular results, we described a new genus in the family *Thelebolaceae*: *Antarctomyces psychrotrophicus* Stchigel & Guarro (Stchigel et al. 2001).

Further studies, using the same techniques, permitted us to recover in pure culture two new ascomycetes: *Apiosordaria antarctica* Stchigel & Guarro (*Lasiosphaeriaceae*, *Sordariales*, *Eurotiomycetes*) and *Thielavia antarctica* Stchigel & Guarro (*Chaetomiaceae*, *Sordariales*, *Eurotiomycetes*) (Stchigel et al., 2003). *Apiosordaria antarctica*, isolated from a soil sample, was characterized by ostiolate ascomata with agglutinated hairs, eight-spored, cylindrical asci, and two-celled, irregularly navicular ascospores, with an upper cell ornamented with very small warts and with an apical germ pore. *Thielavia antarctica*, isolated from a lichen source (*Usnea* cf. *aurantiaco-atra*), was characterized by nonostiolate ascomata, with a thick peridium, eight-spored, cylindrical asci, uniseriate, oblate, ovoid ascospores, a slightly protruding apical germ pore, and a phialidic anamorph.

In addition, we noticed a phytopathogenic fungus *Phaeosphaeria microscopica* (Karsten) O. Erikss (Stchigel et al., 2005) on the grass *Deschampsia antarctica* Desv.. This ascomycete species has been described as a monocotyledonous plant pathogen in other parts of the world (Shoemaker and Babcock 1988).

Finally, during the last year of our survey, we recovered some fungal taxa which represent new reports for King George Island (25 de Mayo Island) and for Antarctica.

Doratomyces nanus (Ehremb. Ex Link) Morton & Smith, 1963, *Mycol. Pap.* 86: 80–82.

Description: Grey to nearly black colonies. Synnemata up to 1000 μm high, with ellipsoidal "heads". Conidia ovoid with a truncate base and a pointed apex, verrucose to tuberculate, produced in chains, 6–9 x 5–6 μm.

Distribution: Maritime Antarctic (first report); Europe.

Common substrates: Dead wood and bark, leaves, herbaceous stems, dung, and soil.

Specimen: ANTARCTICA. King George Island (25 de Mayo Island): Jubany scientific base, from soil (sample M-10), 11-XI-1996, col. W. Mac Cormack, isol. A. M. Stchigel (living culture: FMR 9283).

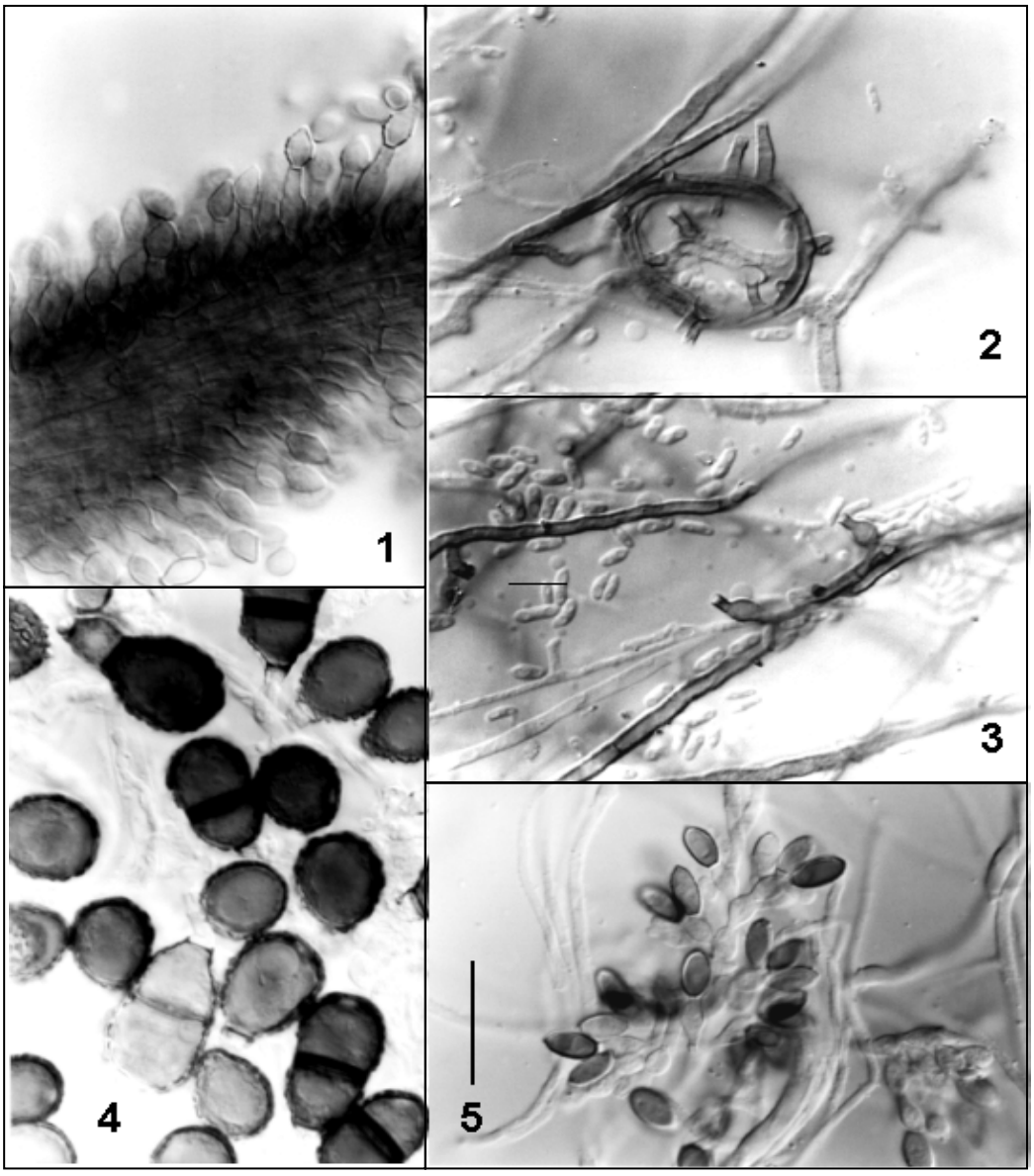

Fig. 1. *Doratomyces nanus*. Detail of a synnema. Figs 2-3. *Phialophora bubakii*. Phialides and conidia. Fig. 4. *Trichocladium asperum*. Conidiophores and conidia. Fig. 5. *Wardomyces humicola*. Conidiophores and conidia. Bar = 30 μm.

Oidiodendron truncatum Barron, 1962, *Can. J. Bot*. 40: 602–604.

Description: Colonies greyish. Conidiophores up to 250 μm high, 3–4 μm wide, brown, smooth-walled. Conidia doliiform, truncate at both ends, spinulose to spinose, produced in chains, 3–6 x 2–3μm.

Distribution: Maritime Antarctic (first report); Canada; Europe; USA.

Common substrates: Soil.

Specimen: ANTARCTICA. King George Island (25 de Mayo Island): Jubany scientific base, from soil (Sample Ant-1), 11-XI-1996, col. W. Mac Cormack, isol. A. M. Stchigel (living culture: FMR 9284).

Phialophora bubakii (Laxa) Schol-Schwartz, 1970, *Persoonia* 6: 66–68.

Description: Colonies greyish-olive to dark brown. Phialides lateral and terminal, 5–10 μm long or larger, 2–3 μm wide, brown to dark brown, smooth-walled, bottle-shaped. Conidia allantoidal, ellipsoidal, or irregularly shaped, smooth-walled, produced in mucilaginous masses, 4–6 x 4–5μm.

Distribution: Antarctica; Canada; Europe; India; USA.

Common substrates: Soft woods, margarine, and soil.

Specimen: ANTARCTICA. King George Island (25 de Mayo Island): Jubany scientific base, from soil (Sample M-15), 11-XI-1996, col. W. Mac Cormack, isol. A. M. Stchigel (living culture: FMR 9285).

Note: This taxon has been previously reported by Abyzov (1993) for Antarctic habitats (ice).

Trichocladium asperum Harz, 1871, *Bull. Soc. impér. Moscow* 44: 125–127.

Description: Colonies grey. Conidiophores 2–5 μm wide, hyaline to pale brown smooth-walled, indistinguishable from the vegetative hyphae. Conidia acrogenous, 1-septate, clavate, ellipsoidal, or obovoid, coarsely verrucose to tuberculate, solitary, 15–40 x 10–15μm.

Distribution: Maritime Antarctic (first report); Americas; Australia; Europe; Mauritius; New Zealand.

Common substrates: Different living and dead plants, mosses, and soil.

Specimen: ANTARCTICA. King George Island (25 de Mayo Island): Jubany scientific base, from soil (Sample M-15), 11-XI-1996, col. W. Mac Cormack, isol. A. M. Stchigel (living culture: FMR 9286).

Wardomyces humicola Hennebert & Barron, 1962, apud Hennebert in *Can. J. Bot.* 40: 1209–1211.

Description: Colonies greyish-olive to dark brown. Conidiogenous cells terminal, doliiform, 3–5 x 2–4 μm, hyaline to sub-hyaline, smooth-walled. Conidia navicular, 1-septate, smooth-walled, slightly constricted at the septum, upper cell greyish-brown to brown, with an equatorial germ-slit, basal cell hyaline to sub-hyaline, 10–14 x 3–4μm.

Distribution: Maritime Antarctic (first report); America; Europe.

Common substrates: Soil.

Specimen: ANTARCTICA. King George Island (25 de Mayo Island): Jubany scientific base, from soil (Sample M-15), 11-XI-1996, col. W. Mac Cormack, isol. A. M. Stchigel (living culture: FMR 9287).

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3. ECOPHYSIOLOGY OF KEY ORGANISMS IN THE ECOSYSTEM

Different flavonoid patterns in *Deschampsia antarctica* and *Colobanthus quitensis* from the marine Antarctic

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Introduction

Life in Antarctica is often seen as being established in an extreme environment. However, evolution has shown that organisms were able to adapt to this environment. In some cases they retained their climate adaptation raised in other environments and thus managed to grow for a long time in Antarctic regions, like both higher plants spread over the Antarctic Peninsula, *Deschampsia antarctica* and *Colobanthus quitensis*. The original natural occurrence is the high altitude region of the Andean mountains. Their distribution over the Antarctic Peninsula is well documented (cf. Moore 1970, Komárková et al. 1990). The ecophysiological survey given by Alberdi et al. (2002) showed, that physiological studies of both plants are scarce, especially if selected for data *raised in the Antarctic environment*. Edwards and Smith (1988) measured photosynthesis and respiration of plants *after transfer* to a lab in England. Experiments on the physiological status of the plants with some measurements performed on Robert Island (not far from King George Island), but mostly under lab conditions, come from Zúñiga et al. (1994, 1996) or Perez-Torres et al. (2006). Studies on cold resistance in Antarctic angiosperms also address anti-freeze proteins (Bravo et al. 2001, Olave-Concha et al. 2005). A field study on CO_2-fixation and Chl fluorescence of *C. quitensis* and *D. antarctica* was published by Xiong et al. (1999).

Many reports on increased UV-irradiation over Antarctica or the Arctic have been published (Hempel 1994, Björn et al. 1999, Huiskes et al. 2003). Marine ecosystems seem to be disturbed by such ozone-hole activities, especially during the Austral spring from October to December, when sea ice cover melts away and UV intensities reach their maximal values (Wiencke et al. 1998, Kirchhoff and Echer 2001, Beyer and Bölter 2002). But a clear conclusion whether terrestrial life is endangered by increased UV-irradiation has not been drawn so far.

Only few papers deal with possible influences of UV-B on both higher plants from Antarctica. Day et al. (1999) performed a UV-B reduction and exclusion experiment in the field and measured growth, bulk plastid pigments and flavonoids. However, UV-B reduction experiments will not allow predictions on plant metabolism under an increase in UV-B. Ruhland and Day (2001) extended these studies to describe influences of reduced versus ambient UV-B on seed banks and total pigments in *C. quitensis*.

Flavonoids are the main shielding compounds against UV-irradiation, but also of importance as antioxidants or in pathogen defence (Smirnoff 2005). Therefore we want to describe the flavonoid patterns in leaves of *D. antarctica* and *C. quitensis*, comparing samplings separated by a time span of 3 years.

The comparison may show whether quantitative or qualitative changes took place with growth time; to our knowledge these analyses are the first direct comparison of flavonoid composition between both plants.

Materials and Methods

During two research expeditions to King George Island (K.G.I., maritime Antarctic, 62°20'S) in 2003 and 2006, samples from *D. antarctica* and *C. quitensis* were collected on the open shores of the island in walking distance from the Dallmann research station. At the selected sampling sites, the plants were abundantly growing. Leaves of 100-150 mg weight were cut from the plants and transferred into vials containing 5 ml methanol per sample. On average, 5 samples per species per sampling date were prepared. Sampling periods were January and February, 2003 and 2006, mostly between 10 am and 4 pm. Additional samplings were done after some plants having been transferred to the Institute of Botany in Innsbruck, Austria. They were kept growing in an unheated glass-house under natural light but only obtaining approx. 10% of the ambient UV. Growth temperature for these plants exceeded average outside temperature by about 3-5 °C.

Climate conditions: during January and February at King George Island in 2003: mean daily temperatures: +2 to +9°C, in wind protected sample plots up to +14°C (in air close to cushion), often sunny, two days with light snow fall, occasionally overcast, seldom fog. Observations for 2006: mean daily temperatures of 0 to +7°C, occasionally sunny (31.1.06), often overcast, windy, several snow falls (19.2.06). Own simple PAR light measurements showed 500 –900 µMol photons on overcast days, but up to 2300 µMol photons PAR on sun exposed cushions in direction of the sun, as often leaf orientation is. Detailed meteorological data, over longer periods of time, accompanied by landscape and geological information, can be found in Wiencke et al. (1998) and Beyer and Bölter (2002).

Flavonoid assays: The samples (leaves in methanol) were stored until transport back to the Institute of Botany at –20°C; later until processing at –53°C. During storage about all flavonoids were extracted into the solvent, therefore two small volumes of methanol for re-extraction removed remaining pigments completely. Separation and quantification was done on an Agilent-HPLC system with diode array absorbance spectra detection according to the method described by Turunen et al. (1999), which also was found to be useful to describe flavonoids in a number of alpine and arctic plants. Semi-quantification of peak amounts were performed as relative absorption units per g fresh weight. In both plants, the special leaf structure did not allow an accurate measurement of leaf surface as an additional reference.

Results and Discussion

The experimental setup allows a good extraction and separation of soluble flavonoids, but the tightly cell wall bound compounds, including most phenylpropanes, could not be assayed. The separation of the vacuolar flavonoids by means of high resolution HPLC with samples harvested in 2003 resp. 2006 showed nearly identical composition in both species, no additional peaks were found in this comparison. In Fig. 1 representative separations are given. Six

main compounds with absorptions in the spectral region of 300-400 nm were selected as possible UV screening pigments for quantification and further identification. Other, always minor peaks recognized at the detection wavelength of 280 nm, but without relevant absorption above 290 nm (lower limit of current UV-B/UV-A irradiation input) were not considered. In *C. quitensis*, these compounds appeared between 25.0 and 31.0 min separation time with B, C and E as main peaks. In *D. antarctica*, the selected peaks separated between 18.0 and 31.5 min, main compounds are C and D. Both separations indicate a quite different, plant specific composition of UV-A absorbing flavonoids.

The insets in Fig. 1 present absorption spectra of different peaks: in *C. quitensis*: only peak A absorbs maximally at 313.5 nm, peak D at 338.0 nm and peaks B, C, E and F at 349.0 nm. The data for *D. antarctica*: peaks A and B: 324.5 nm, C: 350.0 nm, D,E and F: 352.0-353.0 nm. In table 1 the relative amounts of flavonoids are given for three samplings in 2003, two samplings in 2006, and for some samples taken from plants which, after the transfer from Antarctica to the Institute of Botany, were kept in a greenhouse (see Methods).

For samples extracted directly at King George Island, *C. quitensis* showed relative constant amounts of peaks A to E over the sampling period in 2003, only peak F is reduced by about 30%. In 2006 (the first harvest occurred 10 days later compared to 2003) during the 20 days span between samplings most compounds were strongly reduced. Except peak A, the plants from 2003 accumulated more flavonoids than those in 2006. Already from visual observation, *C. quitensis* seemed to develop senescence in February, which may explain the reductions especially for peaks B, C, E and F. Interestingly, the amounts measured at 31.1.2006, before senescence started, represent about the amounts in average from 2003.

Despite the different weather in both years (see methods), there is a remarkable stable equipment of UV-A absorbing flavonoids in this plant. After transfer of such plants to the institute for growth in a greenhouse under warmer and reduced UV conditions, no new compounds could be detected via HPLC. Strong reductions occurred in all peaks except for F, which remained about constant in concentration.

For *D. antarctica*, harvested on the island in 2003, a continuous reduction of all compounds with growth time occurred. In 2006, the 20 days of difference between the two harvests showed reductions only for peaks C and D, while B and F increased strongly, and peak A and E did not change considerably. Generally, this plant accumulated much more compounds in 2006. *D. antarctica* as a typical *Poaceae* seems to be more stable in growth because of continuous development of leaves from their basis compared to *C. quitensis* as a *Caryophyllaceae*. The former adopts to colder weather in February with increasing some flavonoids, as it is well known for several other species, via induction of the phenylpropane metabolism (Grace 2005), while the latter starts to decompose some constituents during the Antarctic autumn. Growth of *D. antarctica* under greenhouse conditions again resulted in strong reduction of all compounds, and in parallel to *C. quitensis*, compound F keeps about the same level after 6 months in the 2003 samples, and is reduced further 2.5 years later. The 6 months glasshouse period for the 2006 samples support the expected reductions because of higher temperature and much less UV-irradiation, but the plants did not visually show any decomposition.

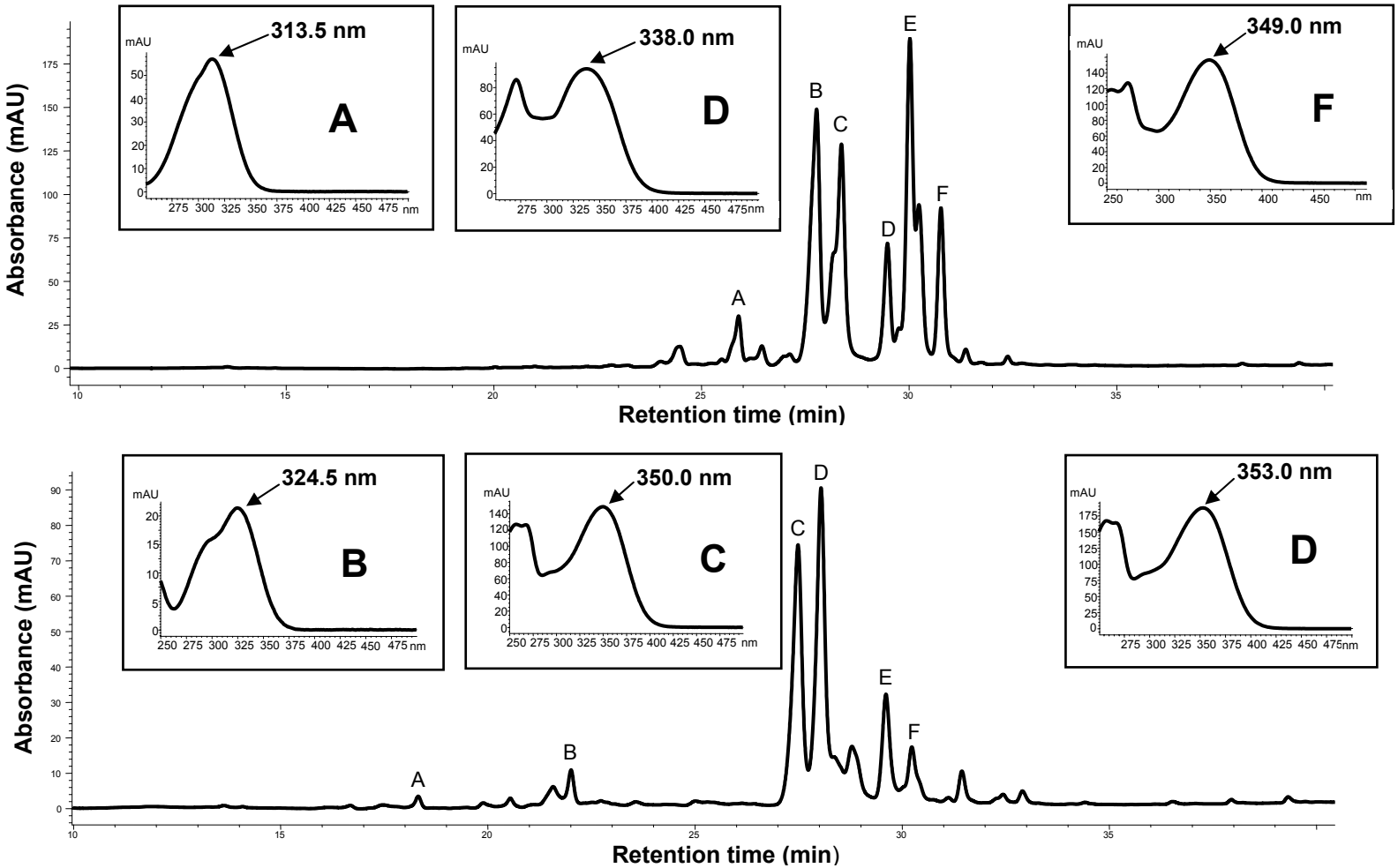

Fig.1. Separation of flavonoids and related compounds from *C. quitensis* (upper) and *D. antarctica* (lower). Peak labelling indicates the compounds as are listed in tab. 1. Detection wavelength: 280 nm; the labelled peaks have additional absorption maxima between 300 and 400 nm (insets).

Colobanthus quitensis

Date	Peak A	Peak B	Peak C	Peak D	Peak E	Peak F
21.01.2003	328 ± 7	1822 ± 171	1443 ± 93	1099 ± 100	2513 ± 261	1066 ± 58
29.01.2003	292 ± 35	1902 ± 375	1455 ± 325	1005 ± 47	2165 ± 167	787 ± 60
08.02.2003	309 ± 11	1778 ± 250	1161 ± 102	1079 ± 86	2377 ± 117	763 ± 89
31.01.2006	373 ± 45	1637 ± 462	1405 ± 411	814 ± 134	2297 ± 371	892 ± 108
19.02.2006	327 ± 14	1214 ± 134	974 ± 99	621 ± 24	1495 ± 109	554 ± 53

Greenhouse – Plants from Antarctica 2003

Date	Peak A	Peak B	Peak C	Peak D	Peak E	Peak F
12.08.2003	84 ± 31	309 ± 4	118 ± 12	396 ± 32	655 ± 59	715 ± 106

Deschampsia antarctica

Date	Peak A	Peak B	Peak C	Peak D	Peak E	Peak F
20.01.2003	169 ± 18	276 ± 45	5571 ± 462	5666 ± 517	867 ± 52	676 ± 66
29.01.2003	117 ± 2	225 ± 25	5278 ± 293	4116 ± 545	777 ± 61	615 ± 57
08.02.2003	106 ± 8	172 ± 33	3708 ± 213	3648 ± 339	519 ± 46	508 ± 48
31.01.2006	248 ± 47	498 ± 102	11884 ± 2037	12447 ± 2123	2283 ± 370	987 ± 277
19.02.2006	205 ± 73	725 ± 316	7076 ± 1814	8914 ± 2608	2248 ± 717	1616 ± 694

Greenhouse – Plants from Antarctica 2003

Date	Peak A	Peak B	Peak C	Peak D	Peak E	Peak F
12.08.2003	44 ± 7	210 ± 45	318 ± 37	367 ± 92	370 ± 24	514 ± 81
26.06.2006	--	28 ± 2	533 ± 43	1055 ± 68	756 ± 1	359 ± 39

Greenhouse – Plants from Antarctica 2006

Date	Peak A	Peak B	Peak C	Peak D	Peak E	Peak F
26.06.2006	--	24 ± 13	672 ± 97	1115 ± 208	704 ± 90	390 ± 80

Tab.1 Relative contents (mAu/g FW) of selected flavonoids from *D. antarctica* and *C. quitensis*, respectively, shown for the years of sampling 2003 and 2006

The chemical identification of individual peaks is not finished, because references for several compounds are not available. For *C. quitensis* comparative runs were made with pure apigenin, kaempferol, luteolin, myricetin and quercetin, as common in European plants (Turunen et al. 1999, Packer 2001, Grace 2005), but the selected peaks did not have identical spectra by means of HPLC. Peak A in *C. quitensis*, and peaks A and B in *D. antarctica*, displayed only one maximum at shorter wavelengths and may indicate phenylpropanes. The remaining compounds have a different peak shape from 260 nm on, and if main absorptions occur higher than 330 nm, derivatives of apigenin and luteolin should be discussed.

The identification of compounds absorbing near 350-353 nm in *D. antarctica* was made possible by comparison with HPLC-standard; they represent luteolin derivatives. A first assay of flavonoids for this plant was given by Webby and Markham (1994) who extracted dried leaves and separated flavonoids by means of 2-D paper chromatography. Spots analysed by 13C NMR revealed mainly different luteolin glycosides like orientin, isoswertiajaponin and lower amounts of tricin and isoswertisin. In our separations, peaks C, D, E and F represent different luteolin glycosides, based on spectral comparisons and thus support the earlier work. Montiel et al. (1999) determined UV-exclusion effects as well as enhanced UV on photosynthesis in both higher plants and in a moss: enhanced UV did not inhibit photosynthesis, and a first separation of flavonoids showed some compounds increasing with additional UV. The HPLC separation method used was different to ours, which does not allow a direct comparison, and the authors did not identify any compound.

D. antarctica and *C. quitensis* did develop enough resistance to grow on the Antarctic continent or the subantarctic islands before human impact developed. They have no commercial value but belong to a highly protected nature, which may now be threatened by global change. However, changing UV environment as part of global changes will not negatively affect both plants in Antarctica: their original occurrence is the world of the Andean mountains, with much higher impact of UV compared to Antarctica: *C. quitensis* can be found up to Ecuador and as high as 4200 m a.s.l , and *D. antarctica* up to Central Chile and adjacent Argentina (Lewis Smith 2003, Gianoli et al. 2004). UV-radiation in high mountain environments is higher due to an increase in altitude, less influenced by ozone hole effects, but reduced at lower latitudes (Caldwell and Robberecht 1980, Blumthaler et al. 1997). In earlier experiments (author CL, unpublished) *D. antarctica* from the maritime Antarctic has been exposed to about 40% more UVB/UV-A (filter cut off 290 nm) than measured at Palmer station for two weeks at about growth site temperatures. The treatment did not have considerable influences on photosynthesis or plastid pigments, but increased the total antioxidant pool (which also contains flavonoids).

The amplitudes of flavonoid accumulation in both Antarctic phanerogames are therefore regarded as sufficient to cope with some increasing UV irradiation. A more negative effect on Antarctic biota and species composition or competition may arise from increasing temperature, resulting already in expansion of vascular plants (Gerighausen et al. 2003, Karentz 2003). Increasing temperatures will also increase a pressure of fungal or bacterial infections on both plants or of invertebrates feeding on them. Again, a good flavonoid accumulation will serve as antioxidant and pathogen-inhibition system, helping *D. antarctica* and *C. quitensis* to survive in their environment.

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Finding the scattered pieces of the mechanisms behind the formation of volatile halogen-containing C_1- and C_2-compounds by polar and cold-temperate macroalgae

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Introduction

To avoid a collapse of the stratospheric ozone layer against solar ultraviolet radiation, the production and consumption of man-made ozone depleting substances, i.e. halogen-containing volatile hydrocarbons, is now controlled by international regulations (UNEP 1987). Beside the industrial emissions, also the natural emissions of volatile organohalogen compounds were investigated and several marine and terrestrial sources of volatile organohalogen compounds were discovered (Laturnus 2003). Extrapolations of global emissions of volatile organohalogen compounds from natural sources into the atmosphere revealed sources strengths comparable to the industrial input (Laturnus 2003). While volatile organohalogen compounds are quite important in atmospheric chemical reactions (Solomon 1999), almost nothing so far is known why and how organisms produce these compounds. Suggestions to explain the function include chemical defence (Fenical 1975), improved resistance against microbial attacks (McConnell and Fenical 1977) and side production in the metabolic system (Küpper et al. 1998). For the formation of volatile organohalogen compounds, an enzymatic mechanism is suggested, based on the halogenation of organic matter by haloperoxidases (Neidleman and Geigert 1986). However, whether or not volatile organohalogen compounds result from the halogenation of organic matter is still unknown. The present study correlates the release rates of different volatile organohalogens determined from various macroalgae to identify similar formation pathways of these compounds, and, thus, to find additional pieces of the puzzle on the mechanism behind the formation of volatile organohalogen compounds.

Material and Methods

Sixteen marine macroalgal species originating from temperate and polar regions were investigated for their release of volatile organohalogens under controlled laboratory conditions. The algae were collected on the shores of King George Island (Antarctica), Spitsbergen (Arctic), and Helgoland (North Sea). Unialgal cultures were obtained from algal reproductive cells and kept in climate chambers. The algal cultures were illuminated by photosynthetic active radiation (PAR) provided by cool-white fluorescent tubes (Osram L58/W19, 23 µmol photons m^{-2} sec^{-1}, 700–400 nm, light-to-dark cycle 16 to 8 hours). Details pertaining to the procedure and the conditions for cultivating marine macroalgae are described by Clayton and Wiencke (1986) and Wiencke and Clayton (1990).

Table 1. Macroalgae of Temperate and polar origin used in this study.

Macroalgal species	Incubation conditions	
	Temp-erature	Photon fluence rate
	[°C]	[µmol photons m^{-2} sec^{-1}]
Brown macroalgae		
Saccharina latissima (L.) Lane, Mayes, Druehl et Saunders (Spitsbergen, Arctic)[e]	0	27-30[a]
Saccharina latissima (L.) Lane, Mayes, Druehl et Saunders (Helgoland, North Sea)[e]	10	12-16[a]
Laminaria digitata (Hudson) Lamouroux (Helgoland, North Sea)	10	12-16[a]
Laminaria hyperborea (Gunnerus) Foslie (Helgoland, North Sea)	10	14-18[a]
Desmarestia antarctica Moe & Silva (King George Island, Antarctica)	0	27-30[a]
Desmarestia anceps Montagne (King George Island, Antarctica)[j]	0	12-16[b]
Green macroalgae		
Lambia antarctica (Skottsberg) Delépine (King George Island, Antarctica)	0	15-20[b]
Ulva clathrata (Roth) C. Agardh (Puerto Williams, Chile)	0	15-20[c]
Acrosiphonia sonderi (Kützing) Kornmann (Disko Island, Greenland)[d]	0	15-20[c]
Acrosiphonia sonderi (Kützing) Kornmann (Disko Island, Greenland)[d]	0	15-20[c]
Enteromorpha bulbosa (Suhr) Montagne (King George Island, Antarctica)[5]	0	15-20[c]
Ulva lactuca L. (Disko Island, Greenland)	0	15-20[c]
Acrosiphonia arcta (Dillwyn) Gain (Disko Island, Greenland)	0	15-20[c]
Red macroalgae		
Phycodrys austrogeorgica Skottsberg (King George Island, Antarctica)	0	15-20[c]
Neuroglossum ligulatum (Reinsch) Skottsberg (King George Island, Antarctica)	0	15-20[c]
Palmaria decipiens (A. und E.S. Gepp) Kylin (King George Island, Antarctica)	0	15-20[c]

a) 18:6 hours light-to-dark cycle
b) fluctuating light-to-dark cycle (17.5:6.5 hours at time of the incubation)
c) fluctuating light-to-dark cycle (18.5:5.5 hours at time of the incubation)
d) different individuals from the same population
e) formerly *Laminaria saccharina* (L.) Lamouroux

Entire macroalgal thalli (3–5 g fresh weight each sample) were incubated for twenty-four hours in closed glass flasks (volume 250–300 ml) equipped with glass stoppers and PTFE collars. The flasks were filled, leaving no headspace, with sterile filtered seawater (0.25 µm filter, Sartorius, Sartobran II) collected from the North Sea and enriched with nutrients after Provasoli. The composition and concentration of the enriched media is described in more detail by Starr and Zeikus (1987). During the incubation period, the algae were exposed to PAR provided by one cool-white fluorescent tube (Osram L58/W19, 23 µmol photons m^{-2} sec^{-1}, 700–400 nm). The photon flux of PAR was measured with a Licor LI-185B quantum radiometer and a Licor LI-190 B 2π quantum sensor. During the incubation period the medium in the glass flask was mixed by a magnetic stirrer to support an even distribution of organohalogens in the medium and to avoid nutrient limitation in the vicinity of the algae. Six replicates of each macroalgal species were investigated in each incubation experiment. In the control experiments, the culture medium without algae was exposed to the same conditions as the algal cultures.

At the end of the experiments, the culture medium containing the volatile organohalogens was poured into 120 mL glass bottles. The bottles were sealed with crimper caps (aluminium caps with PTFE septum, Chromacol Ltd., UK) and stored at 4 °C. Analysis of the first replicate started immediately after termination of the incubation experiment. Prior to analysis, the glass sample bottles were kept for 30 minutes at room temperature in the dark. One hundred

mL of the culture medium in the glass sample bottle was analyzed for volatile organohalogens by automated purge-and-trap gas chromatography and electron capture detection using liquid nitrogen for pre-concentration (Chrompack CP9000 with Chrompack automated purge-and-trap injector).

Table 2. Release rates of volatile organohalogens used in this study for statistical calculations. Negative values corresponded to a decrease of volatile organohalogens in the culture medium. CH_2Cl_2 – dichloromethane, $CHCl_3$ – chloroform, CH_3CCl_3 – 1,1,1-trichloroethane, CCl_4 – tetrachloromethane, C_2HCl_3 – trichloroethene, C_2Cl_4 – tetrachloroethene, $CHBrCl_2$ – bromodichloromethane, $CHBr_2Cl$ – dibromochloromethane, CH_2Br_2 – dibromomethane, $CHBr_3$ – bromoform, 1,2-$EtBr_2$ – 1,2-dibromoethane, CH_3I – iodomethane, CH_2ClI – chloroiodomethane, CH_2I_2 – diiodomethane.

	CH_2Cl_2	$CHCl_3$	CH_3CCl_3	CCl_4	C_2HCl_3	C_2Cl_4	$CHBrCl_2$
	[pmol g^{-1} fresh weight day^{-1}]						
brown							
S. lat.[1]	-1.57	0.46	0.10	0.12	0.48	0.07	3.59
L. sac.[2]	0.75	0.90	-0.02	-0.05	-0.02	0.01	7.63
L. dig.	0.33	0.04	-0.07	-0.08	-0.05	-0.07	1.37
L. hyp.	0.56	0.11	0.07	0.04	0.05	0.01	1.05
D. ant.	-0.18	0.01	-0.02	-0.05	-0.01	-0.03	0.07
D. anc.	1.59	0.20	0.21	0.16	0.09	0.12	4.62
green							
L. ant	0.65	-0.01	0	-0.02	0	-0.02	0
U. cla.	-12.36	0.03	0.02	0.05	0.13	0.26	1.57
A. son.	-12.89	0	0.02	0.04	0.14	0.24	0
A. son.	-12.49	0.02	0.03	0.07	0.14	0.28	0
E. bul.	-12.89	0.02	0.03	0.08	0.16	0.31	0.10
U. lac.	-15.31	0.04	0.05	0.13	0.21	0.45	0.02
A. arc.	-13.55	0	0.04	0.10	0.20	0.44	0.04
red							
P. aus.	7.15	0.37	0.01	0	0.04	0.06	0.54
N. lig.	9.97	0.01	0.01	0.03	0.06	0.11	0.03
P. dec.	12.25	0	0	0.03	0.08	0.13	0.01

	$CHBr_2Cl$	CH_2Br_2	$CHBr_3$	1,2-$EtBr_2$	CH_3I	CH_2ClI	CH_2I_2
	[pmol g^{-1} fresh weight day^{-1}]						
brown							
S. lat.[1]	14.47	16.25	54.92	19.88	1.42	2.83	20.45
L. sac.[2]	15.36	10.95	136.17	2.82	0.75	0.03	11.58
L. dig.	18.10	0.82	62.98	0.01	-0.70	-0.07	-0.31
L. hyp.	12.23	4.64	39.22	1.63	14.91	0.09	0.54
D. ant.	3.13	4.57	11.91	0.86	-0.16	0.01	-0.16
D. anc.	17.78	6.79	85.38	0.32	0.20	-0.01	-0.19
green							
L. ant	0.06	-0.01	0.49	0	0.77	-0.03	-0.11
U. cla.	14.16	1.93	44.01	0.06	4.08	-0.01	-0.14
A. son.	0.16	-0.05	2.23	-0.02	-0.39	-0.03	-0.15
A. son.	0.05	0.03	0.06	0.11	-0.40	0.02	-0.16
E. bul.	6.55	3.07	26.08	0.90	-0.18	0.21	-0.17
U. lac.	1.52	0.05	12.46	0	-0.27	-0.01	-0.19
A. arc.	1.72	0.06	14.45	0.04	-0.49	-0.03	-0.17
red							
P. aus.	0.65	0.07	0.63	-0.01	1.01	0	-0.04
N. lig.	1.12	0.19	6.17	-0.01	0.13	-0.01	-0.06
P. dec.	0.03	0.13	-0.11	-0.01	-0.14	-0.02	-0.08

[1]Spitsbergen, Arctic
[2]Helgoland, North Sea

The sample was transferred with a syringe into the purge-and-trap unit. Helium was used as a purge gas at a purge flow of 40 mL for 15 minutes. The compounds were separated on an Rt_x-volatiles column (Restek, 40 m length, inner diameter 0.32 mm, film thickness 3 µm). The temperature program was set to 50 ^oC isothermal for 10 minutes, a heating rate of 4 ^oC min^{-1}, and 150 ^oC

isothermal for 5 minutes. The total run time was 40 minutes. The compounds detected were methyl iodide, diiodomethane, chloroiodomethane, dichloromethane, chloroform, 1,1,1-trichloroethane, tetrachloromethane, trichloroethene, tetrachloroethene, dibromomethane, bromodichloromethane, dibromochloro-methane, bromoform, and dibromoethane. Each compound was identified and its concentration calculated by adding a calibration standard of the pure compounds (p.a.) in methanol directly to 100 mL of pre-purge Milli-Q water.

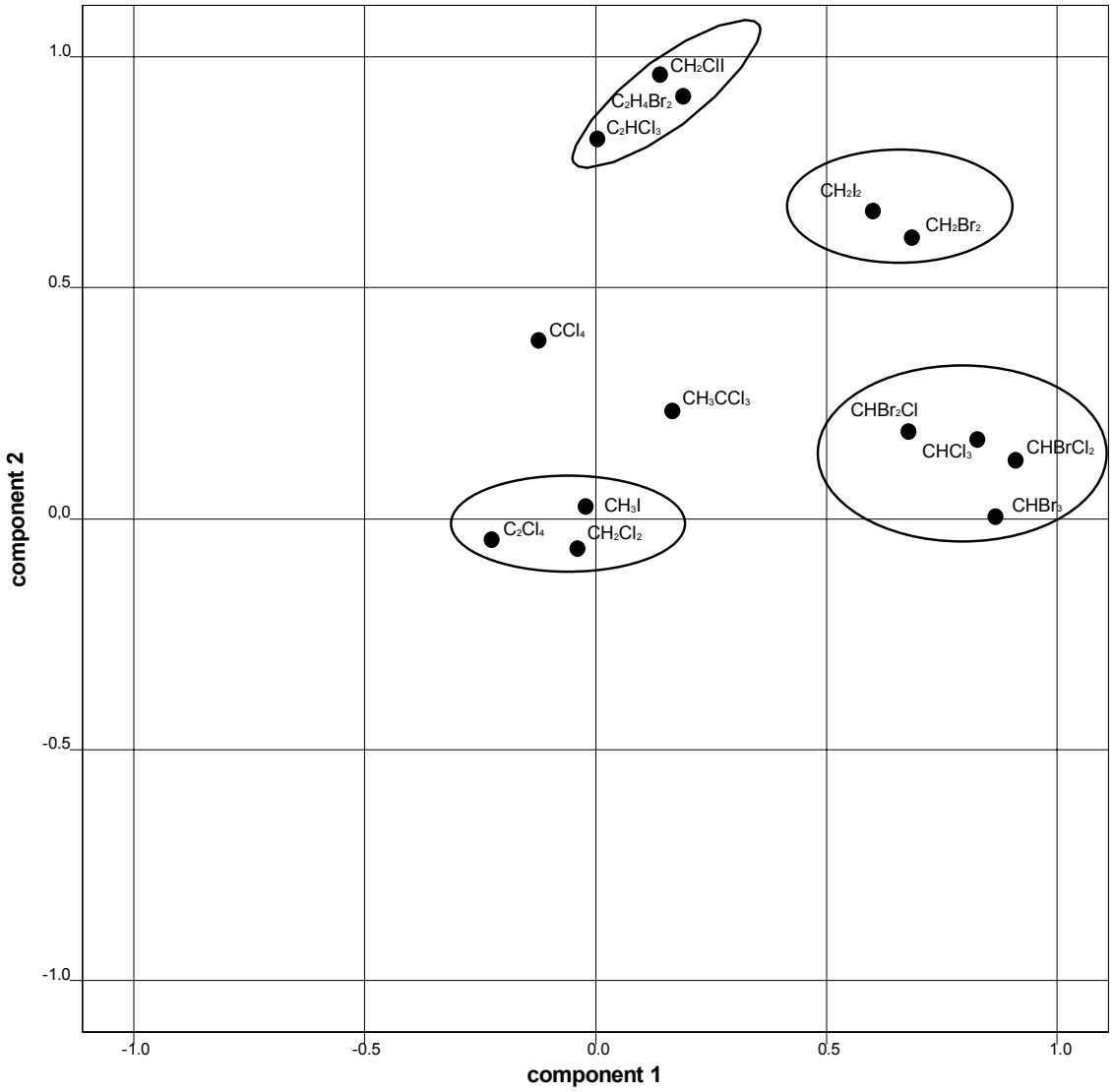

Figure 1. Component matrix of several volatile organohalogens using Principal Component Analysis (PCA). CH_2Cl_2 – dichloromethane, $CHCl_3$ – chloroform, CH_3CCl_3 – 1,1,1-trichloroethane, CCl_4 – tetrachloromethane, C_2HCl_3 – trichloroethene, C_2Cl_4 – tetrachloroethene, $CHBrCl_2$ – bromodichloromethane, $CHBr_2Cl$ – dibromochloromethane, CH_2Br_2 – dibromomethane, $CHBr_3$ – bromoform, 1,2-$EtBr_2$ – 1,2-dibromoethane, CH_3I – iodomethane, CH_2ClI – chloroiodomethane, CH_2I_2 – diiodomethane.

The water containing the standard then was purged for 15 minutes, and the results obtained for the areas were used to calculate the compound concentrations corrected for recovery. Detection limits ranged from 0.02 to 0.12 pmol L^{-1}, and recovery efficiencies ranged from 47 to 100%. Randomly selected

samples were analyzed on a column with a different stationary phase (SPB-624 column, Supelco, length 60 m, inner diameter 0.25 mm, film thickness 1.4 µm). The results were compared with those from a calibration standard run on the same column to increase the reliability of the compound identification.

Results

The release rates used for the statistical calculations are given in Table 2. Further details and discussion of the released amounts can be obtained from Laturnus et al. (2007).

To obtain a general overview on the correlation between the released volatile organohalogens, a non-paramatic Pearson Correlation Tau test was applied and from the results obtained a Principal Component Analysis was performed to identify volatile organohalogens, which may have similar formation mechanisms. The results of the PCA are given in Figure 1. Four groups can be isolated with different significance. The strongest group consisted of diiodomethane and dibromomethane, followed by the group of chloroiodomethane, dibromoethane, trichloroethene, and the group of dibromochloromethane, bromodichloromethane, bromoform, chloroform. The group of methyl iodide, dichloromethane and tetrachloroethene showed the least significance of all in correlation, while tetrachloromethane and 1,1,1-trichloroethene apparently are not connected to any of the other volatile organohalogens.

Discussion

It is known that the general mechanisms behind the natural formation of organohalogen compounds by macroalgae is based on enzymatically controlled reactions of organic matter, halogens and hydrogen peroxide (e.g. Geigert et al. 1984, Yamada et al. 1985). Haloperoxidases, an enzyme group detected in a wide range of marine and terrestrial organisms (Yamada et al. 1985) can catalyse the oxidation of halogens in the presence of hydrogen peroxide to form halogenated organic compounds (1).

$$\text{(1)} \qquad \text{organic matter} + H_2O_2 + X^- + H^+ \xrightarrow[\textit{e.g.}\ \text{haloperoxidases}]{\text{enzymic catalyzation}} \text{halogenated compounds}$$

$$X^- = Cl^-, Br^-, I^-$$

An earlier study already reported a correlation between the release of volatile organohalogen compounds by marine macroalgae and the occurrence of a halogenating activity in the macroalgae (Laturnus et al. 1998). Investigations of different algal parts revealed the blade as the most important place with respects to halogenating activity and release of volatile organohalogens (Laturnus 1996). However, which mechanisms cause the formation of volatile organohalogen compounds is not yet known in detail.

Another pathway may be the reaction of hypobromeous acid, an extremely reactive compound, forming together with organic matter volatile organohalogen

compounds. Hypobromeous acid can be formed by haloperoxidases located near the algal surface and can then be released into seawater (Wever et al. 1991). Van Pée and Unversucht (2003) discussed a novel type of halogenating enzymes called halogenases, which instead of using hydrogen peroxide require NADH, the reduced form of nicotinamide adenine dinucleotide, a co-enzyme involved in biological oxidation-reduction processes. Due to their substrate specificity and regioselectivity, not found in haloperoxidases, halogenases are likely the enzymes involved the in formation of halometabolite..

While the halogenation of organic matter in marine macroalgae is basically understood, the formation mechanisms for halogenated C_1 to C_4 compounds still remains speculative only. Metabolic pathways to synthesize volatile organohalogen compounds, such as bromoform or dibromomethane, have been discussed by some authors (e.g. 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, such as bromoform or dibromomethane. Significant linear correlations between the two compounds were an indication for the occurrence of this mechanism (Laturnus 1995). However, the results of our study revealed no correlations between the dihalogenated organic compounds and the trihalogenated organic compounds. Its looks more like the dihalogenated organic compounds, such as diiodomethane and dibromomethane, are coupled to a similar formation mechanism, while the trihalogenated methanes bromoform, chloroform, dibromochloromethane and bromodichloromethane follow a different formation pathway. It is possible that the mixed halogenated trihaloorganic compounds dibromomethane and bromodichloromethane originate from a nucleophilic substitution of bromoform where bromine is exchanged with chlorine abundantly present in the seawater as chloride (Cl^-) (Class and Ballschmiter 1988).

In macroalgae, 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, an evidence that chloroperoxidases may not be absolutely 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 an enzymic halogenation of alkenes (Geigert et al. 1984). The results of our study gave no indication for this mechanism as the release rates of the halogenated C_2 compounds investigated did not correlate with each other.

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 since no correlations between methyl halide releases and DMSP concentrations in the macroalgae have been found (Laturnus et al. 1998). A third possibility based on the catalysis via a methyltransferase reaction is also discussed by Wuosmaa and Hager 1990, and Saini et al. (1995). The authors suggested that a methyltransferring enzyme isolated from marine macroalgae and higher plants catalyzed the S-adenosyl-L-

methionine-dependent methylation of (except fluorine) to the respective methyl halides. Since we have investigated only methyl iodide and by this it was not possible by the time to identify correlations between different methyl halides, and, thus, find an indication for the formation mechanism via transferase reaction.

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Oxidative stress in Antarctic algae and molluscs

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Introduction

Since the discovery of the importance of radical reactions in normal biological processes, there has been an explosion of research into pro-oxidant and anti-oxidant processes, principally in mammalian systems (Halliwell & Gutteridge 1984). The normal fate of most of the molecular oxygen consumed by animals is tetravalent reduction to water coupled to the oxidation of food and the production of energy. Partial reduction results in the formation of reactive oxygen species, including superoxide anion radical ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$), peroxyl radical ($ROO^{\bullet}$), alkoxyl radical ($RO^{\bullet}$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and peroxynitrite ($ONOO^-$). It has been estimated that about 1–3% of O_2 consumed in animal systems is converted to ROS (Halliwell & Gutteridge 1984). Moreover, Fe can catalyze the conversion of H_2O_2 into $^{\bullet}OH$, via Fenton or Haber-Weiss reactions. Of more recent interest has been reactive oxygen species production and resulting oxidative damage as a mechanism of toxicity in aquatic organisms (Livingstone 1991; Winston & Di Giulio 1991; Regoli et al. 2004). Recently, new information on the formation of reactive oxygen species by mitochondria in aquatic organisms was reported (Heise et al. 2003; Philipp et al. 2005). Reactive species produced in biological systems are detoxified by antioxidant defenses, which are broadly investigated in aquatic organisms (for rev. Abele & Puntarulo 2004; Abele et al. 2007). A physical attribute of low temperature waters is the well-mixed surface that contains higher levels of dissolved oxygen available to coldwater ectotherms than is capable of saturating warmer waters. Metabolic processes are adapted in compensation for reduced energy for enzyme activation at low temperature (Vetter & Buchholz, 1998).The aim of this work was to characterize the oxidative status of both, algae and molluscs, isolated from the nearshore waters around the Antarctic Continent and islands (Potter Cove, King George Island, Antarctic Peninsula).

Materials and Methods

Freshwater Antarctic *Chlorella* sp and *Chlamydomonas* sp. were collected from one of the two permanent lakes located at the base of Three Brothers Hill at the Potter Peninsula, King George Island, South Shetland Islands (62º14'S-58º40'W) (Scientific Base Tte. Jubany). The samples were stored in flasks in

freshwater and taken to the laboratory where *Chlorella* sp and *Chlamydomonas* sp. cells were isolated and analysed as previously described (Stein 1973). Identification of the species was done by specialists from the Laboratory of Phycology and Experimental Culture of Microalgae from the University of Buenos Aires. *Chlorella vulgaris* cells were identified as BAFC CA 4 *Chlorella vulgaris* and *Chlorella kessleri* cells as BAFC CA 10 *Chlorella kessleri* (recently *Parachlorella kessleri*, Krienitz et al. 2004). Cultures of Antarctic *Chlorella* sp., Antarctic *Chlamydomonas* sp., *Chlorella kessleri* and *Chlorella vulgaris* were grown in Bold´s Basal medium supplemented with 1 g l^{-1} glucose and nitrate as nitrogen source (Bold & Wynne 1971). Glucose was added to increase the biomass. Media and nutrients were sterilized. Algae were grown in axenic cultures. *Chlorella* sp cells were grown for 26 days (stationary phase) at 4ºC (natural habitat water temperature -1.8 to 1ºC, Urban 1998) and C*hlamydomonas* sp. cells for 40 days (stationary phase) at 4ºC, whereas *Chlorella kessleri* and *Chlorella vulgaris* cells were grown for 18 days at 20-25ºC (stationary phase). All cultures were grown under light/dark cycles of 12:12 h. The irradiance on the surface of the culture was approximately 176 µmol photons m^{-2} s^{-1} of photosynthetically active radiation (PAR, Philips 40 W day-light- fluorescent light).
Individuals of the bivalve *Laternula elliptica* (Laternulidae) (King & Broderip 1831), important filter feeder key species with circum-Antarctic distribution, were collected by SCUBA divers from 5-17 m depth in Potter Cove, King George Island, Antarctic Peninsula, 62º15'S, 58º44'W. Additionally, the Antarctic limpet *Nacella concinna* was sampled in Potter Cove, King-George Island distinguishing between intertidal specimens from the rocky shores near Jubany Base and a subtidal limpet subpopulation from 10 m water depth. The intertidal limpets colonize rock surfaces and are air exposed in moisty rocky crevices for 2 to 4 hours during ebb tides. They are found in shallow intertidal pools, which have a maximum depth of 0.25 m and remain water covered throughout the whole ebb tide. Shell length of intertidal and subtidal specimens varied from 3.6 to 2.8 mm and from 5.8 to 3.4 mm in both species, respectively.
Data in the text and tables are expressed as mean ± S.E.M. of six independent experiments, with two replicates in each experiment. Statistical tests were carried out using Statview for Windows, ANOVA, SAS Institute Inc., version 5.0.

Results and Discussion

Oxidative stress and antioxidant defenses in Antarctic algae

It was previously reported that the ascorbyl radical/ascorbate ratio ($A^{\cdot}/AH^{-}$) was successfully used to estimate early oxidative transformations in living organisms (Galleano et al. 2002). Data in Table 1 indicate that $A^{\cdot}/AH^{-}$ ratio is significantly lower in Antarctic *Chlorella* sp. as compared to *Chlorella* vulgaris (that reached log phase of growth at temperature of 20-25ºC). This observation suggests that antioxidant protection in the Antarctic algae is more efficient than in the other species. However, also Antarctic *C. vulgaris* cultures showed a better protection against oxidative stress than *C. kessleri* cultures since the ratio $A^{\cdot}/AH^{-}$ in Antarctic *Chlorella* sp. was 7-fold lower than in *C. kessleri*.

Table 1
$A^{\cdot}$: ascorbyl radical, AH^-: ascorbate and $A^{\cdot}/AH^-$: ascorbyl radical/ascorbate ratio in *C. vulgaris*, Antarctic *Chlorella* sp. and C. *kessleri* during log phase (days 6) of algae culture growth, as oxidative stress index.

	$A^{\cdot}$ (pmol $(10^7$ cell$)^{-1}$)	AH^- (nmol $(10^7$ cell$)^{-1}$)	$A^{\cdot}/AH^-$ (10^{-3} AU)
C. vulgaris[1] (day 6)	10.0±2.0	1.3±0.2	7.0±1.0
Antarctic Chlorella sp.[2] (day 6)	2.3±0.7	3.8±0.9	0.8±0.2*
C. kessleri[1] (day 6)	33.0±8.0	0.8±0.4	49.0±20*

[1]Malanga et al. (2001), [2]Estevez et al. (2001). *significantly different at $P \leq 0.05$ from *C. vulgaris* cells.

Furthermore, *C. kessleri* showed a lower content of non-enzymatic antioxidants, i.e. α-tocopherol and β-carotene, as compared to Antarctic *C. vulgaris* cultures (Table 2). Again, Antarctic *Chlorella* sp. showed the highest content of total thiols that could contribute for an appropriate hydrophilic protection, as shown by the low $A^{\cdot}/AH^-$ ratio.

Table 2
Non-enzymatic antioxidants in *C. vulgaris*, Antarctic *Chlorella* sp. and C. *kessleri* cells during log phase of algae culture growth.

	α-tocopherol	β-carotene (nmol $(10^7$ cell$)^{-1}$)	Total thiols
C. vulgaris[1] (day 6)	3.90±0.70	0.17±0.04	0.39±0.08
Antarctic *Chlorella* sp.[2] (day 6)	0.65±0.05	0.21±0.05	9.00±2.00
C. kessleri[1] (day 6)	1.60±0.10	0.07±0.01	0.29±0.06

[1]Malanga et al. (2001), [2]Estevez et al. (2001).

Taken as a whole, these results suggest that oxidative metabolism is different among species, and these differences could be an important factor allowing oxidative stress to operate as a key to control the evolution of the community in response to environmental changes.

Electron paramagnetic resonance spectroscopy (EPR) of Antarctic *Chlorella* sp. was performed with cells supplemented with the spin trap MGD-Fe to assess the content of NO. A sharp increase in cellular NO was detected on day 6 of growth, simultaneously with the beginning of active cellular metabolism during the log phase (Estevez & Puntarulo 2005). To assess the possible contribution of endogenous NO synthase (NOS) activity to the generation of NO, the activity of NOS was assessed as the L-NAME sensitive-NADPH-diaphorase activity (NOS-like) in homogenates from cells upon the initial 22 days of growth. Since NO was identified as the dominant gaseous product from the activity of the enzyme nitrate reductase (NR), this activity was measured in cell homogenates over the same period. A significant increase in both enzymatic activities was observed on day 6 of growth as compare to day 0 of growth (Estevez & Puntarulo 2005). Antarctic *Chlamydomonas* sp. cells showed a similar profile to that described for Antarctic *Chlorella* sp. cells in terms of NO content and NR and NOS-like activities, with a slight extended time frame. Data on Table 3 show the enzymatic activities (NOS and NR) and NO content at log phase of

growth (day 6 for *Chlorella* sp. and day 12 for *Chlamydomonas* sp.). However, *Chlorella vulgaris* cells from temperate climate showed not only a faster growth than algae from Antarctic waters but no changes in NO and NR activity over the growth period and no-detectable NOS-like activity as well (Estevez & Puntarulo 2005).

Table 3
Nitric oxide (NO) content, nitrate reductase (NR) and nitric oxide synthase (NOS) activities in *C. vulgaris*, Antarctic *Chlorella* sp. and *Chlamydomonas* sp. harvested at day 6, 6 and 12, respectively.

	NO[3] (pmol $(10^7$ cell$)^{-1}$)	NR activity[3] (pmol min^{-1} $(10^7$ cell$)^{-1}$)	NOS-like activity[3] (pmol min^{-1} $(10^7$ cell$)^{-1}$)
C. vulgaris (day 6)	0.5±0.1	7±1	nd
Antarctic *Chlorella* sp. (day 6)	156±31	9±1	4.0±0.7
Antarctic *Chlamydomonas* sp. (day 12)	30±5	300±30	45±5

Taken from [3]Estevez & Puntarulo (2005). nd stands for non-detectable.

The significant increase in NO and related enzymatic activities at the triggering of log phase of growth was observed in the two tested species from Antarctica (*Chlorella* sp and *Chlamydomona* sp) but not in *Chlorella* cells from the temperate region. This fact suggested that NO could be part of the signalling network operative under specific growing conditions, such as low temperature stress, and could be an adaptive mechanism to allow cell survival. Since previous data indicated that nitrate uptake by Antarctic phytoplankton and ice algae (Döhler 1998) is decreased by UV exposure, further studies into NO metabolism in Antarctic algae should be considered to explore the effect of the change of environmental conditions (UV, Fe, etc.) on cellular growth.

Oxidative stress and antioxidant defenses in Antarctic molluscs

Iron is a micronutrient and serves in many biological functions in animal tissues. It can participate in many electron transfer reactions, including oxygen transport, activation, and detoxification. In marine invertebrates its content strongly depends on the characteristics of the natural environment (Table 4).

Even though total Fe is stored intracellularly in a *safe* way by its binding to ferritin, any time Fe exceeds the metabolic needs of the cell it may form a low molecular weight pool, referred to as the labile iron pool (LIP), which converts normal by-products of cell respiration, like O_2^- and H_2O_2, into highly damaging $^{\cdot}OH$ or equally aggressive ferryl ions or oxygen-bridged Fe^{2+}/Fe^{3+} complexes. LIP is an effective catalyst for lipid peroxidation (Puntarulo & Cederbaum 1988). The volcanic rocks at King George Island are known to contain around 5% Fe, which is higher than Fe content found in the cold desert soils in Antarctica (0.56-2.5%, Keys & Williams 1981), thus the total Fe content in tissues of the considered molluscs was measured for this study (Table 5).

Table 4
Total Fe content in different molluscs.

Species	Tissue	Fe content (μmol g^{-1} FW)	Ref
Mytilus edulis (Helgoland, North Sea)	whole animal	0.5 ± 0.2	4
Mytilus edulis (New Zealand)	whole animal	0.9	5
Mactra corallina (Mediterranean)	whole animal	0.9 ± 0.3	4
Donax sp. (Mediterranean)	whole animal	0.5 ± 0.2	4
Perna canaliculus (New Zeland)	whole animal	0.6	5
Aulacomya maoriana (New Zeland)	whole animal	0.5	5
Modiolus neozelanicus (New Zeland)	whole animal	2.6	5
Nacella magellanica (Beagle Channel)	digestive glands	1.0 ± 0.1	6
Nacella deaurata (Beagle Channel)	digestive glands	1.9 ± 0.3	6
Mya arenaria (Wadden Sea)	digestive glands	0.6 ± 0.2	7

Taken from [4]Herut et al. (1999), [5]Nielsen & Nathab (1975), [6]Malanga et al. (2004), [7]Estevez et al. (2002).

Table 5
Thiobarbituric reactive substances and total Fe content in Antarctic molluscs. Data are expressed as means ± SD of six independent experiments with three replicates in each experiment for the TBARS, and of fifteen independent experiments with two replicates in each experiment for the total Fe measurements.

	L. elliptica	*N. concinna* subtidal (μmol g^{-1} FW)	*N. concinna* intertidal
TBARS			
digestive glands	0.42±0.07	1.20±0.80	0.50±0.20
mantle	0.50±0.05	0.30±0.20*	0.37±0.08
gills	0.40±0.10	0.04±0.01*,[8]	0.03±0.01*,[8]
Total Fe			
digestive glands	5.00±1.00	3.00±2.00	1.80±0.50
mantle	1.20±0.40*	2.80±0.90	2.60±0.60
gills	4.00±1.00		

*significantly different from digestive glands, ANOVA ($p<0.05$). [8]Malanga et al. (2005).

Both animals, the bivalve *L. elliptica* and the limpet N. *concinna* from King George Island, showed higher Fe content as compared to other phylogenetically related animals from other geographical regions, as previously reported (Estevez et al. 2002; Puntarulo et al. 2004). However, no relevant differences were found among tissues, except for the total Fe content in mantle from *L. elliptica* that is lower than in digestive glands and gills (Table 5).
It was previously reported that lipid radical generation rate in the membranes of cold adapted organisms was higher of that found in related animals from temperate regions (*L. elliptica vs M. arenaria*) (Estevez et al. 2002). For this study, the content of thiobarbituric reactive substances (TBARS), an indicator of lipid peroxidation, was assessed in the digestive glands, mantle and gills from *L. elliptica*, subtidal *N. concinna* and intertidal *N. concinna* (Table 5). No significant differences of TBARS content were detected between different tissues of L. *elliptica*. In subtidal N. *concinna*, the TBARS content in digestive gland was significantly higher than in mantle and gills, whereas in the intertidal specimens only gills had significantly lower TBARS content as compared to

digestive glands (Table 5). However, no-significant differences were observed in the total Fe content of the three Antarctic animal species or between tissues (Table 5), suggesting that only the LIP (and not the total Fe) in these tissues should be responsible for triggering lipid peroxidation.
Moreover, active Fe reduction and lipid radical generation (assessed by EPR measurements) in the digestive glands of *L. elliptica*, were detectable even though lipid soluble and enzymatic antioxidants were present in the tissues (Table 6).

Table 6
Enzymatic and non-enzymatic antioxidants, lipid radicals and Fe reduction rate in digestive glands from *L. elliptica*.

	L. elliptica	Ref
α-Tocopherol (pmol mg^{-1} prot)	10±2	7
β-carotene (pmol mg^{-1} prot)	43±8	7
catalase (U mg^{-1} prot)	99±4	7
Lipid radicals (pmol mg^{-1} FW)	422±47	9
Fe reduction rate (nmol min^{-1} mg^{-1} FW)	188±33	10

[7]Estevez et al. (2002). [9]Puntarulo et al. (2004), [10]Puntarulo et al. (2005).

The activity of catalase in the gills of the Antarctic mollusc *N. concinna* was 44 ± 22 U/mg prot in animals from the subtidal areas, and 140 ± 40 U/mg prot in animals from the intertidal areas, and to 213 ± 57 U/mg prot in animals temporarily air exposed on intertidal rocks. Superoxide dismutase activity was 4 ± 2 U/mg prot in subtidal limpets, 4 ± 3 U/mg prot in intertidal limpets, and 13 ± 2 U/mg prot in highest intertidal limpets.
An integrative study on the activity of the antioxidant enzymes in these animals should be developed to extent the knowledge of the effect of the habitat temperature to the antioxidant ability of the tissues *in vivo*. Intertidal limpets, like *N. concinna*, undergo transient metabolic depression during low tides, a behaviour common to intertidal molluscs during air exposure (Pannunzio & Storey 1998). The shell closure strategy prevents desiccation and predation during low tides and triggers a hypoxic response in the enclosed animal, consisting in metabolic reduction and on switch to anaerobic metabolism. Thus, adaptation to high shore environments involves extended periods of metabolic reduction which may reduce the overall rate of metabolically produced oxygen radicals compared to subtidal limpet species. Moreover, oxidative stress may be enhanced by a frequent shift between low oxygen and normoxic conditions, comparable to ischemia-reperfusion insult. In *N. concinna* specimens, we conjecture that Antarctic high shore conditions, involving regular exposure to air and presumably also thermal stress on sunny days during the Antarctic summer season, cause a necessity to ward off higher oxygen radical species production by increasing its antioxidant defence.
On the other hand, our data shows that the highly abundant polar bivalve *L. elliptica* obviously accumulates significant levels of Fe, thus it might also be prone to oxidative stress and presumably will constitute a sensitive monitoring organism for studies of contamination and human impact on Antarctic coastal waters.

Taken as a whole, the data presented here on oxidative stress conditions in algae and molluscs from King George Island showed that a wide array of complex metabolic pathways could be involved to limit oxidative damage and allow survival of the species adapted to the extreme environmental conditions.

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Growth and age of *Laternula elliptica* populations in Potter Cove, King-George Island

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Introduction

The Antarctic soft shell clam *Laternula elliptica* (*Pholadomyoida*, King and Broderip, 1831) is one of the most abundant bivalves with circum Antarctic distribution. First records of *L. elliptica* at the Western Antarctic Peninsula are from the late Pliocene (5 Ma) (Soot-Ryen 1952; Jonkers 1999). Present day *L. elliptica* populates near shore waters around the Subantarctic archipelagos (Powell 1965) and stocks are found in 3-320 m water depth (Powell et al. 2001) where they form dense beds of up to 300 individuals/m^{-2} (Mercuri et al. 1998). This large sized (maximal shell length > 100 mm) filter feeder burrows up to 50 cm deep into the sediment (Hardy 1972). It occupies a similar ecological niche as the genus *Mya* in Northern Hemisphere temperate coasts (Ralph and Maxwell 1977). In contrast to other *Laternulidae*, *L. elliptica* is able to rebury once removed from the sediment (Peck et al. 2004, and personal observation). This may be crucial for its survival as especially in the shallower areas, clams can be ploughed out from the sediment by scouring icebergs.

In the present study size and age of *L. elliptica* individuals from different water depths in Potter Cove were determined. Lifelong growing hard structures such as mollusc shells or fish otholids can be used to determine chronological age when verified that growth checks are formed at regular time intervals. The formation of such growth checks is based on cyclic changes of environmental factors like temperature or food supply (Richardson 2001). In some bivalves, external growth checks can be read reasonably well by eye on the shell surface (Winther and Gray 1985; Allison 1993; Sukhotin et al. 2003). However, in other species identification of external growth checks is impossible and disturbance marks may further complicate the identification of the “real” growth check pattern. In such cases internal growth band patterns are more reliable. Such patterns are analysed in polished dorso-ventral shell sections, either in the umbo and/or on the whole section from the umbo to the shell margin (MacDonald and Thomas 1980; Brousseau and Baglivo 1987) or in acetate peels of such sections (Richardson et al. 1979). The time interval of growth check formation, e.g. daily, monthly or annually, can be deduced either from mark-and-recapture experiments (Brousseau 1979; Chiantore et al. 2003) or from stable isotope analysis (Krantz et al. 1984; Brey and Mackensen 1997; Heilmayer et al. 2003). In *L. elliptica* the annual formation of growth checks in the umbo was confirmed by stable isotope analysis (Brey and Mackensen 1997).

Marine ectotherms from Polar regions often exhibit extended maximum life spans compared to temperate congeners of similar lifestyle (Brey 1991; Brey et al. 1995; Ziuganov et al. 2000; Cailliet et al. 2001; La Mesa and Vacchi 2001) but rather occupy the slow lane with regard to metabolism, growth and onset of reproduction (Clarke 1988). In this paper we approximate maximum life span of *L. elliptica* and examine

differences of the age-length relationship in different *L. elliptica* populations from shallow and deeper waters in Potter Cove.

Material and Methods

Laternula elliptica individuals were collected by SCUBA diving in different years in Potter Cove, King George Island. 374 individuals were sampled in November-February 2002/2003 at 5-10 m water depth, 25 individuals in 2005 at 30m depth, 15 individuals in 2000 at 15-30m depth, 113 individuals in 1993 at 25m depth and 26 individuals in 1994/95 at 5-30m depth. Altogether 553 individuals were aged by counting the annual growth checks (Brey and Mackensen 1997) in polished cuts through the shell umbo. Umbos were cut with a circular diamond saw (Ø 21mm) along the growth axis, wet-polished with sandpaper (400 and 600 grain size) and growth bands were counted by stereo microscope (Fig. 1).

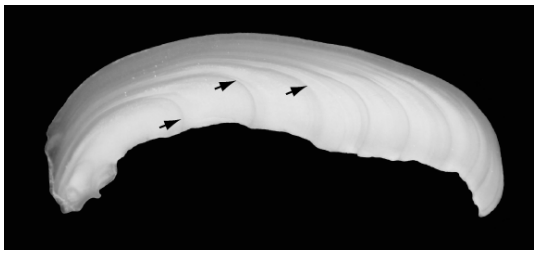

Fig.1 Cut umbo of *L. elliptica* with clearly visible growth rings (arrows). Photo by M. Voigt

A von-Bertalanffy growth model (VBGF):
$L_t = L_\infty * (1 - e^{-K * (t - to)})$
where L_∞ = asymptotic shell length, K = growth constant, t = age and t_0 = age at which length is zero, was fitted to the 553 size-at-age data using the nonlinear iterative "Newton" algorithm. Differences in growth between subsets of clams from different depth ranges (374 individuals from 5-10m versus 153 individuals from 15-30m depth) were evaluated by analysis of variance of the residuals of the common growth model.

Results and Discussion

Age determination from external and internal shell growth bands

In the present study individual age was where determined by growth rings visible in umbo cross cuts. In a previous study Urban und Mercury (1998) aged *L. elliptica* from Potter Cove by counting external growth marks on the shell surface. Their length-at-age key distinctly differs from the one obtained in the present study. While age-at-length is similar in smaller animals up to 4 years, Urban and Mercury (1998) under-estimate age, particularly in larger individuals (Fig. 2). Obviously, shell surface growth bands become less and less separable the larger the animal grows. Thus, for correct age determination of *L. elliptica,* inner growth rings should be used.

Maximum age of L. elliptica

The oldest individuals among the 553 *L.elliptica* from Potter Cove had an age of 36 years (Fig. 2, black dots). No older animals have been reported so far. Other studies investigating the age of *L. elliptica* found maximum ages up to 13 y (Urban and Mercuri 1998), 14 y (Ralph and Maxwell 1977), and 33 y (Voigt 2004), i.e. it is most likely that the maximum lifespan of *L. elliptica* is >= 36 y. With a maximum life span of 36 y, *L. elliptica* ranges at the lower end of the age spectrum recorded for Antarctic bivalves. Maximum ages of 150 y and >100 y have been estimated for the small (<= 45mm) bivalve *Yoldia eightsi* (Peck and Bullough 1993) and the scallop *Adamussium colbecki* (Berkman 1990), respectively.

Both estimates, however, are derived from growth models based on mark-recapture data and are not confirmed by individual aging based on shell growth marks that yield maximum ages of ± 30 y in *Y. eightsi* (unpublished personal observation by T. Brey) and 14 y in *A. colbecki* from Terra Nova Bay (Heilmayer et al. 2003). Apparently, extrapolations of maximum lifespan from growth models have to be viewed with caution, in particular when based on mark-recapture data. In case of *L. elliptica* the largest individual ever reported (Urban and Mercuri 1998) was 120mm in length [anterior-posterior], which is well beyond the size range defined by our growth models (see below). Fig. 2 shows the extraordinary inter-individual variability of the size-age relationship in *L. elliptica*; similar sized individuals can differ > 3fold in age. The increase in age-size variability with increasing size shown in Fig. 2 indicates that the age approximation from shell size is acceptable only for individuals < 40mm length, and still implies +/- 2years of error probability.

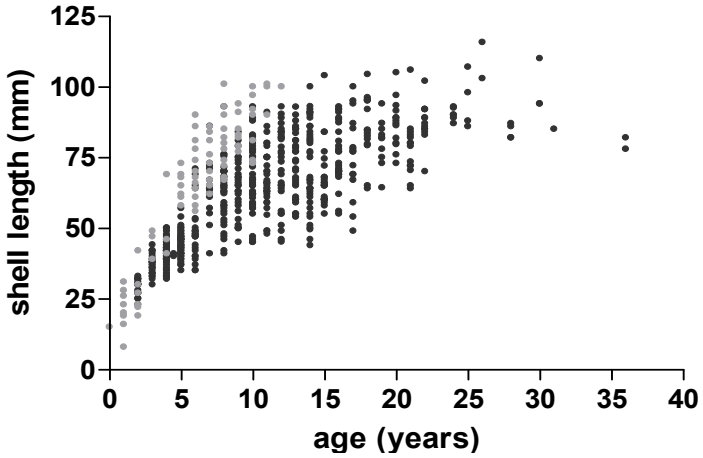

Fig.2: *L. elliptica* individuals aged by internal growth bands (black dots, n = 553) and by shell surface growth marks (grey dots, n = 78, Urban and Mercury 1998).

Individual growth of L. elliptica in Potter Cove

ANOVA of the residuals of the common VBGF fitted to all 527 length-at-age data from animals collected at known depth (L_∞ = 90.08 mm, K = 0.109 y^{-1}, t_0 = -1.665 y, R^2 = 0.683) indicated a significant (P < 0.001) difference in growth between individuals from 5-10m and from 15-30m water depth. Separate growth models indicate distinctly slower growth in shallow water animals (growth constant K is 0.060 compared to 0.112, see Fig. 3).

In Potter Cove the shallow water environment is characterized by iceberg-disturbance, sediment discharges from glacier melting and wave action, as well as higher daily temperature fluctuations compared to deeper waters (Klöser et al. 1994; Schloss et al. 1998b; Schloss et al. 1998a, and personal observations).

Information on the physiological response of *L. elliptica* to frequent disturbance events is yet missing. However, the current state of knowledge supports the idea that intense inorganic sedimentation of melt water discharge, resuspension due to wave action and burial will encumber *L. elliptica* energetically by increasing basal body maintenance costs while most likely decreasing growth rates, scope for activity, and energy investments into reproduction (Widdows et al. 1979; Madon et al. 1998). Iceberg disturbance may have similar effects, because animals must dig in again after being ploughed to the surface, or even become damaged by ice impact.

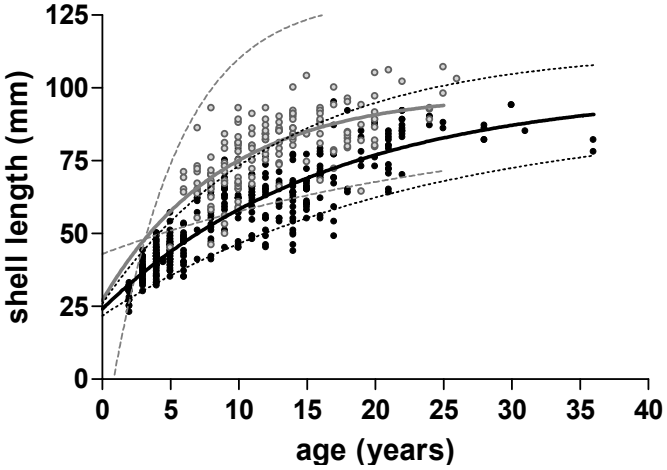

Fig.3: Size-at-age data of *L. elliptica* and superimposed von Bertalanffy growth functions (VBGF) with confidence limits for shallow (5-10m depth, black dots; L_∞ = 99.45, K = 0.060, t_0 = -4.590, R^2 = 0.791, n = 374) and deep (15-30m depth, grey dots; L_∞ = 98.30, K = 0.112, t_0 = -2.864, R^2 = 0.487, n = 153) sites in Potter Cove.

Nevertheless, there is evidence that abundance of *L. elliptica* is much higher at shallow sites compared to deeper sites (Mercuri et al. 1998). At the sampling site of the present study (station tS in Mercuri et al., 1998) *L. elliptica* abundances were high at 5m water depth, (346 ind/m^2) but declined to 5.3 ind/m^2 at 30m depth. We presume that shallow site provide more food, particularly benthic diatoms which are a major food source of *L. elliptica* (Ahn 1997) and can therefore maintain a higher stock of the clam. Obviously there is a trade-off between stress impact and alimentation. At the shallow site, the single “pays” for increased stress through reduced fitness, but the population gains through higher standing stock and most likels higher energy throughput. We might see the environment of *L. elliptica* as a simplified two-dimensional space defined by the two axes stress and alimentation. Then we can expect at each position within this space a typical *L. elliptica* standing stock, age-size structure, individual fitness and activity, which in turn will affect the particle flux from the water column to the seabed by biodepostion (Momo et al. 2002), thereby affecting the entire benthic community (Ahn 1993). Further investigations are currently undertaken to test this hypothesis.

The use for accurate age determination

L. elliptica represents a perfect model for investigations of physiological stress response with age, because age and size are uncoupled in large animals. Individual age determination (by counting growth checks) enables us to distinguish between the effect of size and the effect of age on physiological ageing and stress response parameters.

Because fitness declines with age, the physiological stress response of the species may not be visible when only similarly sized animals are studied and taken as a physiologically homogenous group.

As an example, Figure 4 shows lipofuscin (= age pigment) concentration in *L. elliptica* mantle tissue plotted against individual size (A) and age (B).

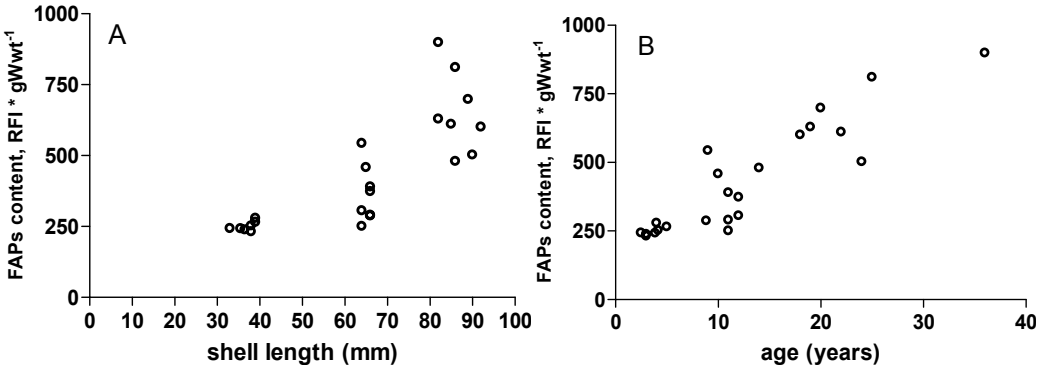

Fig. 4: Lipofuscin content in mantle tissue of *L. elliptica versus* A) shell length and B) individual age (n=23). Figure 6B taken from Philipp et al. (2005).

Lipofuscin (synonym "fluorescent age pigment", FAP) is considered as a physiological marker of ageing. It consists of incompletely degraded damaged cell structures, mainly proteins and lipids and accumulates in the lysosomes, where it is practically indigestible (Terman 2001; Brunk and Terman 2002).The lipofuscin concentration is better correlated with individual age than with size (for detailed statistical analysis see Philipp et al. 2005). This shows that similar sized animals not only differ in chronological (age in years) but also in physiological age and thus might show different responses towards different experimental treatments.

In conclusion, we found a maximum life span of 36 y in *L. elliptica*. Individuals from shallower water depth grow at slower pace compared to individuals from deeper areas, which are less affected by icebergs and sediment import. This finding supports the idea that these two major stress effectors may cause a shift in energy allocation from growth to maintenance in shallow water *L. elliptica*. Individual age determination is essential for a valid interpretation of physiological parameter response to stress in mollusc populations.

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Properties of extracellular proteases produced by psychrotolerant bacteria isolated in the vicinities of Jubany Station

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Introduction

The Antarctic continent is one of the coldest areas on Earth. Microorganisms able to live under such harsh physicochemical conditions have their biochemistry adapted to work optimally in the biotopes where they thrive and their enzymes work more efficiently at low temperatures than the corresponding mesophilic and thermophilic ones (Helmke and Weyland, 1991; Feller et al., 1997; Hoyoux et al., 2001). In several Antarctic environments (like seawater or marine sediments) the temperature is approximately constant throughout the whole year. In other biotopes, like soil and rock surfaces in the Antarctic Peninsula, temperature significantly changes seasonally and bacteria must exhibit a high resistance to freezing and thawing processes (Deming 2002). At Jubany Station, temperatures fluctuate along the year from -20°C in winter to +10°C in summer, with a mean annual air temperature of about –1.5°C. Moreover, soil surface is highly irradiated in summer, with the upper layer of soil sometimes reaching temperatures of about 20°C. The physiological and biochemical adaptations of the microbiota inhabiting those environments mainly involve changes in the structure of their biomolecules to avoid freezing and to support membrane fluidity and nutrient transport systems. Changes in the catalytic efficiency and temperature optimum of their enzymes also are required to compensate the low kinetic energy associated with those environments (Feller 2003). Nevertheless, even when the majority of cold enzymes described in the literature so far are found to have some of the above-mentioned adaptations, it is possible to find psychrotolerant bacteria producing mesophilic-like enzymes, despite the fact that they inhabit cold-climate environments (Oikawa et al. 2003).

Nowadays, extremophiles deserves much interest as they are a source of new biomolecules with activity at extreme conditions. Cold proteases are important for their potential industrial application and also for their role in recycling of organic matter and other ecological processes. Thus, the knowledge about the characteristics of the extracellular proteases produced by Antarctic bacteria is essential to understand the dynamics of the environment where such microorganisms live.

Based on the basic, ecological and industrial interest on cold proteases, in this work we report the characteristics of two groups of proteases produced by psychrotolerant bacteria, previously selected among several proteolytic isolates from the surroundings of Jubany Station, Antarctica.

Methodology

Microorganisms: Isolates were obtained from samples collected from soil, fresh and marine waters, sea sediments and remains of organic matter of animal and vegetal origin. These samples were taken during Argentine summer Antarctic Research Expeditions 1989/90, 1991/92, 1994/95 and 1995/96 near Jubany scientific station (62°14'S, 58°40'W) on King George Island (South Shetland Islands). Protease-producing bacteria were selected at three different incubation temperature (4-6 ºC; 10-13 ºC and 20 ºC) as described previously (Vazquez et al., 1995; Vazquez and Mac Cormack, 2002). All 123 proteolytic isolates were mostly gram-negative rods and psychrotolerant, formerly referred to as "psychrotrophic" by Morita (1975) and defined as those bacteria capable to grow at 0°C but having an optimum temperature higher than 15°C. Two groups of isolates were selected for further characterization: one (Group 1) on the basis of their production of one single band in gelatine-SDS-PAGE and having the lowest optimal temperature for activity found (40 ºC) and the other (Group 2) on the basis of their good enzyme production capacity in submerged cultures. Isolates were identified at genus level using the commercial system Sensident (Merck) and molecular techniques, as described elsewhere (Ruberto et al., 2003).

Protease detection: Proteolytic activity was measured by digestion of azocasein (Sigma). An appropriate dilution of the enzyme solution (400 μl) was incubated with 400 μl of 1% w/v azocasein in 0.1 M Tris, 0.5 mM $CaCl_2.2H_2O$ buffer (pH 8.0) at 20ºC for 30 min. The reaction was stopped by adding 800 μl of 5% w/v trichloroacetic acid and the absorbance of the supernatant was measured at 340 nm after centrifugation of the reaction mixture. One EU was defined as the amount of enzyme that produces an increase of 0.100 in A_{340} under the assay conditions. Two commercial proteases were used as standards: Metalloprotease Type IX P6 141 (Sigma) and Subtilisin Type VIII P5380 (Sigma). Protease profiles were developed in zymograms after SDS-PAGE of cell-free culture supernatants, using gelatine as a copolymerised substrate.

Purification of proteases: Bacteria were grown in submerged culture and the proteases were purified from culture supernatants. Fluid was concentrated by tangential ultrafiltration and then was passed through an S-Sepharose Fast Flow column as described in Vazquez et al., 2004. Total protein content was measured by the bicinchonic acid method using the commercial kit BCA (Pierce).

Effect of temperature and pH on activity and stability: The effect of pH and temperature on protease activity was determined by using the standard protease assay. Determination of the optimum pH value was performed at 20 °C with the following buffer systems (0.1 M each): sodium acetate/acetic acid (pH 5.0); KH_2PO_4/Na_2HPO_4 (pH 6.0-7.0); Tris-HCl (pH 8.0-9.0) and Na_2HPO_4/NaOH (pH 10.0-12.0). The pH stability of the proteases (pH 4 to 12) was investigated in the same buffers (20 mM each). Enzyme solutions were incubated with the buffers at 4 °C for 3 h and the residual activities were measured using the standard protease assay.

For determination of the apparent optimum temperature, the reaction was carried out at several temperatures between 0 °C and 60 °C and pH 8.0. Apparent activation energies (E_{act}) were calculated from the linear portion of Arrhenius plots as described by Pirt (1985). For evaluation of the effect of temperature on enzyme stability the proteases were incubated at temperatures ranging from 10 to 60 °C for 1 h and at pH 8.0 (enzyme assay buffer). In addition, the proteases were incubated at 40 °C for 90 min and at 50 °C for 60 min, monitoring the residual activity every 10 min. After incubation, the mixtures were rapidly cooled and the residual activities were determined using the standard protease assay. The half time of thermal inactivation (t_i) was calculated as the incubation time at which the protease retains 50 % of its maximal activity, at a given temperature (Kärst et al., 1994).

Effect of inhibitors and metal ions: The effect of protease inhibitors (10 mM each): PMSF, 2,2-bipiridil, 1,10-phenantroline, EDTA and cystein, as well as of metal ions (1 mM each): Zn^{2+}, Hg^{2+}, Cu^{2+} and Ni^{2+} was investigated. The proteases were incubated with each of the reagents at 20 °C and pH 8 for 1 h. The residual activities were determined using the standard protease assay.

Results

Identification of the isolates
Both selected groups of bacteria were represented by psychrotolerant, heterotrophic, aerobic, gram-negative motile rods, as was determined by their morphological and physiological characteristics and the comparison of the partial sequence of their 16S rDNA with the available databases, resulting in the following:
Group 1: *Pseudomonas* sp. (Ele-2, Prot-9, Prot-11, P95-1, P95-21, P95-24, P96-18; P96-35).
Group 2: *Stenotrophomonas maltophilia* (ANT-1-1; ANT-7-1; YOA-3) and *Pseudoalteromonas* sp. (P96-47).

Purification of proteases
The eight proteases of Group 1 were purified to homogeneity, corresponding all to a 45 kDa band in SDS-PAGE. After ultrafiltration and S-sepharose purification, proteases of Group 2 remained free from contaminating proteins but the multiple active bands developed in zymograms could not be separated. The characterization was done on the purified solution, with a specific activity of around 4000 U/mg and containing what can be either a mixture of proteases with very similar isoelectric point or a mixture of active autodigestion fragments from one protease, produced during the time of bacterial culture and enzyme production (Vazquez et al., 2005).

Effect of pH on activity and stability of proteases
Optimal proteolytic activity occurred at neutral or moderate alkaline pH for eight proteases from *Pseudomonas* sp. and for P96-47. All retained more than 50% of their maximal activity at pH ranging from 6 to 10 and were inactive at pH 12. Prot-9 and Prot-11 proteases showed the broader pH range for activity. On the other hand, the three *Stenotrophomonas* proteases were active at alkaline pH,

being highly efficient over a broader pH range than subtilisin, with more than 50% of relative activity at pH between 5 and 12.
All Group 1 proteases were equally stable and similar to the commercial metalloprotease when they were exposed to different pH. Except for P96-18, proteases were stable at pH from 5 to 9 and were inactivated at pH 12. Three stability curves are shown in Figure 1.a, as the rest looked similar to P95-1 at pH below 7 and were more stable than P96-35 and less stable than P95-1 at pH above 7. Among Group 2 proteases, P96-47 was slightly more stable at alkaline pH than the commercial metalloprotease was. *Stenotrophomonas* proteases retained more than 65% of activity within a pH range from 4 to 12, with maximal stability at neutrality (Figure 1.b).

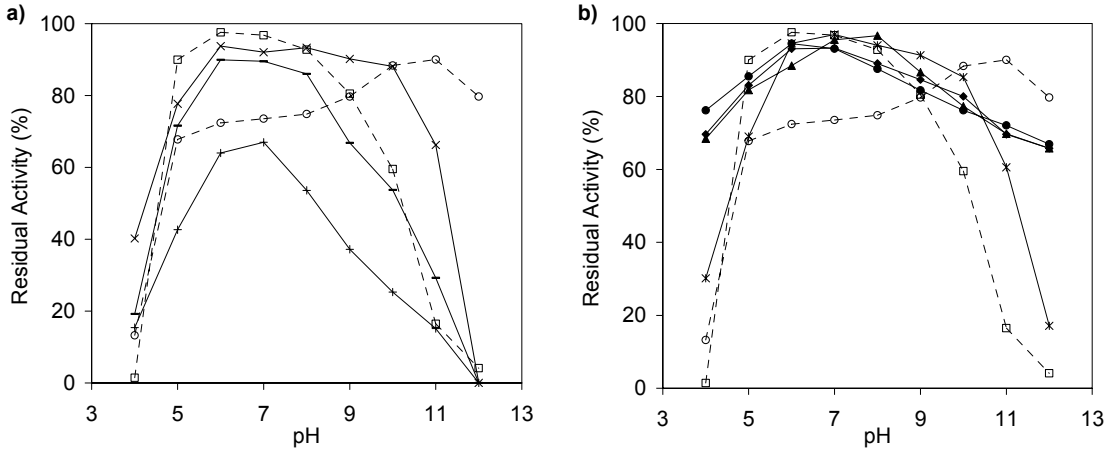

Figure 1: Effect of pH on stability of proteases. Enzymes were incubated at 4 °C for 3 h at different pH and the residual activity was measured at 20 °C and pH 8. a) Group 1: (■) Ele-2, (◆) Prot-9, (▲) Prot-11, (×) P95-1, (✱) P95-21, (●) P95-24, (+) P95-18, (—) P96-35. b) Group 2: (◆) ANT-1-1, (●) ANT-7-1, (▲) YOA-3, (✱) P96-47. (□), dotted line, Metalloprotease Type IX P6 141 and (○), dotted line, Subtilisin Type VIII P5380 (Sigma).

Effect of temperature on activity and stability of proteases

The apparent optimum temperature for activity (OT) of proteases from *Pseudomonas* was 40 ºC and their curves were shifted 10-20 ºC towards low temperatures with respect to those of the mesophilic enzymes. The E_{act} values for the hydrolysis of azocasein were calculated from the linear portion (approximately between 10 ºC and 40 ºC) of the Arrhenius plots of the data. Values of 40 kJ mol^{-1} (proteases Ele-2, Prot-9, Prot-11, P96-35), 42 kJ mol^{-1} (P95-1), 47 kJ mol^{-1} (P95-21), 36 kJ mol^{-1} (P95-24) and 50 kJ mol^{-1} (P96-18) were obtained. The commercial metalloprotease and Subtilisin were tested under the same conditions and showed E_{act} of 58 and 60 kJ mol^{-1} respectively.
For *Stenotrophomonas* proteases, OT was observed at 55ºC (ANT-1-1 and ANT-7-1) and 60ºC (YOA-3). These values and the thermal-dependence profiles were similar to that found for the subtilisin (58ºC). At 70ºC the three proteases exhibited 50-60% of their maximal activity and less than 30% at temperatures below 40ºC. The Arrhenius law was found to be followed between 10ºC and 50ºC and the apparent activation energies were 58 kJ mol^{-1} (ANT-1-1 and ANT-7-1) and 60 kJ mol-1 (YOA-3), similar to those obtained from the commercial mesophilic enzymes. P96-47 protease had a temperature profile more close to that of *Pseudomonas* proteases, with an OT of 45 ºC.

The eight psychrotolerant proteases of Group 1 proved to be thermolabile compared with the commercial enzymes when they were incubated for 1 h at various temperatures. They reached thermal inactivation at temperatures 10 ºC lower than the mesophilic metalloprotease. Four curves are shown in Figure 2.a, the ones not shown were very similar to Ele-2 and P95-1 curves. Their range of half time of thermal inactivation (t_i) at 40 ºC was 20-60 min (Figure 2.b) and at 50 ºC t_i was of only 5 min (figure not shown). On the other hand, Group 2 proteases were less thermosensitive, with t_i values at 50 ºC ranging between 50-70 min, while the commercial metalloprotease had a t_i value of 22 min. Nevertheless, their stability was well below of that of subtilisin (Figure 3.a and b).

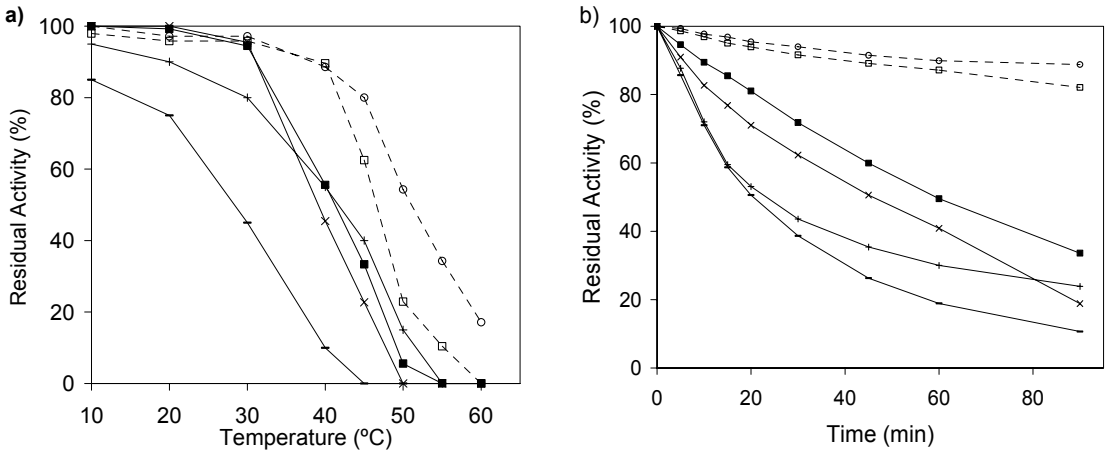

Figure 2: Effect of temperature on stability of proteases of Group 1. a) Enzymes were incubated at different temperatures for 1 h and the residual activity was measured at 20 °C and pH 8. b) Enzymes were incubated at pH 8 and at 40 °C for the indicated periods. (■) Ele-2, (×) P95-1, (+) P95-18, (—) P96-35, (□), dotted line, Metalloprotease Type IX P6 141 and (○), dotted line, Subtilisin Type VIII P5380 (Sigma).

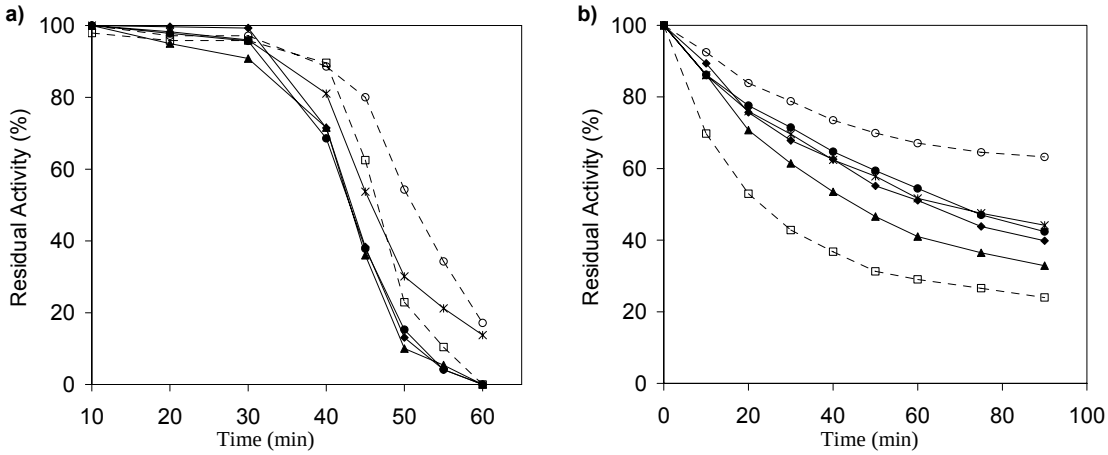

Figure 3: Effect of temperature on stability of proteases of Group 2. Enzymes were incubated at pH 8 and at 40 °C (a) and 50 °C (b) for the indicated periods. The residual activity was measured at 20 °C and pH 8. (◆) ANT-1-1, (●) ANT-7-1, (▲) YOA-3, (✖) P96-47; (□), dotted line, Metalloprotease Type IX P6 141 and (○), dotted line, Subtilisin Type VIII P5380 (Sigma).

Effect of inhibitors and metal ions on activity of proteases

The eight proteases from Group 1 were inhibited in more than 80 % by 10 mM 1,10-phenantroline and EDTA (metalloprotease inhibitors) suggesting that they

are metalloproteases. PMSF (serinprotease inhibitor) was found to be an ineffective inhibitor of all the studied enzymes, except for P96-35, which was slightly inhibited. Cystein caused 50-70 % of inhibition of the activity of Ele-2, Prot-9, P95-1 and P95-24 proteases. 2,2-bipiridil slightly inhibited Prot-9 and P95-24 proteases, while it produced a decrease in activity of P95-21 and P96-35 proteases of 70-80 %.
Proteolytic activities of all the eight enzymes were reduced by more than 50% in the presence of 1 mM Zn^{2+}, Hg^{2+}, Cu^{2+} and Ni^{2+}. Among them, P96-18 and P96-35 were the most sensitive to the addition of the above mentioned metal ions.
Group 2 *Stenotrophomonas* proteases were inhibited by PMSF (ANT-1-1 and ANT-7-1 showed 81% and YOA-3 77% of inhibition). 2,2-Bipiridil did not affect any of the proteases, while *o*-phenantroline, EDTA and cysteine inhibited the three proteases only slightly. All this indicates that the three psychrotolerant-proteases are serine proteases. Cu^{2+} did not affect the activities of proteases and Zn^{2+}, Hg^{2+} and Ni^{2+} reduced proteolytic activity by 74-82%. ANT-1-1 protease was less stable to metal ions than the other two proteases. P96-47 was 91% inhibited by 10mM 1,10-phenantroline and 86 % inhibited by EDTA, indicating the presence of metalloproteases. PMSF and 2,2-bipiridil did not affect the enzyme and cystein caused 84 % of inhibition of the activity, probably due to the presence of disulphide bonds essential for keeping an active enzyme conformation. Tested metal ions affected the activity of the enzyme by 39-78 %, while the commercial metalloprotease was completely inhibited.

Discussion

As a result of a previous screening (Vazquez et al. 1998), the protease-production capacity and the dependence on temperature for activity of the proteases produced by them led to the selection of two groups of psychrotolerant bacteria for their further characterization. Bacteria belonging to the Group 1 all were members of the genus *Pseudomonas.* Members of this genus are known to be enzyme producers and efficient degraders of many organic and toxic compounds (Clarke 1982, Spiers et al. 2000). Although many proteases from cold-adapted bacteria have been characterized (Kärst et al. 1994, Margesin et al. 1992, Oikawa et al. 2003), few studies dealt with comparisons between different protease-producing strains. The eight Antarctic proteases from group 1 share many common features, even when they were isolated from different biotopes and under different incubation conditions and times. All were metalloproteases with optimal activity at 40 ºC and neutral pH and showed a single band in SDS-PAGE, with the same molecular mass. The pH and temperature stability was slightly different, but all were thermolabile compared with mesophilic commercial proteases. These general properties are similar to those reported by Margesin and Schinner (1992) for three psychrotolerant *Pseudomonas fluorescens* strains isolated from cryoconite of Alpine glaciers. The authors postulated that the differences exhibited by proteases obtained from different strains belonging to the same species are in relation to the respective genetic and physiological adaptation of the strains and with the environmental conditions of the sites where the producing strains thrive. Our *Pseudomonas* strains produced proteases with very similar properties compared with the three Alpine proteases, even when the sites

where the producing strains originated from are very distinct and far distant. Nevertheless, both places (Alpine glaciers and Antarctic biotopes nearby Jubany Station) are characterized by cold temperatures but with seasonal fluctuation determining continuous freezing and thawing cycles during summer. This climate situation is likely the responsible for the psychrotolerance but not for the psychrophily observed for the strains isolated from both mentioned cold regions.

Group 2, grouping the producers of the highest levels of proteases in cultures, comprised members of *Stenotrophomonas* (soil isolates) and *Pseudoalteromonas* (sea water isolate) genus. The proteases produced by them had characteristics more related to those showed by mesophilic enzymes and developed a multiple band profile in zymograms. These bands might be the result of active fragments of self-digestion or the activity of more than one protease produced. The likely synthesis of more than one protease could be thought as a strategy to better face the fluctuating supply of nutrients as well as to enhance the uptake of proteins in oligotrophic environments. The only marine isolate from Group 2 produced neutral metalloproteases with optimal activity at 45 ºC whereas the other three isolates produced alkaline serinproteases with optimal activity at 55-60 ºC. Proteases from the four strains of Group 2 had high specific activity with pH and temperature dependence similar to mesophilic commercial proteases. Even when they were much more stable than Group 1 proteases, the thermal stability was not as high as that of the commercial subtilisin. Concerning pH effect, even when the maximal activity occurred at alkaline pH, the three serinproteases were more active than subtilisin at acidic pH. This was confirmed by the pH stability of the proteases, being slightly lower at high pH but considerably higher at low pH (4-5). This broad range of pH activity and stability makes them interesting for industrial application and probably reflects the pH oscillation of the natural habitats from which bacteria were isolated.

It may be stated that the thermolability of the Antarctic proteases we described here is a characteristic widely found in enzymes from cold-adapted organisms. This is mostly due to structural adaptations that confer to these enzymes a high catalytic efficiency at the temperatures of the biotopes where the producing organisms thrive (Feller et al. 1997, Feller 2003). As they never had to confront high temperatures, the enzymes of Antarctic microorganisms could have evolved towards the acquisition of more flexible tertiary structures allowing higher reaction velocities and substrate turnover. From a biotechnological point of view, the thermal sensitivity that these enzymes have could result in an advantage for their selective inactivation, as it was proposed for an Antarctic bacterial alkaline phosphatase (Kobori et al. 1984).

Among the different strategies that bacteria can adopt to acclimatize to cold, adaptations at the enzymatic level are needed to support nutrient degradation and uptake at a rate compatible with life and to compensate for the low kinetic energy at low temperatures (Nichols et al. 1999). For accomplishing that, bacteria can either synthesize cold-efficient enzymes or simply synthesize more enzymes (Feller and Gerday 1997). Taking this into account and considering the higher levels of protease activity found in cultures, as well as the higher specific activities found in Group 2 proteases in comparison with other proteolytic isolates from the same area (Vazquez et al. 1995, 2002), we can infer that these four Antarctic strains are adapted to cold by means of synthesizing more enzymes with high activity. However, the proteases

produced by these strains are somehow more related to mesophilic proteases than to true psychrophilic ones.
On the basis of all the results obtained, it can be possible to consider Antarctica as a promising source of psychrotolerant microorganisms able to produce proteases adapted to efficiently work at low and moderate temperatures. These organisms possess a great potential not only in basic research, for the study of their metabolic capabilities and adaptation to cold, but also for their application in industrial microbiology and biotechnology. They are interesting for their use in processes where it is useful to work at ambient temperature in cold and moderate climate areas.

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4. RESPONSE OF KEY ORGANISMS TO GLOBAL AND REGIONAL CHANGES AND ECOSYSTEM FUNCTIONING

A synthesis of research on biological effects of UV radiation in the water column of Potter Cove

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The studies on the effects of ultraviolet radiation (UVR, 280-400 nm) on bacteria and phytoplankton in Potter Cove started in 1998. A set of publications including PhD theses were produced, while other studies are still ongoing. Here we attempt to synthesize the knowledge gained on this subject along the years in an integral way, additionally including a brief description of the light field in Potter Cove waters. The different methods used to reach the results presented here can be found in the referenced papers.

The light environment in Potter Cove

Potter Cove is an environment strongly influenced by the surrounding land, specially through inputs of organic and inorganic particles from soil and glaciers. As previously described, the cove is characterized by a strong sedimentation of particles, which are in turn exposed to periodic resuspension by winds, representing an additional source of particles entering the water column (Klöser et al. 1994; Schloss et al. 1998). Regarding the influence of suspended particles (SP) on the optical conditions of the water column two contrasting scenarios can be considered. On the one hand, during the spring-summer period when wind speed is < 4 m s^{-1}, a shallow pycnocline is formed, mainly originated by surface freshening. Under these conditions, a high retention of SP can be observed at depths between 0 and 10 m. These SP absorb and scatter a significant portion of incident photosynthetic available radiation (PAR, 400-700 nm). On the other hand, when wind speed is > 4 m s^{-1} the pycnocline is eroded and vertical mixing deepens. This favours resuspension and again PAR is absorbed and scattered (Schloss et al. 2002). These observations led to the hypothesis that phytoplankton in Potter Cove is severely limited by PAR, which could explain the low biomass concentrations in the site (Schloss and Ferreyra 2002).

Beside the effects of PAR in the water column, ultraviolet A radiation (UVAR, 315-400 nm) and ultraviolet B radiation (UVBR, 280-315 nm) effects were studied on the lower levels of the planktonic food web (bacteria and phytoplankton). There is a special concern on UVBR due to the spring reduction of the stratospheric ozone layer in Antarctica, which led to increases in this range of radiation (Frederick and Lubin 1988; 1995; Perin and Lean 2004). UVR is also absorbed and scattered by SP and dissolved materials, including inorganic particles (SIP), dissolved and particulate organic carbon (DOC and POC, respectively) and phytoplankton chlorophyll a (Chla). It should be noted that DOC strongly absorbs in the UVBR range. However, low concentrations of DOC (~14 mg m^{-2}), as well as of POC (~7 mg m^{-2}) and Chla (< 1 mg m^{-2}) were measured in Potter Cove. In contrast, SIP presented concentrations two orders of magnitude higher (~200 mg m^{-2}) (data from Ferreyra et al.: this issue). Consequently, SIP would be the most important factor controlling UVR penetration in the water column at this site. Figure 1 shows the vertical distribution of density, Chla and the depth of the photoactive zone of UVR (Z_{ph}, the depth of 10% of the radiation incident in surface; Neale et al. 2002) in the water column during two days with contrasting SIP concentrations (~96 and ~45 mg m^{-2} for December 27 2000 and January 10 2001, respectively). Z_{ph} was determined for irradiances measured with a PUV-500 spectroradiometer profiler (Biospherical Inc., USA) at 305, 320, 340 and 380 nm,. Maximum Chla values were observed at depths above or immediately below the pycnocline, found at ~5 and ~10 m on December 27 and January 1, respectively. Z_{ph} for UVBR was ~6 and ~8 m while Z_{ph} for UVAR reached ~12 and ~17 m in December 27 and January 1, respectively. These data are consistent with Z_{ph} and diffuse spectral attenuation coefficient (K_d) values described for Potter Cove (Campana et al.: this issue). These observations suggest that, despite the high SIP concentrations, phytoplankton cells may be significantly exposed to both UVBR and UVAR. Consequently, the combination of SIP and the structure of the water column may potentially control the effects of UVR on planktonic organisms. A series of studies were conducted in Potter Cove aiming to understand the effects of UVR on bacterioplankton and phytoplankton, as well as on photochemistry.

UVR effects on bacterioplankton

The study of the effects of UVR on marine bacteria in Potter Cove focused on two strains which were isolated locally. The strains were identified by 16S rRNA gene sequencing as *Arthrobacter* sp. for one of the species, whereas the other one was identified as belonging to the *Flexibacter-Cytophaga-Bacteroides* group (FCB-related species) (Hernández et al. 2004). A series of exposure experiments were done with both species during different surveys (Hernández et al. 2002, 2004, 2006). Cells were incubated under natural solar conditions at the coast of Potter Cove, and exposed to different irradiance treatments using interferential filters (Schott, Germany): Dark, PAR (UVR shielded out), PAR+UVAR and PAR+UVAR+UVBR. Both species showed a high loss of viability after exposure to high UVBR and UVAR doses. However, UVBR effects were more important than

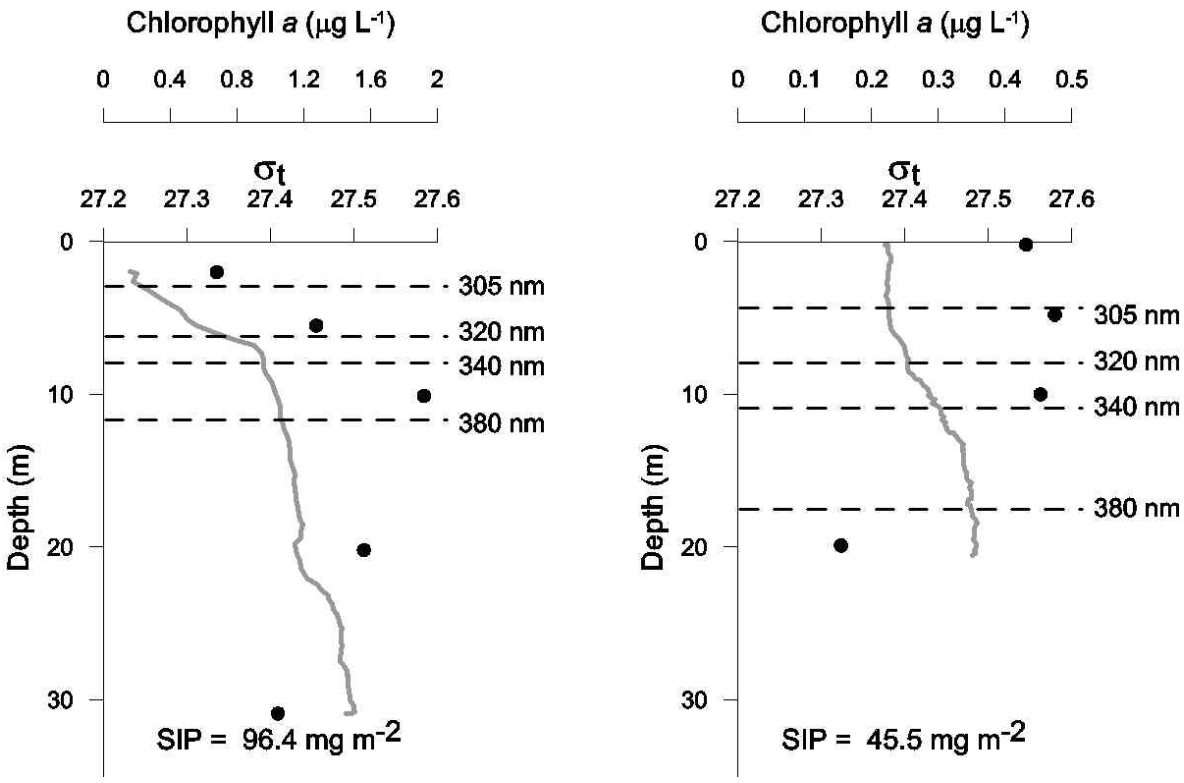

Figure 1. Vertical distribution of water density (continuous line), Chla (filled circles) and Z_{ph} for 305, 320, 340 and 380 nm (dashed lines) during December 27 2000 and January 10 2001. Integrated 30 m SIP concentrations are also shown as inserted text in the figure.

those from UVAR. Mortality > 90 % was observed beyond a threshold UVBR dose ~4 kJ m^{-2}. Moreover, at moderate irradiances FCB-related species showed to be more sensitive to UVBR than *Arthrobacter* sp. This was evidenced during experiments designed to study the dose response of both species to UVBR within the 280-305 nm range (Hernández et al.: 2006, Hernández et al. : this issue). The lethal dose 50 (LD 50) for FCB-related species was 1.2 and 2.8 kJ m^{-2} for 280 and 305 nm, respectively, whereas for *Arthrobacter* sp. they were 2.3 and 4.0 kJ m^{-2}. These results show significant inter-specific differences in the responses of bacterioplankton. Moreover, marked effects on the viability of these organisms can be observed under short-term exposure (2-5 h) to natural insulation, even during cloudy days. Similar differences between both species were observed during incubations performed at fixed depths in the water column (0, 1 and 3 m) (Hernández et al.: this issue). Furthermore, these effects showed some degree of alleviation when the strains were submitted to simulated conditions of vertical mixing (Hernández et al.: this issue). However, in general no complete recovery was observed under such experimental conditions. This could be explained by the low repair capacity after damage found in both *Arthrobacter* sp. and the FCB-related species, as shown during experiments exposing cells to different periods of darkness after solar exposure (Hernández et al. 2004). These observations suggest that repair mechanisms are not effective enough to balance UVR damage in these species.

UVR effects on phytoplankton

Phytoplankton photosynthetic, photoprotective and antioxidant responses to UVR were studied experimentally during several summer missions, on a typical bloom forming diatom species (*Thalassiosira* sp.) isolated from Potter Cove. The role of vertical mixing on UVR effects was additionally tested as a mechanism of photoprotection for photosynthesis and growth of *Thalassiosira* sp. The same light treatments used for the studies of UVR effects on bacterioplankton were used here (at the exception of the Dark one).

A series of short-term field experiments were performed. Frames with the above treatments were moored fixed at surface and 5 m depth. In addition, a moving array of frames was used to simulate vertical movements of the cells (Hernando and Ferreyra 2005). The speed and depth of the simulated mixing incubations were based on previous research on the dynamics of suspended particles in Potter Cove (Schloss et al. 2002; Schloss 1997). Dates of experiments in the field coincided with low and high stratospheric ozone concentration conditions. The same experimental design was used in laboratory controlled experiments using a solar simulator (SONSI) developed by the AWI. In these experiments, UVBR intensities corresponding to low, medium and normal ozone concentrations, as well as vertical mixing, were simulated mimicking field conditions. Results from field experiments were consistent with those from the laboratory, showing that mixing favoured photosynthesis in the three treatments (i.e., PAR, PAR+UVAR and PAR+UVAR+UVBR), compared to the surface- and 5 m-fixed incubations. The only exception was a day when a high absorption of light due to a high load of suspended particles in the water column was observed. On the other hand, a strong reduction in photosynthesis (~40 %) was observed in surface fixed incubations in the SONSI under low ozone simulations and in the field during a day with a strong ozone depletion. Furthermore, simulated mixing failed to completely counteract the effects of increased UVBR during that day (35 and 27 % reduction in the PAR+UVAR+UVBR treatment, as compared to the PAR and PAR+UVAR treatments, respectively). These results suggest that even if vertical mixing in the water column alleviates the negative effects of UVBR, during strong ozone depletion events such process is not completely efficient. Despite this, the application of a mixing index (Hernando and Ferreyra 2005) showed that the relative effect of mixing in alleviating the harmful effects of UVBR on photosynthesis was higher during low than during normal ozone events.

In addition to the study of external protection from UVBR damage by vertical mixing, internal mechanisms reducing these effects were studied at the physiological level. One of them was the induction and accumulation of mycosporine like amino acids (MAAs). During these experiments *Thalassiosira* sp. was exposed to the previously mentioned light treatments in the short-term (hours, photosynthesis) and long-term (days, growth). Cells were exposed to natural solar irradiance at the coast of Potter Cove and to simulated ozone, UVR and mixing conditions in the laboratory using the SONSI. Two MAAs were identified and quantified during both types of experiment: porphyra-334 and shinorine (Hernando et al. 2002; Hernando et al.: this issue). During the exposure

experiments to the natural solar radiation, a net accumulation of both MAAs was observed in the short-term (18 h) in the treatment submitted to PAR+UVAR+UVBR. In the long-term (7 days) a significant accumulation of both MAAs took place in the three light treatments during the first two days of the experiment, reaching a *plateau* during the following days. Maximum concentrations of both porphyra-334 and shinorine were measured in the PAR+UVAR+UVBR treatment. Cell numbers were significantly lower in this treatment than in the others until day 4, reaching similar densities from day 5 to the end of the experiment. The comparison between cell growth rates and the rates of MAAs induction in the different light treatments showed an inverse relationship during the first days of the experiment, reaching similar values from day 4 until the end of the experiment. These results suggest that UVBR retarded algae growth rates at the beginning of the experiment due to the need of cells to synthesize photoprotective compounds, but also that the metabolic cost of this process at the beginning of the exposure to UVR did not affect biomass accumulation in the long-term. An interesting finding from these experiments was the presence of a significant change in the shinorine : phorphyra-334 ratio under the different light treatments from 18 h of exposure up to the end of the experiment. The lowest values were observed in the treatments exposed to UVR compared to the PAR control, with minima in the PAR+UVAR+UVBR treatment. Experimental data from SONSI designed to study the effect of vertical mixing and UVBR on *Thalassiosira* sp. were also consistent with these observations (Hernando et al.: this issue). Given that both MAAs absorb in the same range of UVR, these results suggest the presence of a differential response of these MAAs to UVR, probably related to differences in the rates of induction and photodecomposition of both compounds (Conde et al. 2000, 2004), or to a different enzymatic mechanisms of transformation of porphyra-334 and shinorine in other MAAs (Callone et al. 2006). However, more research is needed to test this hypothesis.

The effects of UVR-induced oxidative stress on phytoplankton were also studied. UVBR may indirectly damage cells via the formation of reactive oxygen species (ROS) like singlet oxygen ($^1O_2$), superoxide radical (O_2-), hydroxyl radical (.OH) and hydrogen peroxide (H_2O_2). Organisms can in part counteract oxidative stress by synthesizing enzymatic and non-enzymatic antioxidants (see the paper from Hernando et al. 2005 and Hernando et al.: this issue, for a more detailed description of these processes). Studies in Potter Cove focused on the responses of two non-enzymatic antioxidants (α-tocopherol and β-carotene) in *Thalassiosira* sp. and in a phytoflagellate (Hernando 2006). These responses were compared to those of one sub-Antarctic phytoflagellate (*Asteromonas* sp.). One of the most significant results emerging from these studies was the fact that both Antarctic species showed significantly lower antioxidant responses than the sub-Antarctic phytoflagellate. In contrast, MAAs induction was much higher in *Thalassiosira* sp and *Asteromonas* sp. than in the phytoflagellate . These results suggest the presence of different adaptive mechanisms to UVR in both environments.

UVR and hydrogen peroxide (H_2O_2) accumulation in the water column

Indirect effects of UVR on bacteria and phytoplankton can originate from the formation of oxidant photoproducts in the water column. DOC can react photochemically with UVR, particularly UVBR, producing H_2O_2 in surface waters (Moffet and Zafiriou 1990; Karl et al. 1993). Net accumulation of this photoproduct in the water column depends on the balance between formation and biological enzymatic breakdown (Abele-Oschger et al. 1997). A main concern with this compound is that H_2O_2 is a ROS highly toxic for organisms, directly entering through the cell membrane and producing damage (Halliwell and Gutterigde 1985). Its dynamics was studied in Potter Cove in October – December 1995, during the period of spring ozone minimum (Abele et al., 1999). A series of vertical profiles down to 30 m depth and in the scale of 1 m were done under the seasonal sea ice and in open waters, as well in tidal ponds and sea ice. In addition, water exposure experiments were also carried out. Surface waters (0 m) presented the highest H_2O_2 concentrations (average for the whole study 247 ± 64 nmol L^{-1}), decreasing as a function of depth (141 ± 24 nmol L^{-1} 5 m). Tidal pools presented concentrations in the same range than surface waters (220 ± 40 nmol L^{-1}). These values are low compared to results from temperate areas, but were close to the ranges reported for Antarctica (Weller and Schrems 1993). On the other hand, exposure of filtered seawater to different ranges in the ultraviolet showed the presence of some photochemical breakdown of DOC with formation of H_2O_2 (66 ± 29 nmol L^{-1} h^{-1}), particularly by UVBR. Low H_2O_2 concentration and photoproduction in Potter Cove were explained by the low DOC concentrations in the water column during the study (mean concentrations 121 ± 24 µmol C L^{-1}), which are consistent with values reported for the same area during a different survey (Ferreyra et al.: this issue). Contrasting with the previous results, relatively high H_2O_2 concentrations were measured in surface waters during periods of strong rainfall (1450 nmol L^{-1}), as well as in freshly deposited snow (> 10000 nmol L^{-1}) and in tidal pools (2000 nmol L^{-1}). Only during these events concentrations in the micro-molar range were observed, suggesting that the most important input of H_2O_2 to this coastal system is snowfall, more than DOC breakdown by UVR. An interesting point to be highlighted is the fact that none of the two bacterial strains studied were affected by H_2O_2, even at concentrations by far higher than those measured in the natural environment in Potter Cove, suggesting the presence of a strong antioxidant response in these organisms (Hernández et al. 2002; 2004).

Concluding remarks

Research on UVR effects on bacteria and phytoplankton suggest that these organisms can be significantly affected. Species studied in Potter Cove were limited in number, and consequently results are difficult to extrapolate to natural communities. However, the detailed observations obtained allow some general conclusions, suggesting that several factors can act together in controlling UVR effects on plankton. These are mainly vertical mixing, the structure and optical properties of the water column, and internal photoprotective mechanisms of the cells.

Global warming and glacier retreat may induce significant changes in the above environmental variables (Cook 2005; Schloss 2003; Schloss et al.: this issue) increasing the stratification of the water column, which could start even earlier in the season than before. Decreasing and increasing trends in surface salinity and temperature, respectively, were documented for Potter Cove during the last 15 years, which support these ideas (Schloss et al.: this issue). This may in turn lead to an augmentation in the residence time of cells in shallow surface waters, and consequently increase their exposure to UVR, particularly to UVBR during the spring ozone hole period. This could be in part counterbalanced by changes in the optical properties of the water column, due to an increased input of suspended particles from glaciers. However, how both processes (i.e. changes in stratification and in optical conditions) will act in the long term in the context of global warming remains an answered question. In this sense, more long term monitoring of UVR and the physical and optical characteristics of the water column in coincidence with eco-physiological studies on the plankton community are necessary. Such type of research should also consider a wider range of species, as well as experiments with natural communities.

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A comparative study of defense strategies against UV-induced damage in an Antarctic diatom (*Thalassiosira* sp.) and a sub-Antarctic phytoflagellate (*Asteromonas* sp.)

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Introduction

During the past decades, spring-time stratospheric ozone depletion over the Antarctic and the Southern Ocean has resulted in enhanced levels of ultraviolet-B (UVB, 280-315 nm) radiation reaching the earth´s surface. One of the main difficulties to evaluate the potential ecological impact on natural phytoplankton of increased in UVB is that UV sensitivity is species specific (Hernando & San Román 1999; Hernando 2006). UVR induced effects on phytoplankton include the reduction of growth and photosynthetic rates (Villafañe et al., 2003). Some studies have demonstrated that the of ultraviolet-A (UVA, 315 – 400 nm) were generally higher than that of UVB (Villafañe et al., 2003). This is generally attributed to the fact that the amount of UVA energy that reaches the Earth's surface is much higher than that in the UVBR region (Villafañe et al., 1999).

Several biological effects of UVB involve endogenous photosensitization and formation of reactive oxygen species (Martin & Burch 1990). There are a variety of sensitizers within cells which absorb UVB. Interaction between excited sensitizers and triplet oxygen produces active oxygen intermediates consisting of singlet oxygen ($^1O_2$), superoxide radical (O_2^-), hydroxyl radical ($^{\cdot}OH$) and hydrogen peroxide (H_2O_2) (Ichiki et al. 1994). One of the possible mechanisms that could counteract the damage generated by UVB radiation induced oxidative stress is the synthesis of both enzymatic and non-enzymatic antioxidants (Davidson 1998; Niyogi 1999). Moreover UVB can destroy the natural lipid soluble antioxidants and promote the formation of lipid peroxidation products (Estévez et al. 2001). The non-enzymatic antioxidants are generally small molecules, such as ascorbate and glutathione acting in the aqueous phase, whereas the lipophilic antioxidants (such as α-tocopherol and β-carotene) are active in the membrane environment. Especially α-tocopherol is known for its protective effect against lipid peroxidation of biological membranes via peroxyl and alcoxyl radicals scavenging. In contrast, the main function of β-carotene is photoreceptive, because it acts as a pigment antenna in the photosynthesis process.

Another strategy to minimize the effects of UVR (ultraviolet radiation) is through the presence of UV screening compounds. The most studied compounds are those collectively named mycosporine like amino acids (MAAs), which are found in many marine and freshwater algae (Karentz et al. 1991; Banaszak 2003). MAAs have been proved to be an effective protection mechanism (Neale et al. 1998). The synthesis of MAAs was found in some natural populations and cultures of phytoplankton (Hernando et al. 2002; Zudaire & Roy 2001). How-

ever, the synthesis of MAA´s is not a general response, and several species do not show an increase of MAAs content even after several weeks of exposure to UVR (Villafañe et al. 2000).
The aim of this work was to study immediate effects on growth rate and photo-protection parameters in response to UVB and UVA on a Sub-Antarctic phyto-flagellate (*Asteromonas sp.*) and an Antarctic diatom (*Thalassiosira* sp.) under culture conditions.

Materials and Methods

Culture conditions

The experiments were carried out in the Beagle Channel (CADIC, Ushuaia, 54º 52'S, 68º 18'W) and Potter Cove (South Shetland (25 de Mayo) Islands, Antarctica, 62°14'S, 58°38'W) . Surface water samples were taken using a five-liter Niskin bottle and maintained in the laboratory at 8°C and 3ºC for Sub-Antarctic and Antarctic species, respectively. Phytoflagellate (*Asteromonas sp*.) and diatom (*Thalassiosira* sp.) single cells were isolated and inoculated into 200 ml flasks with filtered seawater plus F/2 culture medium (Guillard 1975).Before the experiments, cultures were grown at an irradiance of 210 $\mu mol.m^{-2}.s^{-1}$ photosinthetically active radiation (PAR), provided by ´cool-white´ fluorescent bulbs on 12/12 h at 8ºC and 3ºC, respectively. Once exponential growth was reached, aliquots of the mono-specific cultures were inoculated into a series of 500 ml vessels and then exposed to solar radiation from February 24 to March 2, 1998 (*Asteromonas* sp.) and January 4 to 9, 1998 (*Thalassiosira* sp.). For chlorophyll-a (chl-a) analyses 20 ml water was filtered through Whatman fiberglass filters (GF/F, 25 mm) followed by the extraction of pigments in absolute methanol (Holm-Hansen & Riemann 1978). Sub-samples for cell counts were kept in dark bottles and fixed with formalin previously neutralized with sodium borate (final concentration 0.4% w/v). All the samples were counted with an inverted microscope using a Sedgwick-Rafter counting slide according to Villafañe & Reid (1995).

Experimental design

Cells were exposed in parallel to three irradiance treatments: PAR (control, 400-700 nm), UVA (PAR + Ultraviolet-A radiation, UVA, 315-400 nm) and UVB (PAR + UVA + UVB). Cylindrical UV-transparent quartz flasks were used for phytoplankton exposure. For the UVA treatment, the bottles were covered with Mylar foil (DuPont country, which has 50% transmission at 323 nm) and PAR controls were performed with cylindrical Plexiglass flasks (UF3) (Röhm & Haas Darmstadt, Germany) which cutt off all UV radiation (Hernando & San Román 1999). Three replicate samples were used for each of the treatments and controls. Culture medium was added to the different treatments at the beginning of the experiment. Samples (80 ml) were taken out daily at 9 a.m. to determine chl-a concentration, cell number and the content of MAA´s for *Asteromonas* sp. where samples were analysed at the beginning and at day 5 of experiment. The content of α-tocopherol and β-carotene was measured at the beginning of the experiment, and at days 1, 3 and 5 of exposure.

Irradiance measurements
Incident solar radiation was monitored continuously during the experiment using a spectroradiometer (model GUV 510, Biospherical Instruments, Inc.), which records irradiances at four wavelengths in the ultraviolet region (305, 320, 340 and 380 nm), and Photosynthetic Available Radiation (PAR, 400-700 nm). Data were recorded at a frequency of one per minute in a site close to the experimental setup. To calculate UVB and UVA doses the appropiate equation from Orce et. al. (1997) was used.

Growth measurements
Cell instantaneous growth rate was determined according to the following equation:

$$\mu = \ln (N_{t1}/N_{tn-1})$$

Where μ is a specific rate constant (d^{-1}), t_1 is the time of measurement and t_{n-1} is the time of the previous one, N_1 is the cell concentration at time t_1, and N_{n-1} is the cell concentration at time t_{n-1}.

Lipid soluble antioxidants
The content of α-tocopherol and β-carotene in the cell homogenates was quantified by reverse-phase HPLC with electrochemical detection using a Bioanalytical Systems LC-4C amperometric detector with a glassy carbon working electrode at an applied oxidation potential of 0.6 V (Desai 1984). Extraction from the samples was performed with 1 ml of methanol and 4 ml of hexane. After centrifugation at 600*g* for 10 min, the hexane phase was removed and evaporated to dryness under N_2. Extracts were dissolved in methanol:ethanol (1:1 v/v) and injected for HPLC analysis. d,l- α-tocopherol from synthetic phytol (Sigma) and β-carotene were used as standards.

UV-absorbing compounds (MAA´s)
Samples from the fixed-depth experiments were extracted during two hours in 7 ml of methanol at 8ºC. After extraction, samples were centrifuged; the supernatant was scanned from 250 to 750 nm with a Shimadzu (model UV-1203) spectrophotometer. The peak height at 334 nm (OD, height in mm of peak maximum from spectrophotometric scan of methanol extract) was considered as an estimate of the concentration of UV-absorbing compounds (Mycosporine-like amino acids, MAAs; Dunlap et al. 1995).

Statistical analyses
All the statistical analyses were carried out with the program statistica and they correspond to the exponential instantaneous growth phase.
Parametric repeated measures analysis of variance was applied to test the significance of the observed differences between treatments. Data were tested prior to the analyses in order to verify the homoscedacity and normality requirements of the ANOVA. When such requirements were not satisfied, a standard transformation of data was applied. When the interaction was significant, the differences between treatments for every day were analyzed. In all cases the exponential phase of the growth rate of PAR treatment was taken as control.

In order to compare the protection efficiency in the *Thalassiosira* sp. cells, a parametric multiple regression model was applied for each treatment. In this model, the growth rate was considered as dependent variable dependent as a function of MAA´s and antioxidant as independent variables.

Results and Discussion

On *Asteromonas* sp. an inhibition of growth rate by UVA radiation was observed during the exponential phase (**Figure 1 A**, Hernando et al., 2005). In *Thalassiosira* sp. there was an inhibition of growth by UVB and UVA on day 1 and only by UVB on day 2 of the exponential phase. Afterwards, no growth inhibition was observed from day 3 to 5 (**Figure 1 B, Table 1 A**).

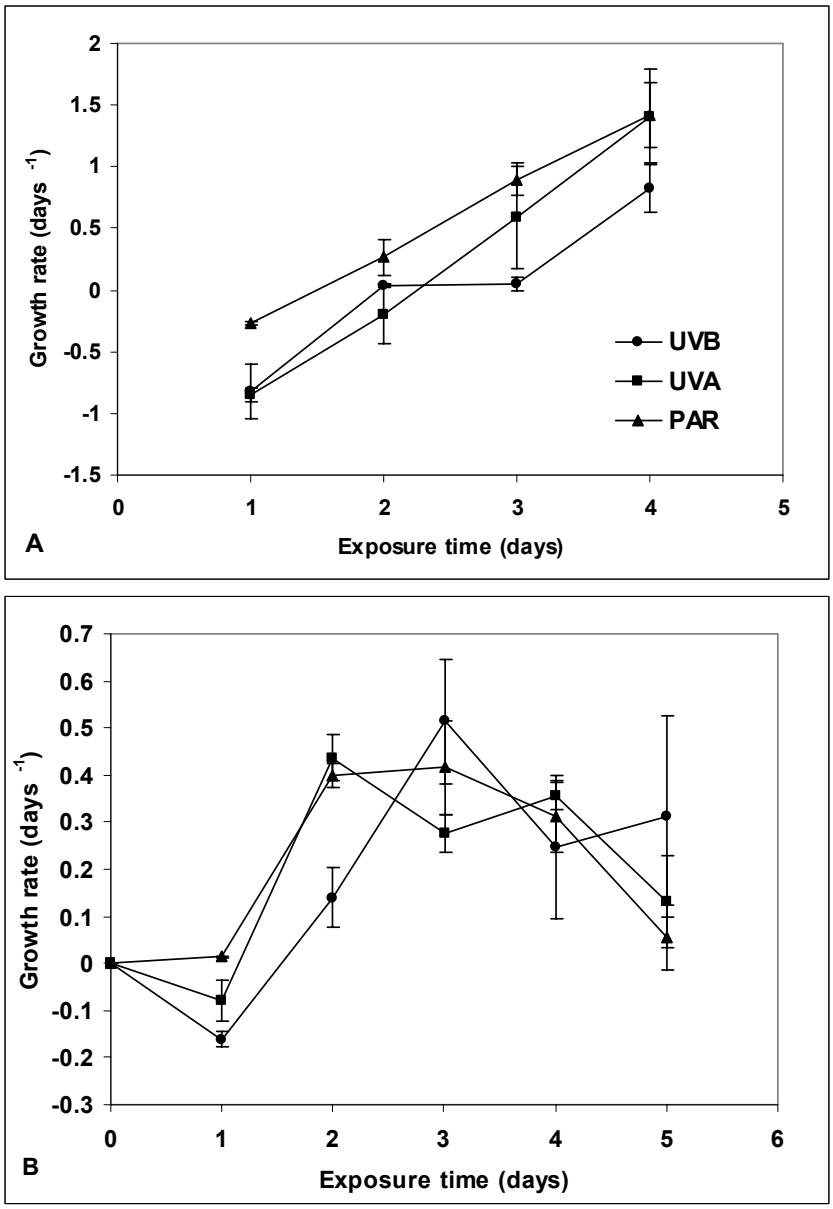

Figure 1
Exponential instantaneous growth rate of A) *Asteromonas* sp.; B) *Thalassiosira* sp. exposed to UVB treatment (UVB+UVA+PAR), UVA treatment (UVA+PAR) and PAR treatment (PAR). Each point represent the mean ± SD.

The values were significantly lower than those of PAR (**Figure 2 A, B; Table 1 B**). However, the content of α-tocopherol in *Asteromonas* sp. was significantly higher than those observed in *Thalassisira* sp. (**Figure 2**, P< 0.01). α-tocopherol in *Thalassiosira* sp. reached maximum values around 5 pmol $(10^4 \text{ cell})^{-1}$ without significant differences between days 1, 3 and 5 for UVB and UVA treatment.
No significant differences were observed between days in the content of lipid soluble antioxidants (α-tocopherol and β-carotene) neither for *Asteromonas* (Hernando et al. 2005) nor for *Thalassiosira* sp. exposed to PAR (P> 0.05, **Figure 2 and 3 C**). The decrease in α-tocopherol in the studied diatom species (respect to initial day of exposure) and the increase in α-tocopherol and β-carotene in *Asteromonas* (after day three, Hernando et al. 2005), suggests an

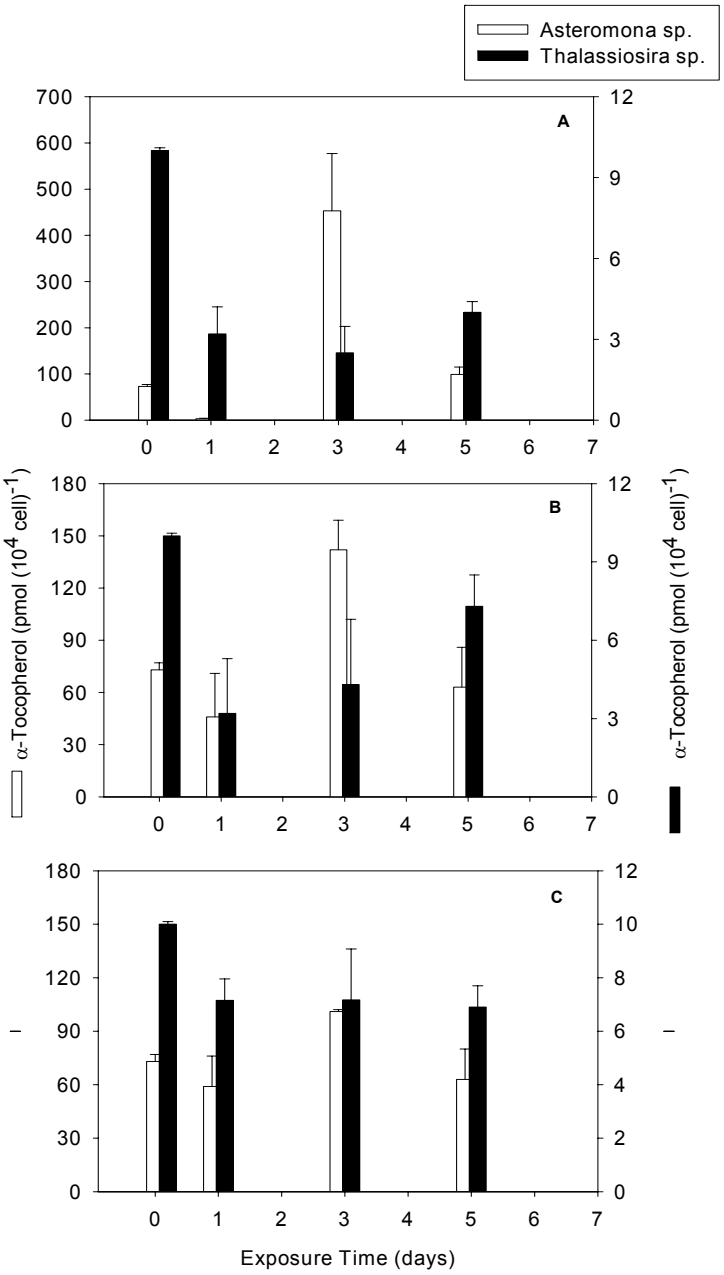

Figure 2
Effect of UV radiation on content of α-tocopherol in *Asteromonas sp.* and *Thalassiosira sp.* as a function of exposure time. A: UVB treatment (UVB+UVA+PAR), B: UVA treatment (UVA+PAR) and C: PAR treatment. Each point represents the mean ± SD.

A	Growth rate	
Factor	F	Sign. Level
Day	7.4	< 0.01
Treatment	45.03	< 0.001
Interaction	7.06	< 0.001
Day	F	Homog. Group
1	27.27	< 0.0001
UVB		**a**
UVA		**b**
PAR		**c**
2	28.18	< 0.001
UVB		**a**
UVA		**b**
PAR		**bc**
3	2.42	> 0.05

B	α-tocopherol	
Factor	F	Sign. Level
Day	19.39	< 0.01
Treatment	22.94	< 0.01
Interaction	2.24	> 0.05
	Means	Homog. Group
Day 0	10	**a**
Day 1	4.52	**b**
Day 3	4.77	**b**
Day 5	6.06	**b**
UVB	4.94	**a**
UVA	6.85	**ac**
PAR	8.2	**b**

C	β-carotene	
Factor	F	Sign. Level
Day	2.33	> 0.05
Treatment	2.05	> 0.05
Interaction	1.27	> 0.05

D	MAA´s	
Factor	F	Sign. Level
Day	96.7	< 0.0001
Treatment	24.47	< 0.001
Interaction	9.42	< 0.001
Day	F	Homog. Group
1	1.36	> 0.05
3	20.11	< 0.001
UVB		**a**
UVA		**b**
PAR		**bc**
5	19.29	< 0.001
UVB		**a**
UVA		**b**
PAR		**c**

Table 1
Results of parametric analysis of variance repeated measures showing the significance of UV effects on the exponential instantaneous growth rate of Antarctic *Thalassiosira* sp. Note: The factors are Day (1, 2, 3 and 4) and Treatment (UVB, UVA and PAR) and the variable was μ (instantaneous growth rate). The same letter means no significant differences (Tuckey test).

active generation of active oxidant species during exposure of growing cultures, leading to the consumption of antioxidants. In *Thalassiosira* sp., the content of α-tocopherol showed a significant decrease on the exponential growth period (until day 3) of the exposure for algae exposed to the UVB and UVA treatment.
The connection between oxidative damage on the one hand, and antioxidant defense mechanisms on the other, has been postulated in both animal and plant cells (Kingston-Smith 2000). This observation could explain the reduction of growth rate inhibition in UVA treatment, as resulting from a high decrease of α-tocopherol in *Thalassiosira* sp. Cells (**Figure 1 B, Table 1 B**).
Lipophilic molecules as α-tocopherol are able to deactivate $^1O_2$, reduce O_2 and terminate lipid radical chain reactions (Polle & Rennenberg 1994), and are regenerated by ascorbate. The content of β-carotene in *Asteromonas* sp. increased significantly (Hernando et al. 2005) in cultures exposed to UVB and (contrasting with α-tocopherol results) UVA on day 3 of incubation. These results could be explained considering that there was a significant increase of the lipoperoxidation (TBARS) in the UVA treatment on day 1 and 2 of incubation (Hernando et al. 2005). A significant decrease of β-carotene was observed on

days 4 and 5 in the UVA treatment. These results could indicate little consumption or a decreased synthesis, suggesting a significant role for this antioxidant on protection against the damage produced by UVA radiation. This fact could explain the tendency to decrease the inhibition on UVA treatment for the growth rate on days 3 and 4 (35% and 5% compared with PAR treatment, respectively; Hernando et al. 2005). In the Antarctic diatom, however, the content of β-carotene did not show differences between days or treatments (P> 0.05) (**Figure 3, Table 1C**). Like for the case of α-tocopherol, the content of β-carotene in the diatom was significantly lower than for *Asteromonas* sp., reaching maximum values around 3 pmol $(10^4 \text{ cell})^{-1}$. An Antarctic phytoflagellate (Hernando, 2006) and *Thalassiosira* sp. produced only 10 and 1.5% of β-carotene as compared with the *Asteromonas* from Beagle Channel. These reduced values suggest a stronger antioxidant response to UVR from the Sub-Antarctic phytoflagellate species studied in this work.

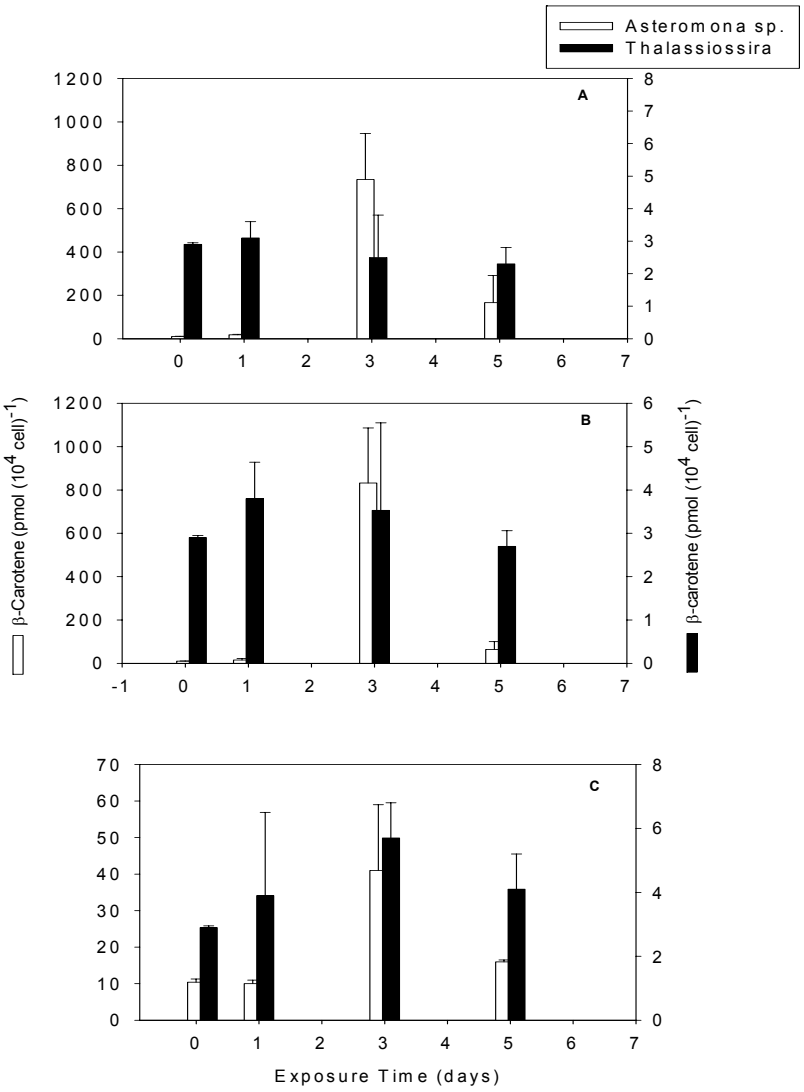

Figure 3
Effect of UV radiation on content of β-carotene in *Asteromonas sp.* and *Thalassiosira sp* as a function of exposure time. A: UVB treatment (UVB+UVA+PAR), B: UVA treatment (UVA+PAR) and C: PAR treatment. Each point represents the mean ± SD.

On the other hand, probably other defense mechanisms may help the cells to cope with UVR damage, like the synthesis of MAA´s, which probably play a significant role in photoprotection in Antarctic phytoplankton (Hernando et al. 2002). Indeed, MAA´s concentration per cell of *Thalassiosira* sp. was maximum and significantly higher in the UVB treatment on day 3 (**Table 1 D**, **Figure 4 A**), when growth inhibition was not observed. The cellular concentration of MAA´s did not show significant differences between treatments neither between day 1 and 5 of exposure of phytoflagellate (**Figure 4 B**).

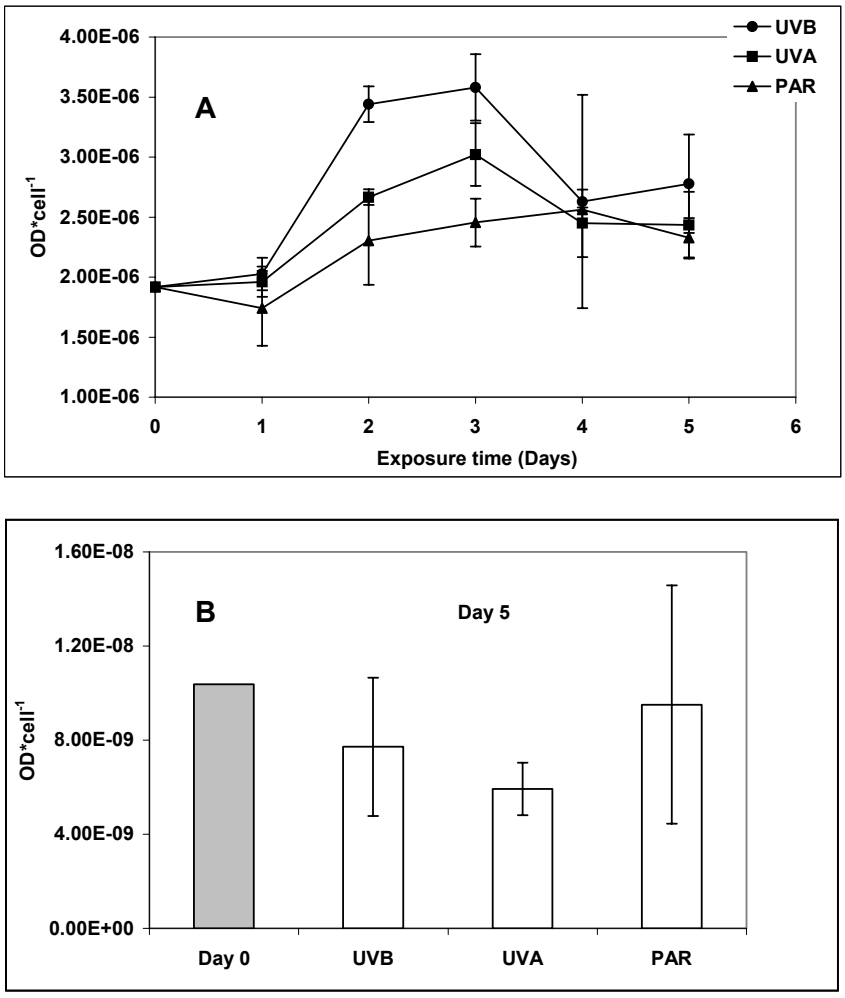

Figure 4
Absorbance representative of photoprotective mycosporine-like amino acids (MAAs) for the different radiation treatments, normalized to total cell number/mL. a: *Thalassiosira* sp. at every day of exposure. b: Asteromonas sp. at the beginning and at 5th exposure day. Means and standard deviations of means are presented.

The cellular concentration was significantly higher in *Thalassiosira* sp. as compared with that observed in *Asteromonas* sp. When was compared the protection efficiency in *Thalassiosira* cells, the MAA´s concentration was significant in the UVB and UVA treatments in relation to α tocopherol (**Table 2, A, B**). These results showed a positive relation between MAA´s and higher growth rate. In

PAR treatment there was no relation between both defense strategies and growth (**Table 2 C**). In *Asteromonas* sp., despite the fact that on UVA treatment the inhibition showed a tendency to be lowest at the end of the exponential growth, on UVB treatment the inhibition remained high (95% and 42% for days 3 and 4 respectively, Hernando et al. 2005). Clearly, the damage (TBARS) was low on days 4 and 5, so protection was somehow present, but maybe not in the form of lipid antioxidants. Perhaps by that time the cells have developed other ways to cope with harmful UV. These results could be explained considering that other stress conditions, like DNA damage, could be related to UVB radiation in algae (Karentz et al. 1991).

A

R	F	P	Variable	B	t	P
0.83	5.46	< 0.05	Intercept	-1.1	-2.76	< 0.05
			Antioxidant	0.1	1.6	> 0.05
			Maa´s	292257	2.81	< 0.05

B

R	F	P	Variable	B	t	P
0.76	4.11	< 0.05	Intercept	-0.8	-2.22	< 0.05
			Antioxidant	-0.1	-1.14	> 0.05
			Maa´s	487398	2.69	< 0.05

C

R	F	P	Variable	B	t	P
0.52	0.74	> 0.05	Intercept	0	0.003	> 0.05
			Antioxidant	-0.1	-0.66	> 0.05
			Maa´s	309926	1.15	> 0.05

Table 2
Multiple regression results showing the significance of α-tocopherol and MAA´s in exponential phase of growth rate for treatment: (A) UVB (UVB+UVA+PAR); (B) UVA (UVA+PAR) and (C) PAR.

Conclusions

A significant result coming forth from this experiment is that UVR-induced damage affected the starting time of exponential growth, but not final biomass accumulation nor growth during the exponential phase in neither of the studied species. Observation suggests that cells were able to cope with UV, and that the only significant effect was the delay in the timing of exponential growth.

Overall, our results support the idea that UVR damage/repair balance involves the combined action of several internal factors in the cell. However, our results showed different mechanisms in the algae groups studied. On the Sub-Antarctic *Asteromonas* sp. the production of tocopherols plays a key role in UV protection. On the other hand, in the *Thalassiosira* sp Antarctica the MAA´s synthesis was the most effective internal factor for photoprotection

Acknowledgements
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Photosynthetic performance and impact of ultraviolet radiation on the reproductive cells of Antarctic macroalgae

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Introduction

Macroalgal species inhabiting the polar regions of both Hemispheres are generally classified as being low light adapted (Kirst and Wiencke 1995). The radiation regime in high latitudes is subject to strong seasonal variation (polar days and nights) and also to changes caused by sea-ice conditions (Svendsen et al. 2002). This consequently affects algal productivity and population dynamics in the course of the year. The degree of UV-B (280- 315 nm) exposure is accordingly highly seasonal as affected by the sea-ice, prevailing weather conditions and the turbidity of the water column (Hanelt et al. 2001).

A yearly net springtime stratospheric ozone loss of 60- 70% over Antarctica has been a recurring phenomenon since its detection in the early 1980s that intensifies ambient UV-B radiation on the biosphere (Crutzen 1992; Herman *et al*. 1996). The area affected by ozone depletion has also expanded to 5 fold over the past decades in the continent. In this regard, it is necessary to study stress physiology on Antarctic primary producers.

The adverse effects of UV-B exposure on photosynthesis result from the absorption of high-energy radiation by biomolecules such as proteins and nucleic acids. The D1 protein in the core complex of photosystem II and the carbon dioxide-fixing enzyme RubisCO have been identified as major targets of UV exposure (Vass 1997; Bischof et al. 2000). Exposure to UVR may also generate reactive oxygen species contributing to the photooxidation of compo-nents of the photosynthetic machinery (e.g. pigments such as chlorophylls) (Bischof et al. 2003).

Existing Antarctic phycological studies show the lack of information on the effect of UVR on seaweeds (Wiencke 1996; Wiencke et al. 2006). The advance in Antarctic macroalgal research is primarily constrained by logistic difficulties.

This is in contrast to the more accessible Arctic locations where recent studies have shown that early life stages of macroalgae are most sensitive to UVR and their sensitivity is related to the depth distribution pattern of the adult sporophytes (Roleda *et al*. 2007).

No study has yet been conducted on the structural, biochemical and physiological responses of reproductive cells of Antarctic macroalgae exposed to UVR. This paper presents the physiological aspect with emphasis on the photosynthetic performance of the propagules of several macroalgal species exposed to light stress.

Materials and methods

Algal material

Fertile thalli of several macroalgal species (2 greens, 2 browns, and 3 reds) were collected in Peñon Uno and Peñon de Pesca around King George Island, Antarctica (62° 14'S, 58° 42'W) (Table 1). Blades with reproductive structures (n= 5) were thoroughly cleaned of epiphytes, washed with filtered seawater and processed for release of reproductive cells. Propagules released were maintained under low light condition (1- 2 µmol photon m^{-2} s^{-1}).

Table 1. Species collected around King George Island, their distribution zone, reproductive cell types isolated from different life-history stages and corresponding sizes of their propagules.

Class/Species	Distribution zone	Reproductive cell type	Cell size (µm)
ULVOPHYCEAE			
Monostroma hariotii Gain	eulittoral	gamete	7‡
Urospora penicilliformis (Roth) Areschoug	eulittoral	zoospore	6; 20‡
PHAEOPHYCEAE			
Adenocystis utricularis (Bory de Saint-Vincent) Skottsberg	eulittoral	zoospore	4‡
Ascoseira mirabilis Skottsberg	sublittoral	gamete	3
BANGIOPHYCEAE			
Porphyra endiviifolium (A. Gepp & E.S. Gepp) Y.M. Chamberlain	supralittoral	monospore	15
FLORIDOPHYCEAE			
Iridaea cordata (Turner) Bory de Saint-Vincent	eulittoral	tetraspore	22
	sublittoral	tetraspore	20
Gigartina skottsbergii Setchell & N.L. Gardner	sublittoral	tetraspore	23
	sublittoral	carpospore	27

Cell sizes are in diameter, ‡ cell length

The initial density and cell size of reproductive cells was counted and measured by use of a Sedgewick-Rafter Cell S50 spore counter (Graticules Ltd., Tonbridge, England) observed under a light microscope (Zeiss Axioab, Ger-

many). Stock suspensions were diluted with filtered seawater to obtain cell densities necessary for the desired background fluorescence for photosynthetic measurements among the five replicates.

Irradiation treatments

Photosynthetically active radiation (PAR, 400- 700 nm) was provided by white fluorescent tubes (Osram, L65 Watt/25S, Munich, Germany). Ultraviolet radiation (UVR, 280- 400 nm) was generated by UVA-340 fluorescent tubes (Q-Panel, Cleveland,OH, USA). Cell culture dishes were covered with one of the following filters to cut off different wavelength ranges from the spectrum emitted by the fluorescent tubes: Ultraphan transparent (Digefra GmbH, Germany), Folanorm (Folex GmbH, Germany) or Ultraphan URUV farblos corresponding to the PAR + UV-A + UV-B (PAB), PAR + UV-A (PA) and PAR (P) treatments, respectively. Ultraviolet radiation was measured using a Solar Light PMA 2100 radiometer equipped with the UV-A sensor PMA 2110 and the UV-B Sensor PMA 2106 (Solar light, Philadelphia, USA). Ultraviolet radiation below the UV-transparent filter was 4.34 W m^{-2} UV-A and 0.40 W m^{-2} UV-B. Photosythetically active radiation was adjusted using a cosine quantum sensor attached to a LI-COR data logger (LI-1000, LI-COR Biosciences, Lincoln, Nebraska, USA) to be 22 µmol photon m^{-2} s^{-1} (~ 4.73 W m^{-2}).

Chlorophyll fluorescence measurements

Photosynthetic parameters were measured as variable fluorescence of photosystem II (PSII) using a Water Pulse Amplitude Modulation fluorometer (Water-PAM) connected to a PC with WinControl software (Heinz Walz GmbH, Effeltrich, Germany). After propagule release and adjustment of cell density (not exceeding 1 h after spore release), optimum quantum yield (F_v/F_m, n= 5) and photosynthesis-irradiance curve (P-I curve in terms of relative electron transport rate, rETR = PFR x $\Delta F/F_{m'}$, n= 3) were measured at time zero as described by Roleda et al. (2006a). The hyperbolic tangent model of (Jassby and Platt 1976) was used to estimate P-I curve parameters described as:

$$rETR = rETR_{max} * \tan h (\alpha * I_{PAR} * rETR_{max}^{-1})$$

where $rETR_{max}$ is the maximum relative electron transport rate, tan h is the hyperbolic tangent function, α is the initial slope of the curve at pre-saturation irradiance (as a measure for the electron transport efficiency) and *I* is the photon fluence rate of PAR. The saturation irradiance for electron transport (I_k) was calculated as the intercept between α and the ETR_{max} values. Curve fit was calculated with the Solver module of MS-Excel using the least square method comparing differences between measured and calculated data.

Controls measured at time zero were filled into corresponding culture dishes. To evaluate the effect of different radiation treatments and exposure times, 5 ml of fresh reproductive cell suspension were filled into each 35mm x 10mm cell culture dish (Corning™, Corning Inc., NY, USA) and exposed under

the three radiation conditions for 4 hours (n= 5) at 2 ± 1.5 °C. After exposure treatment, F_v/F_m was determined and the suspension was returned to the same culture dish and cultivated under dim white light (4 ± 1 µmol photons m^{-2} s^{-1}) at the same temperature for recovery. Time zero control was also maintained at the same condition. Measurements of photosynthetic recovery were made after 24- 48 hours in dim white light condition. Settled and germinating zygotes were slowly re-suspended by sucking and jetting the medium against the bottom of the culture dish using Eppendorf pipettes. Optimum quantum yields were expressed as percent of control.

Results

The I_k values of propagules investigated varied between species, reproductive cell type and habitat. Saturating irradiance (I_k) was highest in the zoospores of the eulittoral green macroalga *Urospora penicilliformis* and lowest in the monospores of supralittoral red macroalga *Porphyra endiviifolium* (Table 2). Comparison between eulittoral and sublittoral *Iridaea cordata* showed comparable I_k values (38- 39 µmol photon m^{-2} s^{-1}), while diploid carpospores of *Gigartina skottsbergii* have higher a I_k value compared to its haploid tetra-spores. Comparison between different groups of algae showed a generally higher I_k in green (83- 87 µmol photon m^{-2} s^{-1}) followed by brown (52- 64 µmol photon m^{-2} s^{-1}) and lower in red (33- 54 µmol photon m^{-2} s^{-1}) macroalgae.

Table 2. Photosynthesis-irradiance curve parameters estimated using the hyperbolic tangent equation of Jassby and Platt 1976, and mean optimum quantum yield (F_v/F_m) of propagules immediately after release and after post cultivation in dim white light (4 ± 1 µmol photons m^{-2} s^{-1}) for 24- 48 h.

Class/Species	P-I curve parameters			Photosynthetic efficiency (F_v/F_m)	
	I_k	Alpha	$rETR_{max}$	After release	After post cultivation
ULVOPHYCEAE					
M. hariotii	83	0.06	5.41	0.288±0.04	0.397±0.15
U. penicilliformis	87	0.16	14.14	0.501±0.04	0.511±0.04
PHAEOPHYCEAE					
A. utricularis	64	0.14	9.04	0.462±0.11	0.601±0.04
A. mirabilis	52	0.10	4.99	0.400±0.06	0.446±0.05
BANGIOPHYCEAE					
P. endiviifolium	33	0.12	4.07	0.488±0.04	0.249±0.02
FLORIDOPHYCEAE					
I. cordata (eulittoral)	39	0.15	6.01	0.476±0.04	0.448±0.07
I. cordata (sublittoral)	38	0.11	4.28	0.445±0.04	0.523±0.02
G. skottsbergii (tetraspore)	44	0.13	5.60	0.307±0.07	0.371±0.05
G. skottsbergii (carpospore)	54	0.13	6.87	0.403±0.03	0.434±0.02

I_k (µmol photon m^{-2} s^{-1}) is the light intensity at which the initial slope of the curve ($\propto$) intercepts the horizontal asymptote of the maximum relative electron transport rate ($rETR_{max}$).

The slope alpha (α), a parameter for the performance of both light-harvesting and photosynthetic conversion efficiency, is characterized by a gradual increase (α= 0.06) of rETR in gametes of *Monostroma hariottii* and a steep increase (α= 0.16) in zoospores of *U*. *penicilliformis* at lower photon flux density (PFD). Photosynthetic capacity, expressed as $rETR_{max}$, was highest in *U*. *penicilliformis* and lowest in *P. endiviifolium* (Table 2).

Optimum quantum yield of the PSII (F_v/F_m) of freshly released reproductive cells was likewise highest in *U*. *penicilliformis* (0.501 $\pm$ 0.04) and lowest in *M. hariotii* (0.288 $\pm$ 0.04). In brown and red macroalgae, higher F_v/F_m was observed in supra- and eulittoral (*Adenocystis utricularis*, *P*. *endiviifolium* and *I*. *cordata*) compared to sublittoral (*Ascoseira mirabilis* and *G*. *skottsbergii*) species (Table 2). Post cultivation in dim white light (4 µmol photon m^{-2} s^{-1}) generally showed an increase in the photosynthetic efficiencies of germinating cells except for *P. endiviifolium* (Table 2).

Exposure to 4 hours of different light treatments consisting of PAR only (P), PAR+UV-A (PA) and PAR+UV-A+UV-B (PAB) showed species-specific response in F_v/F_m, expressed as percent of control (Fig. 1). All species, except *U*. *penicilliformis* and *A*. *utricularis* (Fig. 1B-C), were photoinhibited after exposure to a PAR fluence of 6.8 x 10^4 J m^{-2}. A 50% decrease in F_v/F_m was observed in other supra- and eulittoral species (*M*. *hariotii*, *P*. *endiviifolium* and *I*. *cordata* [int]; Fig. 1A, 1E-F), and 70- 87% decrease in F_v/F_m of sublittoral species (*A*. *mirabilis*, *I*. *cordata* [sub] and *G*. *skottsbergii*; Fig. 1D, 1G, 1H-I).

Relative to PAR treatment, light supplemented with UVR further reduced photosynthetic efficiency of propagules by 18- 65% in the PA and 26- 78% in the PAB treatment. The monospores of the supralittoral species *P*. *endiviifolium* were most tolerant to UVR with minimal additional photoinhibition of 18% and 26% in PA and PAB treatments respectively (Fig. 1E). Tetraspores of the subtidal *I*. *cordata* were most susceptible to PA treatment with additional 65% photoinhibition (Fig. 1G) while gametes of the sublittoral *A*. *mirabilis* were most susceptible to the impact of PAB with additional 78% reduction in their photosynthetic efficiency (Fig. 1D).

Post cultivation in dim white light (4 µmol photon m^{-2} s^{-1}) allowed repro-ductive cells of all species investigated to recover their photosynthetic efficiencies. Recovery was relatively more efficient in supra- and eulittoral species (Fig. 1A-C, E-F) compared to sublittoral species (Fig. 1D, G-I). Moreover, photosynthetic recovery was also higher in diploid carpospores compared to haploid tetraspores of *G*. *skottsbergii* (Fig. 1H-I).

Discussion

This study reports on the latest advances of phycological research in Antarctica with emphasis on the photosynthetic activity of reproductive cells of several macroalgae. The impact of exposure to varying spectral composition on the photosynthesis of propagules showed a species-specific relation to the zonation pattern of the parental plants and the corresponding life-history cell types.

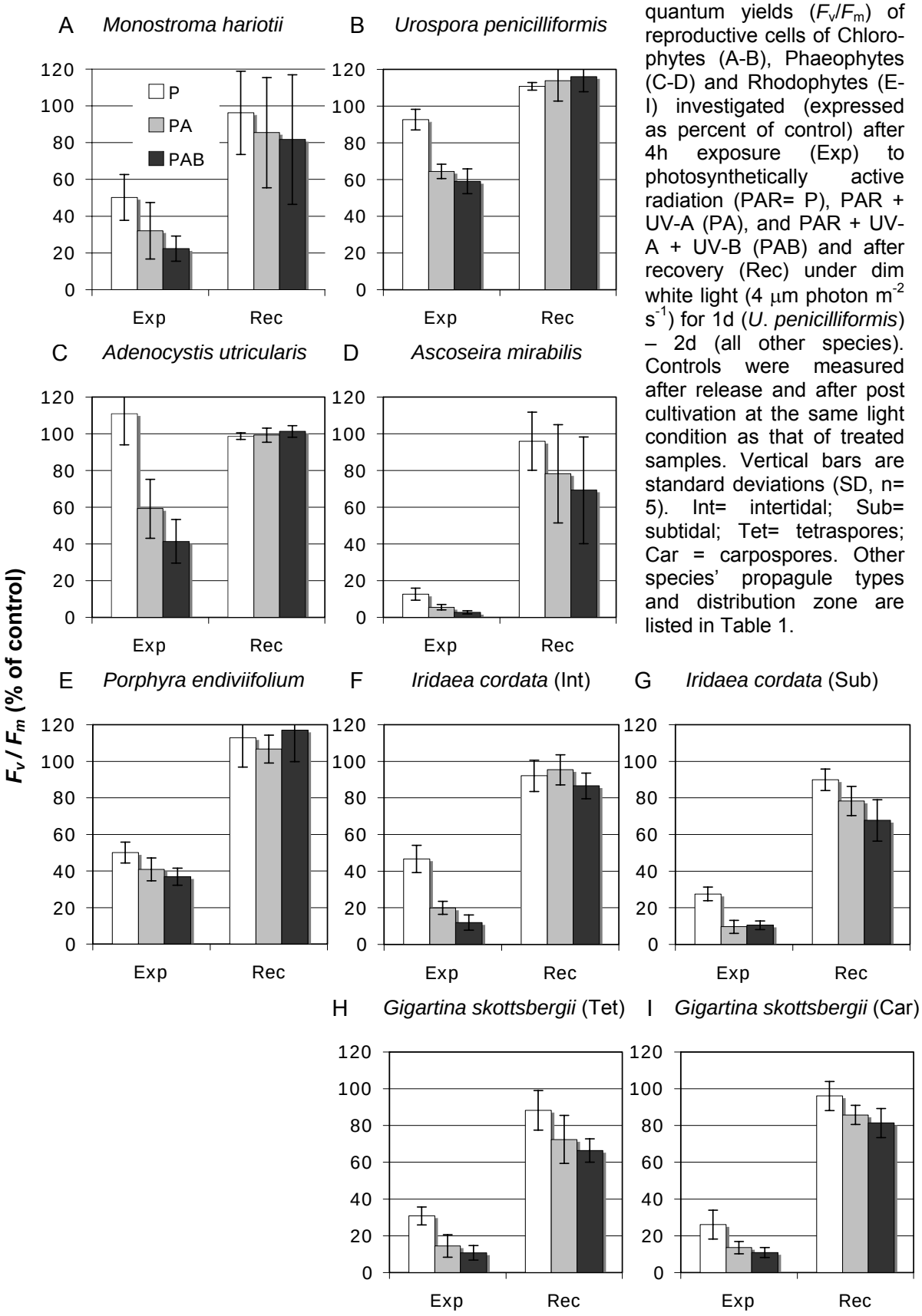

Figure 1. Mean optimum quantum yields (F_v/F_m) of reproductive cells of Chlorophytes (A-B), Phaeophytes (C-D) and Rhodophytes (E-I) investigated (expressed as percent of control) after 4h exposure (Exp) to photosynthetically active radiation (PAR= P), PAR + UV-A (PA), and PAR + UV-A + UV-B (PAB) and after recovery (Rec) under dim white light (4 μm photon m^{-2} s^{-1}) for 1d (*U. penicilliformis*) – 2d (all other species). Controls were measured after release and after post cultivation at the same light condition as that of treated samples. Vertical bars are standard deviations (SD, n= 5). Int= intertidal; Sub= subtidal; Tet= tetraspores; Car = carpospores. Other species' propagule types and distribution zone are listed in Table 1.

The estimated slope (α) and saturating light intensity (I_k) derived from P-I curves showed that photosynthesis of reproductive cells of Antarctic macroalgae are shade adapted compared to adult plants (e.g. *U. penicilliformis*; Roleda and Campana, unpublished data). Low light adaptation of photosyn-thesis is observed to be the general characteristic feature of reproductive cells of macroalgae (Amsler and Neushul 1991; Roleda et al. 2004, 2005, 2006a, 2006b; Zacher et al. 2007). This might be related to the chlorophyll antenna size and number of chloroplast present in reproductive cells compared to multicellular macroscopic stages. Survival of propagules will therefore be dependent on their immediate settle-ment on substrate at depths or under algal canopies where the prevailing low-light microenvironment is suitable for their germination.

A photon fluence of 6.8 x 10^4 J m^{-2} PAR did not affect the F_v/F_m of zoospores of eulittoral species *U*. *penicilliformis* and *A*. *utricularis*. In spores and gametes of other species, the reduction of photosynthetic capacity and quantum efficiency when exposed to fluence of PAR exceeding their requirement is a protective strategy to dissipate excess energy absorbed by the photosystem II as heat to avoid photodamage. This is a regulative protective mechanism against excessive radiation also known as dynamic photoinhibition (Osmond 1994). This process may also be regulated by an increase in the zeaxanthin content of the PSII antenna (Adams and Demming-Adams 1992) and/or by increasing the amount of inactive PSII centres which dissipate a surplus of absorbed energy as heat to protect the photosynthetically active centres (Öquist and Chow 1992). In contrast, impairment of D1 protein leading to decrease in photosynthetic capacity is called chronic photoinhibition. This occurs in shade-adapted macroalgae growing in the lower sublittoral zone when exposed to high irradiances. These species have a lower ability to down-regulate photosynthesis through the protective dynamic photoinhibitory process (Hanelt et al. 2003).

Additional reduction of the photosynthetic efficiency was observed in all species exposed to light supplemented with UV-A and UV-A+UV-B. Mono-spores of supralittoral species *P*. *endiviifolium* were observed to be the most tolerant to UVR. Although the measurable effects of both PAR and UVR in the reduction of photosynthetic efficiency are similar, the mechanisms behind PAR- and UVR-induced inhibition of photosynthesis are different (Franklin et al. 2003). Photosynthetic performance may be additionally depressed in light treatments supplemented with UVR by possible damage to the oxidizing site and reaction center of PS II (Grzymski et al. 2001; Turcsányi and Vass 2002).

After photoinhibition, recovery of photosynthesis often requires dim white light condition (Hanelt et al. 1992). Full recovery of photosynthetic capacity was observed in supra- and eulittoral species after 24- 48 hours post-cultivation in low white light. Incomplete recovery was observed in sublittoral species especially in propagules exposed to UVR. Recovery of photosynthetic efficiency of zoospores of different kelp species varies between 8 and 24 hours in upper and lower sublittoral species respectively (Roleda et al. 2006a). Exposure to UVR was further observed to delay photosynthetic recovery of Arctic kelp zoospores (Roleda et al. 2006a). Comparison between species showed that intertidal *I*. *cordata* tetraspores were more tolerant to all light treatments compared to

tetraspores isolated from subtidal sporophytes. Higher recovery rates were also observed in spores of intertidal *I*. *cordata* pre-exposed to UVR. Depth related sensitivity of reproductive cells was previously reported in kelp zoospores isolated from sporophytes collected at different depth gradient (Swanson and Druehl 2000). Although the measured photoinhibition of photo-synthesis are similar between the diploid carpospores and haploid tetraspores of *G*. *skottsbergii*, more efficient recovery was observed in diploid carpospores compared to the haploid tetraspores.

The sensitivity of photosynthesis of reproductive cells of Antarctic macroalgae to PAR and UVR is related to the observed zonation pattern of the adult plants. This response was also reported in the early life stages of macroalgae from the northern Hemisphere (Roleda et al. 2007). The prevailing environmental factors in different habitats along the vertical gradient of the shore are important in conditioning the physiological optimum and conferring fitness for the survival of the organism. An increase in stratospheric ozone depletion and the corresponding increase in irradiance of UV-B on the biosphere might, however, re-shape seaweed community structure along coastal environments (cf Bischof et al. 2006).

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UV-susceptibility of photosynthesis of adult sporophytes of four brown Antarctic macroalgae (Phaeophyceae)

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Introduction

The Antarctic ozone hole is reported to develop annually in Austral spring since the late 1970s and results in increasing irradiances of solar UV-B radiation on the earth's surface and in the aquatic environment (Farman *et al.* 1985, Häder *et al.* 1998). Exposure of organisms to enhanced UV-B radiation results in multiple damage, e.g. dimerisation of DNA molecules, formation of reactive oxygen species, inhibition of photosynthesis and growth (Roleda 2006, Lesser 2006, Rautenberger & Bischof 2006, Mansilla *et al.* 2006). Due to a lower solar zenith angle over Antarctica, the irradiance of ultraviolet radiation is usually lower in comparison to the tropics. Therefore, Antarctic macroalgae are generally considered to be adapted to these low UV irradiances and may thus exhibit a higher UV-susceptibility than temperate or tropical macroalgae. In this study we aimed to evaluate the susceptibility of photosynthetic efficiency of four abundant field-grown macroalgae from Antarctica, exposed to artificially increased irradiances of UV-radiation.

Material and methods

In January and February 2005, individuals of four brown macroalgal (Phaeophyceae) species, *Adenocystis utricularis* (collected from the eulittoral), *Ascoseira mirabilis* (collected 1.50 m below low tide level), and *Desmarestia menziesii* (collected 1 m below low tide level; see also Klöser *et al.* 1996) were collected at "Peñon Uno" (Potter Cove, King George Island). Individuals of *Desmarestia anceps* (sublittoral) were obtained by SCUBA diving between 5 to 6 meters depth at "Peñon de Pesca". Upon collection, algal material was immediately covered with black plastic bags and transferred to a climate chamber set to a temperature of 2 °C near the Dallmann Laboratory, Jubany Base. Until experimental exposure, algae were maintained at 15 µmol photons m^{-2} s^{-1} PAR. From these cultures the experimental material was distributed into Petri dishes and exposed to 15 µmol photons m^{-2} s^{-1} of PAR (L58W, Osram, Germany). After 12 hours of pre-acclimation in PAR alone, this radiation regime was supplemented by 9.5 W m^{-2} UV-A (UV-A-340, Q-Panel Lab Products, USA) and 0.87 W m^{-2} UV-B (TL20W/12RS, Philips, The Netherlands), measured by an UV/VIS spectroradiometer (Ramses ACC, TriOS GmbH, Germany). The petri dishes were covered by different cut-off filters in order to generate three light/UV conditions: PAR alone ($\lambda \geq 400$ nm, Ultraphan URUV, Digefra, Germany), PAR+UV-A ($\lambda \geq 320$ nm, Folanorm SF-AS, Folex, Germany) or

PAR+UV-A+UV-B ($\lambda \geq 295$ nm, Ultraphan URT, Digefra, Germany). Under these conditions, the specimens were exposed for four hours and were subsequently transferred to dim PAR (15 µmol photons m^{-2} s^{-1}), to observe recovery from UV-exposure after 24 or 28 hours. Photosynthetic activity was measured as optimum quantum yield of photosystem II ($Fv/Fm=(Fm-F_0)/Fm$) according to Schreiber *et al.* (1994) using a portable PAM-2100 chlorophyll fluorometer (Walz, Germany). Variable chlorophyll fluorescence is a well suited and rapid technique to detect UV-induced stress in algae (Clendennen *et al.* 1996, Hanelt *et al.* 1997, Bischof *et al.* 1998a,b). Before measurements were performed, samples were exposed for 5 minutes to darkness. The protocol of Fv/Fm measurements in brown macroalgae followed the procedure described by Hanelt (1998). Measurements of photosynthetic activity were performed after 4 hours of exposure to PAR alone and UV radiation and after 4 and 24 under recovery conditions. For controls (non-photoinhibited cultures), Fv/Fm ratios for *A. mirabilis* (0.691±0.035), *A. utricularis* (0.755±0.006), *D. menziesii* (0.780±0.007) and *D. anceps* (0.753±0.004) were determined and remained constant during the whole experimental time. The means of the respective Fv/Fm values of controls were normalized to 100% photosynthetic efficiency and all the following readings were calculated as a percentage of these. Statistical analyses were performed using JMP IN 5.1 (SAS Institute Inc., USA) after arcsin-transformation. One-way analysis of variance (ANOVA) with repeated measurements and a subsequent post-hoc test according to Tukey's HSD was conducted in order to identify significant differences between treatments. A level of probability of $p \leq 0.05$ was applied.

Results and Discussion

In all specimens of tested brown macroalgae, photosynthetic efficiencies (Fv/Fm) were affected by the incident ultraviolet radiation. In all species, 4 hours of exposure to either UV-A or UV-B radiation led to a decrease of the optimum quantum yield of PS II (Fig. 1). In *A. mirabilis* exposed under PAR+UV-A, Fv/Fm did not decrease significantly from that of its control (PAR alone) and consequently there was no recovery. Thus, photochemistry of the photosynthetic process was apparently not inhibited by UV-A radiation. Furthermore, the reduction of optimum PS II-quantum yield by only 10% after 4 hours of PAR+UV-A+UV-B exposure and subsequent complete recovery within 4 hours after removal of UV-B radiation might suggest a down-regulation of photosynthesis as a possible strategy of protection against ultraviolet radiation like evidenced for PAR exposure (Osmond 1994, Franklin & Forster 1997). Another effective UV-protection in macroalgae might be based on thallus morphology: thicker algae show less sensitivity to UV radiation than filamentous species (Halldal 1964, Franklin & Forster 1997). *A. mirabilis* consists of optically dark-pigmented leathery fronds (Wiencke & Clayton 2002), and, thus incident UV radiation might be reflected, attenuated or absorbed by the thallus itself to elongate the optical path (Caldwell *et al.* 1983). Furthermore, UV absorbing compounds like pholorotannins may provide cellular protection (Pavia *et al.* 1997), but at present it is not known whether *A. mirabilis* contains UV absorbing components in sufficient quantities.

In the other three species, both PAR + UV-A and PAR+ UV-A + UV-B radiation also caused photoinhibition and, thus, a significantly stronger decrease of Fv/Fm by 6 to 13% and 16 to 21%, respectively. In specimens of *Adenocystis utricularis*, which were collected in the eulittoral, Fv/Fm exhibited a reduction by 9 and 16% after 4 hours of exposure to PAR + UV-A and to PAR + UV-A + UV-B, respectively. Subsequently to these exposures, this species recovered almost completely within 4 hours, as indicated by an increasing Fv/Fm (Fig. 1). Again, such a fast recovery from UV-A and UV-B radiation suggests that photosynthesis in *A. utricularis* might also rather down-regulated (rapidly-reversible) than damaged as suspected in *A. mirabilis*. Hanelt *et al.* (1994) also observed similar kinetics of recovery in field-grown *A. utricularis,* but exposed to natural solar radiation. The authors concluded that PAR-induced photoinhibition was rather a dynamic, regulatory process than photodamage of photosystem II. Thus, this species might be able to acclimate to strong white light (Hanelt *et al.* 1994) and as well as to UV-A and UV-B radiation, as demonstrated in this study.

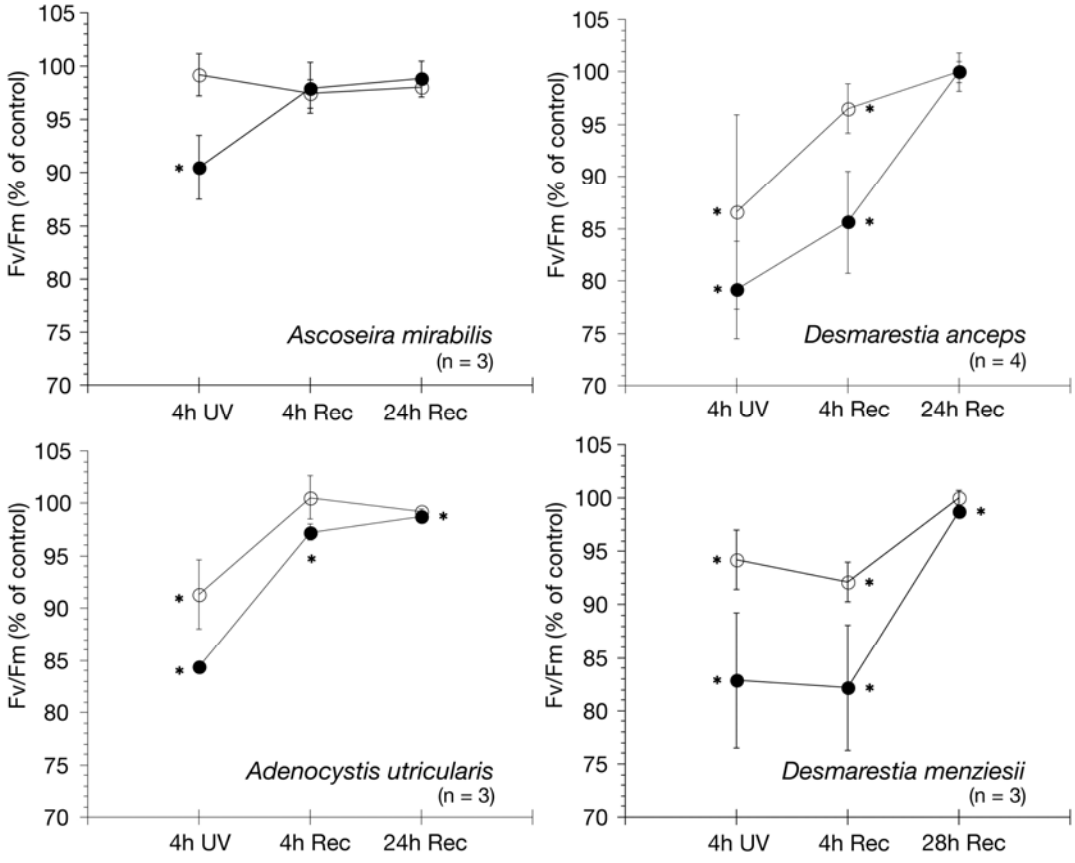

Fig. 1: Impacts of a 4 hours exposure to artificial PAR + UV-A (open circles) and PAR + UV-A + UV-B (closed circles) radiation on optimum quantum yield of PS II (Fv/Fm) of adult sporophytes of four brown macroalgae *Ascoseira mirabilis*, *Adenocystis utricularis*, *Desmarestia anceps* and *Desmarestia menziesii* and their recovery after 4 and 24 hours in dim PAR light. Note that *D. menziesii* was measured after 28 hours of recovery. Error bars represent the coefficients of variation. Asterisks represent significant differences from control values (=100%).

In contrast, *Desmarestia anceps* collected in the sublittoral between 5 and 6 meters depth was a more sensitive species than *A. mirabilis* obtained from 1.5 meters depth with respect to exposure to both, UV-A and UV-B radiation, and

recovery from UV-B radiation (Fig. 1). Fv/Fm in *D. anceps* was reduced by 13% and 21% due to PAR + UV-A and PAR + UV-A + UV-B exposure, respectively. Similar results were also obtained after exposure of *Desmarestia menziesii* to PAR + UV-A (6% of control) and PAR + UV-A + UV-B (17% of control) radiation for 4 hours (Fig. 1). The depression of photosynthetic efficiency was not statistically different between both species. Fv/Fm after 4 hours of recovery from PAR + UV-A + UV-B exposure was not statistically different between both *Desmarestia* species and significantly lower than in *A. mirabilis* and *A. utricularis*. A complete recovery was only measured 24 and 28 hours after of PAR + UV-A and PAR + UV-A + UV-B-exposure. Thus, UV-exposure caused a strongly delayed recovery in both *Desmarestia* species. Hence, such a delayed recovery may reflect a slowly reversible photoinhibition indicating that proteins in photosystem II might be damaged (Osmond 1994) due to incident ultraviolet radiation. Although phlorotannins were found in both *Desmarestia* species (Fairhead *et al.* 2005), Fairhead *et al.* (2006) could not provide any evidence for phlorotannins as UV-screens recently.

In various studies on macroalgae, Fv/Fm was a more sensitive parameter for UV-induced stress to photosynthesis rather than the maximum electron transport rate ($ETR_{max.}$) because light capture by the antennae system might be more affected than photosynthetic reaction centers which may still remain active (Bischof *et al.* 1999, Hanelt *et al.* 1997). Furthermore, besides the degree of inhibition of photosynthetic efficiency, the velocity of recovery in dim PAR after removal of UV radiation can be regarded as an even more significant parameter for evaluation of UV-susceptibility (Bischof *et al.* 1999, Hanelt *et al.* 1997). Many studies comparing the UV-susceptibility in macroalgae from different shore levels revealed lower sensitivity of intertidal species and a higher UV-sensitivity with increasing growth depth (reviewed by Franklin & Forster 1997, Bischof *et al.* 1998a,b). This general pattern could not be confirmed by this study on field-grown macroalgae because neither exposure to UV radiation nor recovery from ultraviolet radiation has revealed an UV-susceptibility in relation to depth distribution. Recapitulatory, *A. mirabilis* from 1.5 meters water depth seems to be a less sensitive species with respect to UV-A and UV-B radiation than *A. utricularis* occurring in the eulittoral. Rapidly reversible UV-induced photoinhibition is applied by *A. mirabilis* and *A. utricularis* to protect them from enhanced UV radiation. A similar pattern of reduction of photosynthetic efficiency and a delayed recovery due to UV-exposure was measured in both species of *Desmarestia*: *D. menziesii* obtained from 1 meter below tide level and *D. anceps* collected between 5 and 6 meters in the sublittoral. The similarity of results from these closely related species (Peters *et al*. 1997) might be due to similar morphological characteristics (Wiencke et al.1995, 1996) of lateral branches and probably by a comparable physiological potential of acclimation. Therefore, probably both *Desmarestia* species could occupy the same zone of the phytal and could exist up to the same depths considering UV-susceptibility. Delépine (1966) and DeLaca & Lipps (1976) reported that both species form mixed stands at their southern distributional limit (Melchior Island) whereas *D. anceps* mainly occurs in the central sublittoral due to lower turbulence resistance and feeding preference by the fish *Notothenia neglecta* for *D. menziesii* growing in the lower sublittoral at King George Island, the center of their geographical distribution (Klöser *et al.* 1996).

Hence, in this study, the decrease of Fv/Fm and the velocity of recovery in dim PAR after UV-exposure were highly species-specific and not ruled by tidal distribution.
In most previous studies on UV-susceptibility of Antarctic macroalgae, laboratory-grown material raised from stock cultures was used (e.g. Bischof *et al.* 1998b), while the present study used field-grown macroalgal material. In contrast to laboratory-grown algal material, which should be used to study mechanisms of adaptation, field-grown material is normally exposed to a broad spectrum of environmental factors and, thus, it is suitable for investigating mechanisms of acclimation.

In summary, these results support the importance of UV-B radiation in structuring seaweed communities from Antarctica.

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Interactive effects of temperature and radiation on UV-absorbing mycosporine-like amino acids in two red macroalgae from Antarctica

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Introduction

Because of stratospheric ozone springtime reduction in Antarctica the ultraviolet B-radiation waveband (UVB: 280-315 nm) markedly increased, and serious concerns have arisen about its impact on the biosphere (Franklin & Forster 1997). In many organisms UV-B is predominately absorbed by DNA, RNA and proteins causing photodamage that can subsequently disturbing vital metabolic functions such as transcription, replication and translation (Buma et al. 1997). Damage of these biomolecules results in a loss of viability such as a reduced or inhibited cell division, reproduction, photosynthesis and growth, or even enhanced mortality (Franklin & Forster 1997; Wiencke et al. 2000). The ecological consequences of these negative changes in cellular and physiological processes are not fully understood, particularly in marine macroalgal communities, but many seaweeds living in the intertidal as well as in the upper subtidal zone of Antarctica are strongly affected (Zacher & Campana, this issue).

However, many macroalgae living in high-radiation habitats are capable to counteract UV-radiation stress by repair and/or protective mechanisms. A very important biochemical strategy against biologically harmful UV-radiation is the biosynthesis and accumulation of mycosporine-like amino acids (MAAs). Typically absorbing in the UVA (315-400 nm) and UVB region, MAAs were invoked to function as passive sunscreen substances by dissipating the absorbed radiation energy in form of harmless heat (Bandaranayake 1998). MAAs are widely distributed in red macroalgae (Karsten et al. 1998; Hoyer et al. 2001), and their photoprotective role has been inferred from cyanobacteria and microalgae that are capable to synthesise and accumulate MAAs under UV-exposure and thus show UV-insensitive growth and photosynthesis compared to cells lacking MAAs (García-Pichel et al. 1993, Neale et al. 1998).

Based on the MAA concentrations and the induction patterns after exposure to different radiation conditions Antarctic Rhodophyta can be classified in 3 categories (Hoyer et al. 2001): Type I - no MAAs at all; Type II – MAAs inducible in variable concentrations, and Type III - permanently high MAA values. While Type I occurs in deep-water red algae, Type II and III species are growing in the supra- and eulittoral zone. Experiments under defined radiation sources indicate that the induction, biosynthesis and accumulation of MAAs is a very flexible and species-specific process. While some taxa synthesise MAAs particularly under UVB, in others MAA synthesis is stimulated under UVA or high PAR (Hoyer et al. 2002).

Besides the stimulating effect of increasing solar radiation on the MAA metabolism in macroalgae other environmental factors such as nutrient availability (Korbee et al. 2005) may also act as controlling factor. Antarctica is characterised by pronounced seasonal variations of the radiation conditions, and their effect on UV-absorbing compounds are well studied in seaweeds (Hoyer et al. 2001, 2002 and references therein). In contrast, the impact of temperature, the other ecological key factor of polar regions, on the MAA metabolism in macroalgae has not been studied so far. Therefore, the present study explores for the first time the interactive effect of temperature and various radiation conditions on the biosynthesis of MAAs in two abundant Antarctic red macroalgae typically inhabiting shallow waters in the Potter Cove (King George Island).

Material and Methods

Algal material

Specimen of the red algal species *Iridaea cordata* (Turner) Bory de Saint Vincent and *Palmaria decipiens* (Reinsch) Ricker were collected in the intertidal of Potter Cove, King George Island, South Shetlands, Antarctica (62°14´S, 58°40´W) and were established as unialgal cultures at the Alfred-Wegener Institute by the last author. Stock cultures were grown in the laboratory at 15 µmol photons m^{-2} s^{-1} PAR, 16:8 h light:dark cycle and 0 °C, and kept in 0.2 µm filtered North Sea water (32 psu) enriched after Provasoli (Starr and Zeikus 1987).

Experimental set up

Both macroalgal species were transferred from stock cultures to the experimental temperatures of 5 and 10°C, and acclimated for 23 days at 15 µmol photons m^{-2} s^{-1} PAR and a light:dark cycle of 16:8 h. The same media as above were used. After this period both seaweeds were treated with three different radiation conditions; PAR (400 to 700 nm, 36 µmol photons m^{-2} s^{-1}), PAR+UVA (320 to 400 nm, 5.0 W m^{-2}), and PAR+UVA+UVB (295 to 320 nm, 0.41 W m^{-2}). The applied UVA and UVB spectral ranges differed from the CIE definition because of the cut-off filters used (see below) and because of the calibration of the available radiometer (PMA 2100, Solar Light Co., Philadelphia, USA) Daylight fluorescent lamps (Lumilux Deluxe, Osram, Germany), in combination with Q-Panel UVA-340 fluorescent tubes (Cleveland, USA) emitting a spectrum similar to solar radiation in the UVR range were used.

During the experiment, plants were kept in glass beakers filled with filtered PES-enriched North Sea water supplemented with 2.1 mM sodium hydrogen carbonate as an additional inorganic carbon source. Glass vessels were covered with specific filters to cut-off UVA+UVB (400 nm cut-off: Folex PR, Folex, Dreieich, Germany), only UVB (320 nm cut-off: Ultraphan URUV, Digefra, München, Germany), and with a 295 nm cut-off filter (Ultraphan UBT, Digefra, München, Germany).The algae were kept for 5 days under these conditions before harvesting. The samples were oven-dried at 50 °C overnight, and then stored in Eppendorf tubes under dry and dark conditions prior to MAA 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

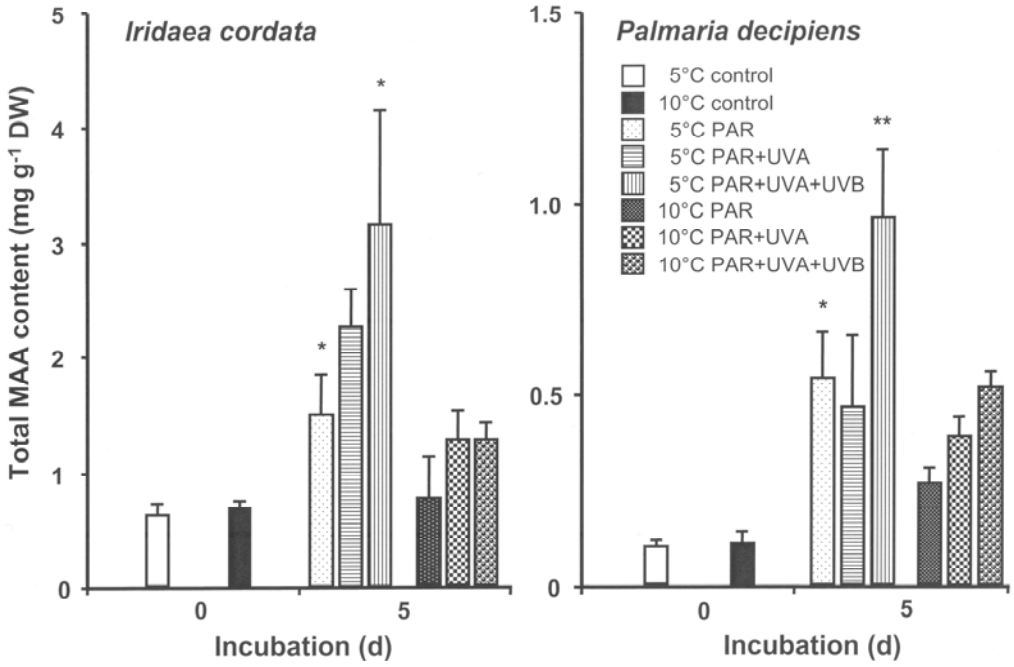

Figure 1 Interactive effect of different radiation conditions (PAR, PAR+UVA, PAR+UVA/B) and temperatures of 5 and 10 °C on the total MAA concentrations in *Iridaea cordata* and *Palmaria decipiens* after 5 days incubation. Given are the mean values ± SD (n=4). Asteriks indicate significant differences in MAA amounts between both temperatures (*: p<0.05; **: p<0.01; ***: p<0.001).

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 5% 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} according to Hoyer et al. (2001). 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*, *Mastocarpus stellatus* and *Porphyra umbilicalis*, which originated from the rocky island Helgoland, North Sea, Germany. Quantification was made using the molar extinction coefficients listed in Karsten et al. (1998b).

All MAA concentrations are given as means of 4-5 replicates (±SD), expressed as concentration on a dry weight basis (mg g^{-1} DW). Differences in the MAA content under the distinct filter treatments and at the different temperatures were statistically verifiyed using a two-way ANOVA followed by a multiple comparison test (Tukey-Kramer HSD - test). When no homogeneity of variances could be obtained a Kruskal-Wallis test was assessed. Significances occured when the probability were at $p < 0.05$.

Results

Both *Iridaea cordata* and *Palmaria decipiens* acclimated for 23 days to dim light (15 µmol photons m^{-2} s^{-1}) and 5 and 10 °C contained similarly low total MAA concentrations (control, Fig. 1). Treatment with higher PAR, PAR+UVA and PAR+UVA/B led in both species to an induction of total MAA content within 5 days. In *I. cordata* all samples at 5 °C contained under each radiation conditions at least 2-fold higher total MAA concentrations than plants kept at the same wavenband at 10 °C ($p < 0.001$) (Fig. 1). In addition, the radiation waveband applied had a strong effect on the MAA amount ($p < 0.01$). However, the response was temperature-dependent. At 5 °C the strongest inductive effect was observed in *I. cordata*, i.e. the shorter the wavelengths the higher the MAA concentration synthesised and accumulated (Fig. 1; $p < 0.01$). PAR+UVA/B treatment was accompanied by a more than 4-fold rise in total MAA amount compared to the control. In contrast, at 10 °C enhanced PAR did not affect the MAA values, while PAR+UVA and PAR+UVA/B led both to a 2-times increase in total MAA content only compared to the respective control ($p < 0.01$).

Palmaria decipiens showed a similar response, however, with some marked differences (Fig. 1). Although total MAA concentration of this species was about 3-times lower, the principal temperature effect was similar, i.e. MAA amounts at 5 °C were about double as high compared to samples kept at 10 °C (Fig. 1; $p < 0.01$). Treatment with enhanced PAR and PAR+UVA at 5°C led to a similar 3-3.5-times accumulation of MAAs, while exposure to PAR+UVA/B resulted in even 7.5-fold higher MAA values in comparison to the control ($p < 0.001$). At 10 °C the response pattern in *P. decipiens* slightly changed, i.e. all treatments led to much lower, but increasing total MAA concentrations towards shorter wavelengths (Fig. 1).

In *I. cordata* the main individual MAAs exhibiting temperature dependent differences in the induction patterns are shinorine and palythine (Abb. 2). After 5 days at 5 °C palythine was present in significantly higher concentrations under all radiation conditions than in the corresponding 10 °C samples ($p < 0.001$). The same was true for the quantitatively dominant shinorine (Fig. 2; $p < 0.01$). In addition, both palythine and shinorine contents increased with decreasing wavelengths.

In contrast to *I. cordata, P. decipiens* contained in addition to shinorine and palythine as main MAA porphyra-334 (Fig. 3). While shinorine and palythine in *P. decipiens* showed the same temperature and radiation response as in *I. cordata*, porphyra-334 exhibited a different pattern. Compared to the control the latter MAA increased about 4-fold at 5 °C already under enhanced PAR ($p < 0.001$). While treatment with UVA did not further stimulate accumulation of porphyra-334, UVB led to a minor additional increase in the concentration. In contrast to the shinorine and palythine values at 10 °C, porphyra-334 contents at this temperature exhibited almost similar, but slightly lower concentrations than at 5 °C. In addition, the shorter the wavelengths the more porphyra-334 was synthesised and accumulated (Fig. 3; $p<0.01$).

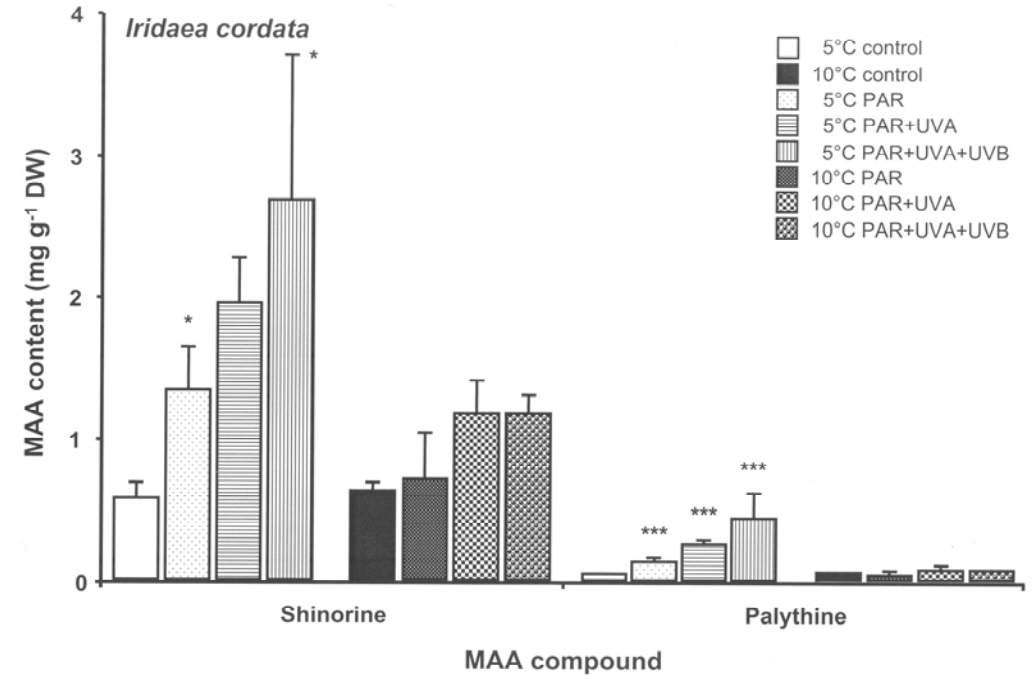

Figure 2 Interactive effect of different radiation conditions (PAR, PAR+UVA, PAR+UVA/B) and temperatures of 5 and 10 °C on individual MAA concentrations in *Iridaea cordata* after 5 days incubation. Given are the mean values ± SD (n=4). Asteriks indicate significant differences in MAA amounts between both temperatures (*: p<0.05; **: p<0.01; ***: p<0.001).

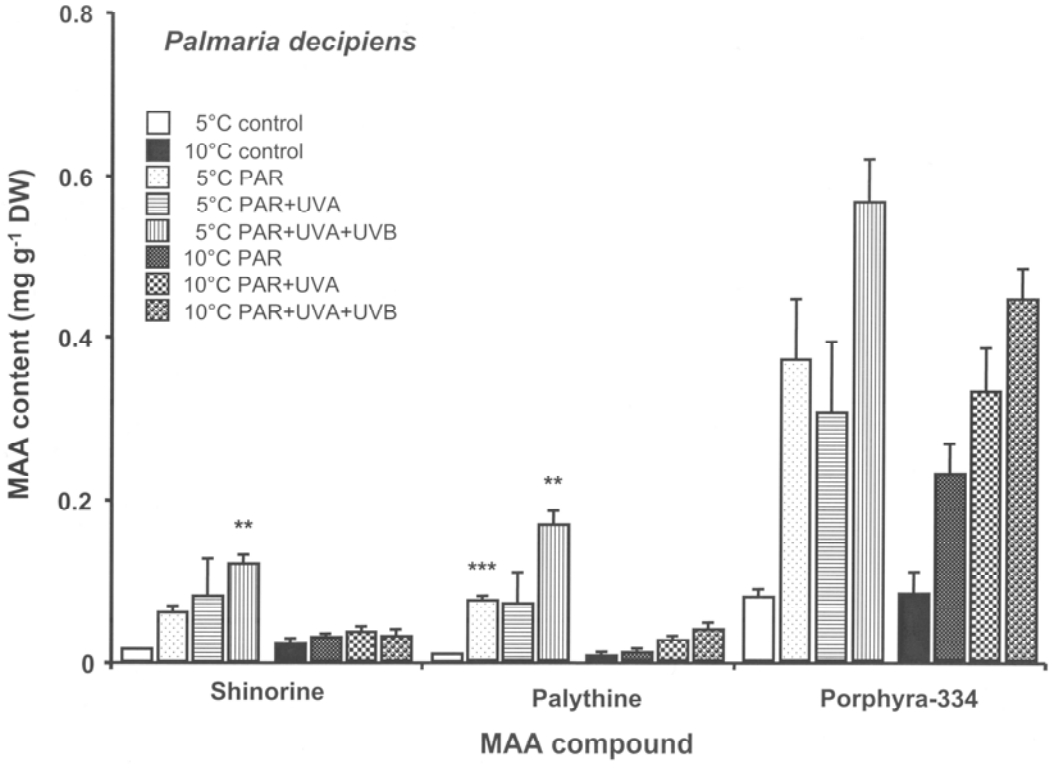

Figure 3 Interactive effect of different radiation conditions (PAR, PAR+UVA, PAR+UVA/B) and temperatures of 5 and 10 °C on individual MAA concentrations in *Palmaria decipiens* after 5 days incubation. Given are the mean values ± SD (n=4). Asteriks indicate significant differences in MAA amounts between both temperatures (*: p<0.05; **: p<0.01; ***: p<0.001).

Discussion

The simultaneous action of temperature and irradiation on macroalgae has been investigated in several studies. Poll et al. (2002) looked at the temperature dependence of UVR-induced DNA damage, while Pakker et al. (2000) studied the photo-reactivation of this damage. Effects of temperature and irradiation on photosynthesis (Gómez et al. 2001), growth rate (Major & Davison 1998) and release, attachment and survival of carpospores (Orduna-Roja & Robledo 1999) were also investigated. All these studies clearly indicate that both factors must be considered to understand the observed effects.

The present study explores for the first time different temperature and radiation regimes on the induction and accumulation of MAAs in *Iridaea cordata* and *Palmaria decipiens* from Antarctica. In both red algal species investigated already enhanced PAR led to a strong accumulation in total MAA concentration, and particularly the addition of UVB resulted in a further stimulation. Therefore, both *I. cordata* and *P. decipiens* can be classified as Type II MAA inducer according to the classification of Hoyer et al. (2001).

The most important result of the present study is the strong temperature effect on the MAA induction pattern. Particularly low temperatures stimulate the concentrations of shinorine and palythine in *I. cordata* and of shinorine, palythine and porphyra-334 in *P.decipiens*. Comparison of the induction patterns at 5 and 10 °C clearly indicate that shinorine and palythine are much less synthesised at the higher temperature, while this effect was less pronounced for porphyra-334. We hypothesize that the higher accumulation rates of MAAs at lower temperatures can be explained by enhanced anabolic enzyme activities which are related to more efficient enzyme variants, perhaps isoenzymes, or increased enzyme contents under these conditions. It seems to be a general phenomenon in polar algae, that many of their physiological processes, such as growth or photosynthesis, exhibit temperature optima very similar to those of temperate species (Kirst & Wiencke 1995). Instead of the development of specific adaptive (genetical) mechanisms to the cold-water environment, seaweeds from Antarctica and the Arctic seem rather to exhibit a broad potential for physiological acclimation. The increase in MAAs under low temperatures in both Antarctic red algae can be attributed to such an acclimation strategy.

While juvenile lateral fronds of the red alga *P. decipiens* collected in Antarctic winter contained low concentrations of MAAs, mature plants from late spring and summer exhibited significantly higher contents indicating strong seasonal effects (Post & Larkum 1993), which may be related to the changing daylengths and radiation conditions. Although experimental evidence for a particular trigger mechanism as well as details for the biosynthetic pathway for individual MAAs are still missing, it is reasonable to assume that a signal transduction pathway must be involved in the overall process leading to high MAA concentrations. Due to the different types of MAA induction patterns the presence of various photoreceptors, most probably between the blue light and UVB wavelengths, have to be taken into consideration (Kräbs et al. 2002).

Temperature, but also seasonally fluctuating irradiance and daylengths, can exert strong effects on photosynthesis and respiration in Antarctic macroalgae (Gómez 1997). However, the presumed interactions between photosynthethic/ respiratory pathways and MAA metabolism are not well understood. Nevertheless, in many ecophysiological studies with different abiotic treatments on

macroalgae respiration appeared to be typically more insensitive than photosynthesis.
The induction data presented strongly support the function of MAAs as sunscreen compounds in response to a more stressful situation as it may occur in shallow waters where macroalgae are exposed to increasing UVB and higher PAR. Therefore, the biochemical capability to synthesise and accumulate MAAs contributes to the adaptation of *I. cordata* and *P. decipiens* against the prevailling radiation conditions in Antarctica. In addition, preliminary data on the photosynthetic performance of both algal species indicate under identical experimental conditions only minor responses, thus supporting their broad tolerance of many physiological processes (Hoyer et al., unpublished results).

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Effects of UV radiation and grazing on biomass and primary production of subtidal benthic algae in Antarctica

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Introduction

Marine benthic macro- and microalgae play a key role in Antarctic coastal ecosystems. They are important primary producers that contribute to the coastal food webs either directly (Iken et al., 1998) or indirectly, as a source of particulate and dissolved organic carbon (Fischer and Wiencke, 1992). Furthermore, macroalgae serve as habitat and shelter for various organisms (Momo et al., 1998) and provide a substrate for microalgal epiphytic communities (Klöser, 1998). Benthic microalgal assemblages are also found colonizing hard bottom substrata and are thought to affect the marine successional process, particularly at the early stages (Neushul et al., 1976).

Ultraviolet radiation (UVR, 280-400 nm) has harmful effects on the aquatic biota. Ultraviolet B (UVBR, 280-315 nm) and ultraviolet A (UVAR, 315-400 nm) penetrate in the water column down to ecologically significant depths, directly or indirectly affecting several targets in the organisms (Häder, 2000). Over the past decades, Arctic and Antarctic organisms in particular have been exposed to seasonally increased levels of UVBR due to the spring and summer ozone depletion phenomenon (Perin and Lean, 2004). UVBR is known to be a stress factor that can negatively affect marine primary producers (Villafañe et al., 2003; Bischof et al., 2006). In this scenario and particularly at the beginning of a colonization process, species-specific or life stage-specific algal susceptibility to UVR are expected to alter the structure of the coastal systems (Lotze et al., 2002).

Besides environmental factors, primary producers can be strongly controlled by their consumers (Lotze and Worm, 2002). Grazing has been shown to produce drastic changes in benthic algae physiognomy and cause reductions of the community biomass in freshwater (Steinman, 1996) and marine systems (Nicotri, 1977; Hillebrand et al., 2000).

Studies focusing on the effects of UVR on benthic algae-herbivores interactions are particularly scarce for Antarctica (Wahl et al., 2004; Zacher et al., in press) and - to our knowledge - have not been performed on subtidal benthic hard bottom assemblages (see Zacher and Campana, this issue). In the present study we report the first results of long term field studies addressed to analyze the combined effects of ambient UVR and grazing on a subtidal benthic algal community in Antarctica, one of the regions that have been most seriously affected by the ozone depletion. We will focus this communication to report the effects of both factors (UVR and grazing) on the community biomass and net primary production and we discuss the possible consequences of the observed effects on trophic interactions.

Materials and methods

Study site. The field experiment was carried out in Potter Cove, Isla 25 de Mayo (King George Island), South Shetland Islands, Antarctica in a sheltered subtidal area located at Peñón de Pesca (62° 14´S, 58° 40´W). For details of the experimental site and experiment location see Zacher and Campana and Campana et al. (this issue).

Experimental design and set up. A two-factorial design was performed to test the effects of UVR and grazing on the structure of the benthic primary producers community. Experimental units consisted of PVC cages of 50 cm wide and 10 cm deep. Four ceramic tiles (10 x 10 cm) were placed in each cage and served as settlement substrata. In order to manipulate the light conditions, different cut-off filters were used, including the following treatments:
1) PAR (Photosynthetically active radiation, 400 to 700nm), by use of Plexiglass GS 231(Röhm, Germany) which blocked radiation < 400nm and allowed only PAR transmission,

2) PAR + UVA (320 to 700nm), by use of Plexiglass GS 2458 (Röhm, Germany) covered by Folanorm SF/AS 130 µm, that together blocked radiation < 325 nm and allowed transmission of PAR and UVAR,

3) PAR + UVA + UVB (280 to 700nm), covered by Plexiglass GS 2458 which transmitted the whole spectrum, and

4) Full sunlight (control treatment), a treatment without filter that served as a filter control treatment.

In order to evaluate the effect of grazing, the following treatments were performed:

1) Open cages, that had all sides open and allowed free access of grazers,

2) Closed cages, where the presence of grazers was limited through a lateral closure of the cages, using a 1mm mesh size to allow the exclusion of most of the meso- and macroconsumers, and

3) Half cages (control treatment), cages containing holes in the lateral closure in order to test for cage artifacts.

Thus, eight treatments were performed with four replicates of each treatment, so a total of 32 cages was used for the complete design (Table 1).

Table 1. Experimental design. Shown are the numbers of replicates (cages) for each treatment.

Treatment	PAR + UVA + UVB	PAR + UVA	PAR	Full Sunlight
Open	4	4	4	4
Closed	4	4	4	
Half	4			

Field work. The field experiment was started on December 20th 2003 and samplings were performed after 23, 40, 59 and 73 days of colonization. The filters and cages were cleaned bi-weekly and the Folanorm film was exchanged every 15 days to prevent aging effects. Underwater solar light climate was determined by a multiband spectroradiometer PUV 510 (Biospherical, USA). Grazer abundance was determined visually by SCUBA diving every three to four weeks. Each sampling date, a tile was randomly selected from each cage by SCUBA diving and transported to Dallmann Laboratory, where estimates of biomass were performed.

Biomass, net primary production and grazing rate. Algae were removed by scraping and rinsing the tiles and biomass was determined as dry weight (mg DW cm^{-2}) (48h at 80° C). Area-specific net primary production was estimated for each colonization time (mg DW cm^{-2} day^{-1}) and grazing rates were estimated as the difference between the biomass in ungrazed and grazed tiles, as:

$$\text{grazing rate} = \frac{B_u - B_g}{\Delta t}$$

where B_u is the biomass of the ungrazed treatments (closed cages), B_g , the biomass in the grazed treatments (open cages), and delta t the time period (Southwood & Anderson, 2000).

Statistical analyses. Two way MANOVA/ANCOVA analyses were performed using irradiance (three levels, PAR, PAR+UVA and PAR + UVA + UVB) and grazing (two levels, grazed and ungrazed) as the independent variables and time as covariable. Post-hoc multiple means comparisons were performed using Fisher's LSD test at α=0.05 significance level. Homogeneity of variances was checked using Cochran's Test. Cage and filter artifacts were tested by means of t-tests (Zar, 1996).

Results

Radiation measurements. On average, 30±14% of incident UVBR (305nm); 49±19% (340nm) and 60±21% (380nm) of UVAR and 65±20% of PAR penetrated down to the experimental units at 2 metres depth (N=5; Fig. 1). For additional radiation measurements, see Richter et al. (this issue).

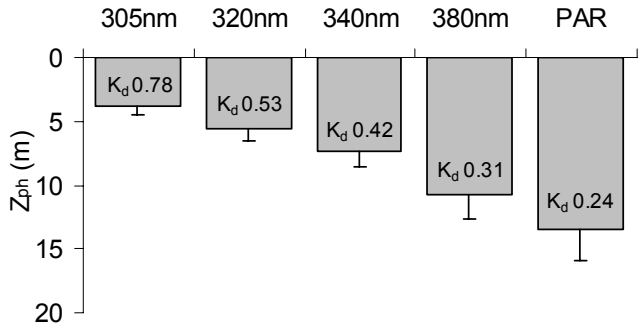

Fig. 1. Photoactive zone depth (Z_{ph}) -representing 10% of incident radiation - for the different measured UVR and PAR wavelengths (means + SE are shown, N=5). Diffuse vertical attenuation coefficients (K_d) are indicated inside the bars.

Grazer abundance. The macroconsumers found in the cages were amphipods, (mostly *Gondogeneia antarctica*) and gastropods (the limpet *Nacella concinna* and the snail *Laevilacunaria antarctica* as the most abundant).

Biomass, net primary production and grazing rate. Biomass was not affected by the light treatment whereas grazing significantly reduced the community biomass (ANCOVA, $p<0.05$). Furthermore, grazed treatments (open cages) exhibited a significantly lower biomass when UVR was absent, in the PAR treatment (Fischer Test, $p< 0.05$) (Fig. 2a). Similarly to biomass, net primary production was significantly reduced by grazing (ANCOVA, $p< 0.05$) and was unaffected by the light treatment (Fig. 2b). Algal growth reached maximum

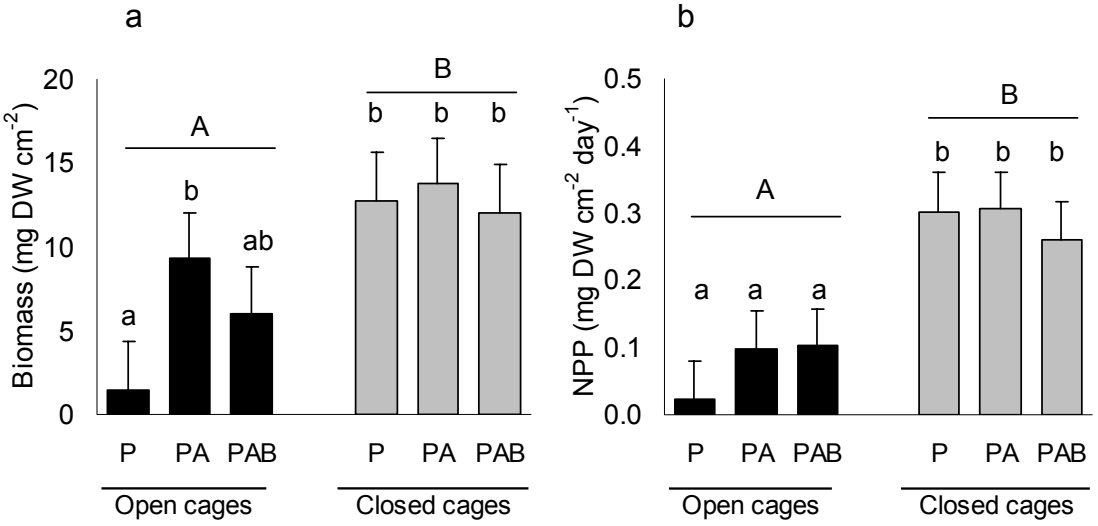

Fig. 2. Effects of UVR (PAR = P, PAR + UVA = PA and PAR + UVA + UVB = PAB) and grazing (open and closed cages) on biomass (a) and net primary production (b). Shown are means + SE, n=12. Capital letters indicate the significant grazing effect, as means of the grazer treatments (ANOVA/ANCOVA, $p<0.05$). Lower case letters show significant differences detected by Fisher's multiple means comparison Post-Hoc test. Different letters indicate significant differences between treatments.

values after 40 days of colonization in the closed cages (Fig. 3). Grazing rate was higher in the UV-shielded communities after 40 and 59 days of colonization (Fig. 4). No cage or filter artefacts were detected in the studied parameters.

Discussion

The effects of UVR on assemblages of marine primary producers were mostly studied in intertidal and shallow-waters environments (Lotze et al., 2002; Roux et al., 2002; Wahl et al., 2004; Dobretsov et al., 2005). In most studies, communities have shown to be more sensitive at the beginning of the successional process. In particular, important structural variables such as species composition and biomass were strongly affected by UVR (Santas et al., 1998; Lotze et al., 2002). In the present study, algal biomass was unaffected by UVR. The standing crop of the studied community was mostly comprised by benthic colonial diatoms (Campana et al. this issue), that have shown high resistance to UV radiation (Peletier et al., 1996; Sundbäck et al., 1997; Wulff et al., 1999, 2000).

Generally, grazers are capable of causing strong declines in the algal biomass (either by direct consumption or by dislodgement) in freshwater (Steinman, 1996) and marine habitats (Nicotri, 1977, Hillebrand et al., 2000). As a result, less photosynthetic tissue per unit of area is available for fixing carbon (Steinman, 1996). In our study, grazers controlled benthic primary producers biomass. Consequently, the mean net primary production was reduced by grazing in all the irradiance treatments.

It is important to point out that the observed grazing effects were stronger in the UVR-shielded communities. Solar UVR was shown to affect trophic interactions in freshwater environments by a decrease of the invertebrate grazing (Bothwell et al., 1994; De Nicola and Hoagland, 1996).

In the present study, it can be hypothesized that UVR exerts a control on grazing either indirectly, by reducing algal palatability or directly, by less activity of grazers or reducing their density. As a matter of fact, grazing rates were different according to the colonization time and were affected by the irradiance treatment. Communities that received only PAR showed the highest grazing rates when the communities reached the higher biomass. However, complementary laboratory and field studies should be carried out in order to explain the observed trends.

In conclusion, grazers exert a control on the studied community that may affect its structure and function. UVR may cause an attenuation of grazing that can, therefore, result in a positive effect on the community net primary production (i.e. biomass productivity) (Bothwell et al., 1994) (Fig. 5). However, other factors should be also taken into account such as direct UVR effects on the algal community structure and palatability.

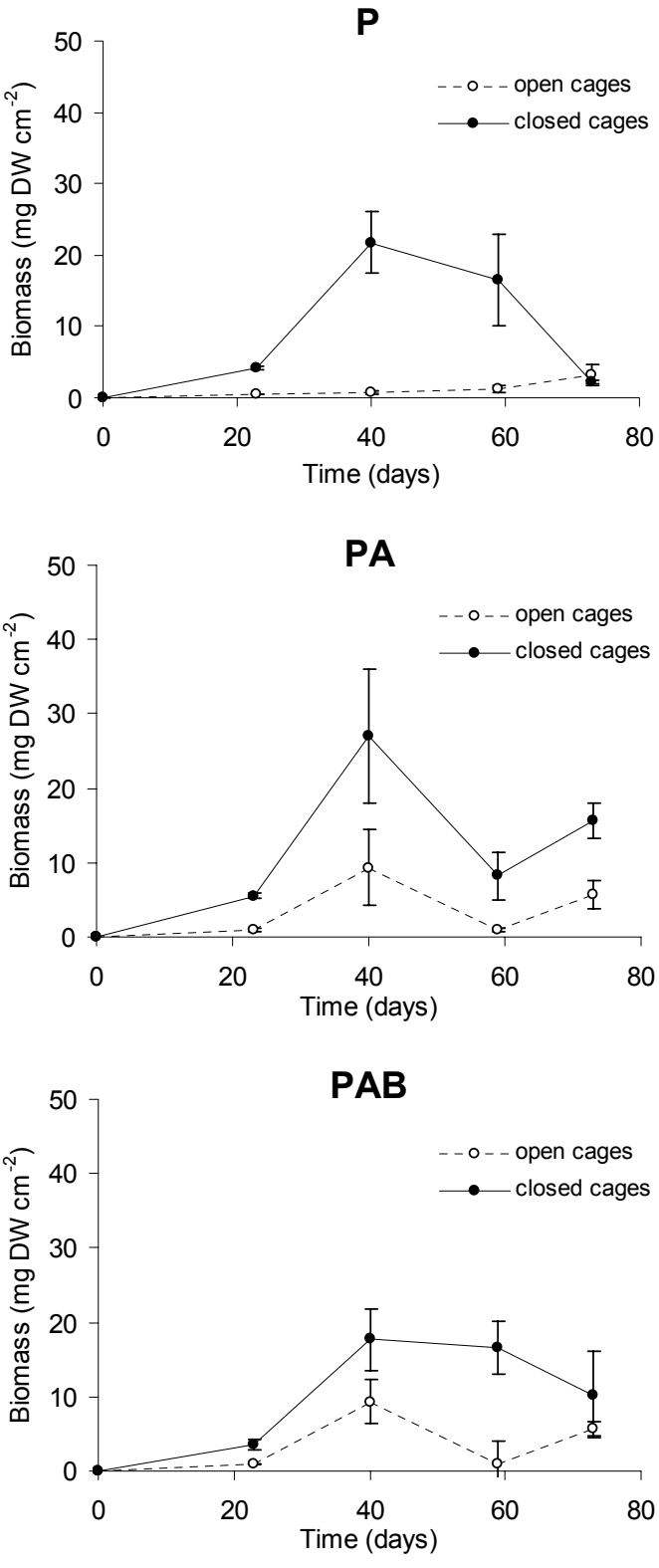

Fig. 3. Effects of radiation treatment (PAR, PAR+UVA and PAR+UVA+UVB) on biomass along the succession in grazed and ungrazed communities. Shown are means +/- SE.

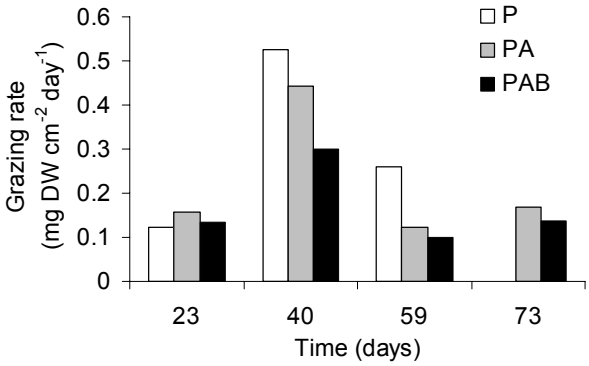

Fig. 4. Grazing rate for each colonization time.

Our findings suggest that UVR plays a key role in structuring the benthic producers community, exerting both direct and indirect effects. Thus, the result of the interaction of both factors are expected to affect the structure and dynamics of the benthic system and may be capable of causing modifications of the energy and matter flows through the coastal food web.

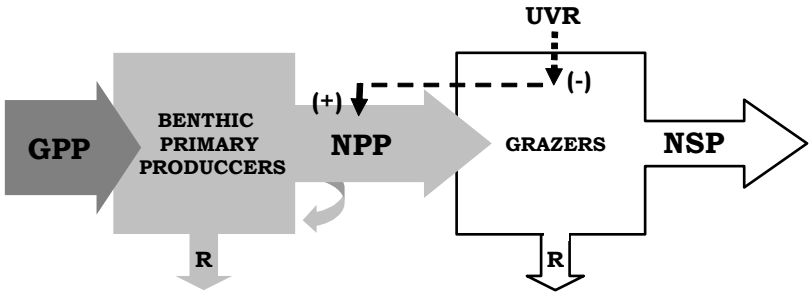

Fig. 5. Possible energy flux between the first (benthic primary producers) and second (benthic herbivores) trophic levels. UVR may cause a decreased grazing activity that can result in a positive effect on the community NPP. (GPP: gross primary production; NPP: net primary production; R: respiration; NSP: net secondary production).

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UV radiation and grazing effects on an intertidal and subtidal algal assemblage: a comparative study

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Introduction

Benthic marine macro- and microalgae are the major primary producers in coastal ecosystems, providing food and shelter for a variety of associated species (Iken 1996; Pinckney and Zingmark 1993). Therefore, changes in algal productivity or diversity can severely affect the structure of coastal marine food webs (Santas et al. 1998). A well known phenomenon over the Antarctic continent is the strong decline of stratospheric ozone (>50%) in spring (Farman et al. 1985; WMO 2003), thereby increasing the irradiance of ultraviolet-B radiation (UV-B 280-315 nm) on the Earth's surface. The timing of the ozone depletion over Antarctica is crucial as it coincides with the break up of sea ice, i.e. the phase of highest water transparency and highest growth rates of most algal species (Karentz 2003). Thus, tidal algal assemblages can be exposed to high UV-B radiation during the austral spring and summer months.

UV radiation (UVR, 280-400 nm) can negatively effect algae in various ways (reviewed in Franklin and Forster 1997; Wulff 1999; Bischof et al. 2006). Most harmful effects were found in laboratory studies, using artificial irradiance and focusing on physiological effects at the single organism level. However, autecological studies with single species are not able to detect synergistic or indirect UV effects on community level and predictions of ecosystem response to UVR cannot be made on single trophic-level assessments (Bothwell et al. 1994). In the marine environment, only few studies on interactive effects exist, demonstrating the significance of climatic (e.g. temperature, UVR) and ecological factors (e.g. grazing) as important drivers on algal recruitment (Lotze and Worm 2002).

Field-studies regarding UV effects on marine intertidal communities have been carried out mostly in temperate and tropical regions. In these experiments, UVR was identified as a significant driver on community structure during early succession (Lotze et al. 2002; Molis and Wahl 2004; Dobretsov et al. 2005).

Studying UV effects on Antarctic algal assemblages is particularly important due to the severe ozone depletion over this region (WMO, 2003). However, to our knowledge only one field-study on UV effects on an Antarctic microalgal assemblage exists (Wahl et al. 2004). To date, experiments studying interactions between UV effects and other ecologically important factors, e.g. grazing are missing. Furthermore, comparisons of benthic assemblages at different tidal

zones can help us identify a more general impact of UVR and grazing on the colonization process in marine hard bottom benthos in Antarctica.

In the light of this we designed a two-factorial field-experiment to study the effects of UVR and grazing on an intertidal and a subtidal algal assemblage in Antarctica. The main questions were (1) whether UVR and consumer treatments influence dry weight and recruit density at the two sites, (2) whether the developing assemblages at the two sites react differently to UVR and grazing.

Materials and Methods

Study sites. The field experiments were conducted close to the Dallmann Laboratory/Jubany Base, King George Island, Antarctica at two different sites. One on a rocky intertidal platform (lower eulittoral, Peñón Uno, 62°14 S, 58°41 W) and another one in a sheltered, hard bottom subtidal area (Peñón de Pesca, 62° 14´S, 58° 40´W) at 2 m water depth (Fig. 1).
In our study area the intertidal algal communities are characterized by Rhodophyta (e.g. *Iridaea cordata* Turner (Bory), Phaeophyta (e.g. *Adenocystis utricularis* (Bory) Skottsberg) and Chlorophyta (e.g. *Monostroma hariotii* Gain, (Iken 1996) and the subtidal communities by Phaeophyta (*Ascoceira mirabilis*, *Desmarestia sp.*), Rhodophyta (e.g. *Iridaea cordata* Turner (Bory), *Gigartina skottsbergii*) and benthic diatoms (Klöser et al. 1996; 1998).

The gastropod *Nacella concinna* Strebel, among other smaller gastropods like *Laevilacunaria antarctica* Martens and *Laevilitorina umbilicata* Pfeffer, was found very frequently at both sites (Kim 2001; Ferraz Nonato et al. 2000). Dominant amphipod species in the area are *Gondogeneia antarctica* Chevreux and *Djerboa furcipes* Chevreux (Momo et al. 1998; Obermüller et al. 2003). During the sampling period, the maximal tidal range was around 2 m at a sea surface temperature between -1.8°C (spring) and 2°C (summer). Water transparency is strongly variable, depending on glacial freshwater input and wind direction. UV-transparency of the water body was highest in spring (e.g. 28 November 2003) with a maximal 1 % depth at 16 m for UV-B radiation (280-315 nm), 19 m for UV-A radiation (315-400 nm), and >20 m for PAR (400-700 nm). Minimum concentrations of nitrate, phosphate, and silicate were recorded in February at non-limiting algal growth levels of 15, 2, and 47 µM, respectively (Schloss et al. 2002).

Experimental design and set-up. Using a randomized block design, we studied in a two-factorial experiment the effects of grazers (two levels, grazer vs. no grazer) and UVR (three levels, PAR+UV-A+UV-B, PAR+UV-A and PAR only,) on the succession of algal assemblages (n = 4) in the intertidal and the subtidal. For details of the design see Campana et al. (this issue).The experiments were run from 20 December 2003 to 9 March 2004 (74 days) in the intertidal and from 20 December 2003 to 8 March 2004 (73 days) in the subtidal. In this study we only refer to the third sampling date after 8 weeks of exposure.

Radiation measurements. For detailed radiation measurements and the differences of the two sites see Richter et al. (this issue).

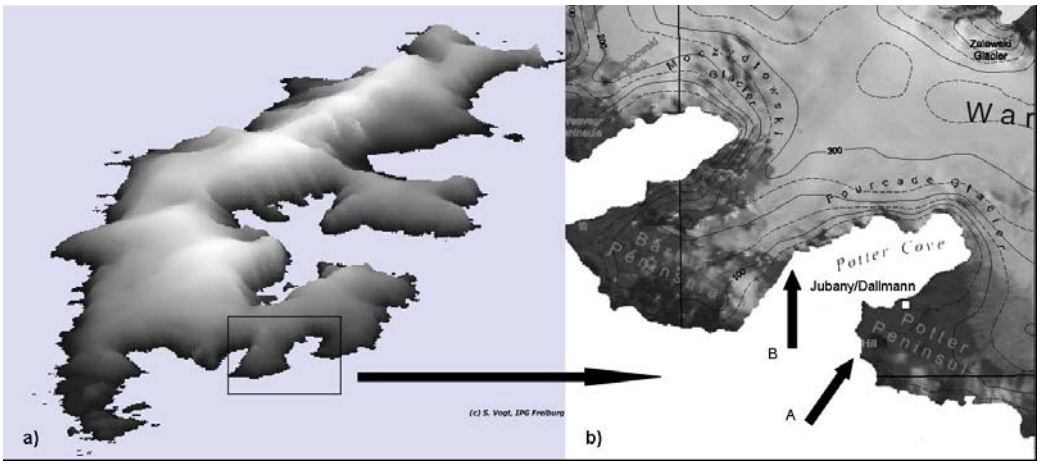

Fig. 1 a) King George Island, Antarctica (S. Vogt, IPG Freiburg). b) Experimental sites close to the Dallmann Laboratory. A: Peñón Uno; B: Peñón de Pesca.

Sampling of macroalgae. The collection of the tiles took place on 16 February (i.e. 58 days after starting the experiment) at Peñón Uno (lower intertidal) and 17 February 2004 (i.e. 59 days after starting the experiment) at Peñón de Pesca (subtidal). All tiles were processed at the Dallmann Laboratory immediately after collection from the field. Photosynthetic efficiency as optimum quantum yield (F_v/F_m) was measured with a PAM 2000 (Walz, Germany) after 5 minutes dark adaption directly on the tiles. Percent cover of all species (>2 mm length) was estimated with a Plexiglas sheet marked with 50 random dots (1 dot = 2 % cover). Recruit density (number cm^{-2}) of macroalgae < 2 mm was determined by counting individual germlings in four sub-samples per tile (~50 mm^2) using a stereomicroscope (16x magnification), leaving a border of 1 cm unsampled to avoid edge effects. Dry weight (as dry mass) of the community was measured by removing and drying (48 h at 80 °C) all organisms from the tile.

Data analysis. A t-test was performed to test for differences between two independent groups (e.g. test for cage or filter artefacts). A two-way ANOVA was performed to test for the effects of consumers and UV radiation on biomass, density of red and green algae recruits and photosynthetic efficiency with a significance level of P <0.05. Prior to analysis, data were tested for homogeneity of variances (Cochran´s test). Heteroscedastic data after ln- or square-root transformation were analyzed by the non-parametric Kruskal-Wallis test. Post-Hoc comparisons were performed with Newman-Keuls test using Statistica ™ 6.0 software package.

Results

Overall, four macroalgal species were found in the intertidal and three in the subtidal after 58 to 59 days of exposure, respectively (Table 1). At Peñón Uno (intertidal) the new developing assemblage was formed by very small stages of the green alga *Monostroma hariotii* Gain (more than 95% of the total macroalgal recruits on the tiles), pennate diatoms and red algal recruits. At Peñón de Pesca (subtidal) the assemblage was dominated by green algal filaments of *Urospora penicilliformis* (Roth) Areschoug and colony-forming diatoms, accompanied by fewer red algal recruits Table 1).

Table 1. Algae found after 58 and 59 days of exposure in the inter- and subtidal. Dominant species are bold.

	Intertidal	Subtidal
Chlorophyceae	***Monostroma hariotii***	***Urospora penicilliformis***
	Ulothrix sp.	
Rhodophyceae	*Palmaria decipiens*	*Palmaria decipiens*
	Red int	Red sub, Delesseriaceae
Bacillariophyceae	**Diatoms**	**Diatoms (colonies)**

In general, no cage or filter artefacts were detected (t-test P >0.05, between open and half cages and PAB and full sunlight treatment, respectively).
In the closed cages optimum quantum yield (F_v/F_m) was significantly lower at the intertidal site (P = 0.0001, Table 2). Optimum quantum yield was not significantly influenced by UVR neither in the intertidal nor the subtidal site.
The dry weight was generally higher at the subtidal than at the intertidal site. Both sites showed a significant higher dry weight in the closed cages (intertidal: P <0.0001; subtidal: P = 0.012). No significant effects of UVR on dry weight were found in any of both sites (Table 2).

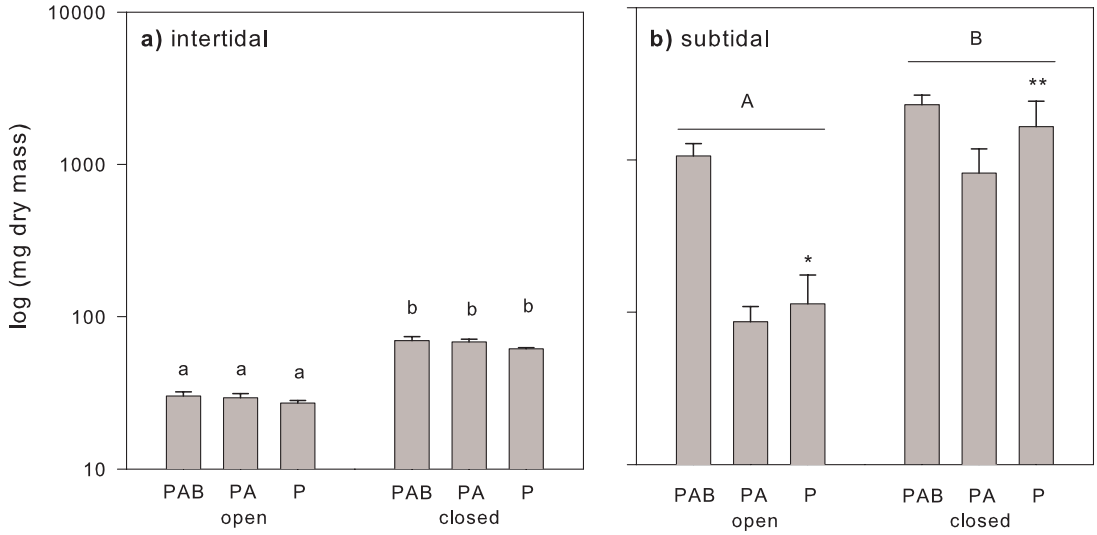

Fig. 2 Effects of UV (PAB = PAR + UV-A + UV-B, PA = PAR + UV-A, P = PAR) and grazers (open and closed cages) on dry weight at the two samplings sites (mean ± 1 SE, n = 4). Note logarhythmic scale. Different letters indicate significant differences between grazer treatments. Lower case letter were used to indicate significant differences in the intertidal and capitals for significant differences in the subtidal (as mean of the grazer treatments). (*,**) indicates the significant difference found between P open and closed treatment (Newman-Keuls-test)

Overall, at the intertidal site only microscopically identifiable green algal recruits (<2 mm) were present (mostly *Monostroma hariotii* and few *Ulothrix sp.*) while at the subtidal site bigger green algal filaments (>2 mm) of *Urospora penicilliformis* were identified.
UVR and grazers significantly reduced green algal recruits in the intertidal (UV: P <0.002; grazer: P <0.0001). Highest effects (minimal green algal recruits) were found in the treatment with the ambient spectrum and grazers present (PAB open) resulting in an UV*grazer interaction (P <0.024; Table 2, Fig. 3).

In the subtidal site no significant UV or grazer effects on the green algal filaments were found after 8 weeks of exposure (Table 2, Fig. 3).

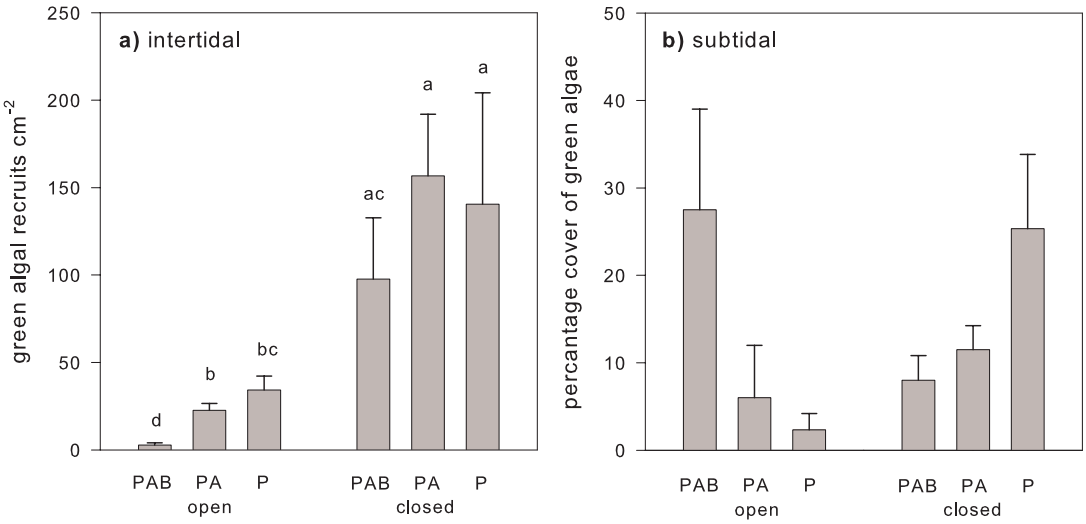

Fig. 3 Effects of UV (PAB = PAR + UV-A + UV-B, PA = PAR + UV-A, P = PAR) and grazers (open and closed cages) on green algal recruits at the two samplings sites (mean ± 1 SE, n = 4). Note that in the intertidal only small recruits were found (<2mm) using individuals cm^{-2} whereas in the subtidal the percent cover of bigger filaments (>2mm) was used for the analysis. Different letters indicate significant differences between treatments.

Table 2. Two-factorial ANOVA or non-parametric Kruskal-Wallis test on UV radiation and grazer (G) effects on photosynthetic efficiency, biomass, density of Chlorophyta and Rhodophyta at both sampling sites.+ significant; - not significant, nf not found.

	Intertidal			Subtidal		
	UV	G	UV*G	UV	G	UV*G
Photosynthetic efficiency	-	+	-	-	-	-
Dry mass	-	+	-	-	+	-
Green algae density	+	+	+	-	-	-
Palmaria decipiens density	+	-	-	+	+	+
Red int density	-	-	-	nf	nf	Nf
Red sub density	nf	nf	nf	-	+	-

In general more red algal recruits grew in the intertidal than in the subtidal (Fig. 4). At both sites two species of red algal recruits settled. Dominant among these was *Palmaria decipiens* (Reinsch) Ricker in the intertidal and *P. decipiens* as well as a member of the family Delesseriaceae in the subtidal. However, *P. decipiens* was only growing in the open cages in the subtidal.
UVR significantly reduced the density of *P. decipiens* in the intertidal and in the subtidal (intertidal: $P = 0.037$; subtidal: $P = 0.042$; Table 2). The density of *P. decipiens* was not affected by grazers in the intertidal but was favoured by herbivores in the subtidal ($P = 0.024$) revealing in a UV*grazer interaction ($P = 0.042$; Table 2). The density of a second red algal recruit (Red int) from the intertidal did not show any treatment effect whereas the species from the Delesseriaceae was depressed due to grazer presence ($P = 0.044$, Table 2, Fig. 4).

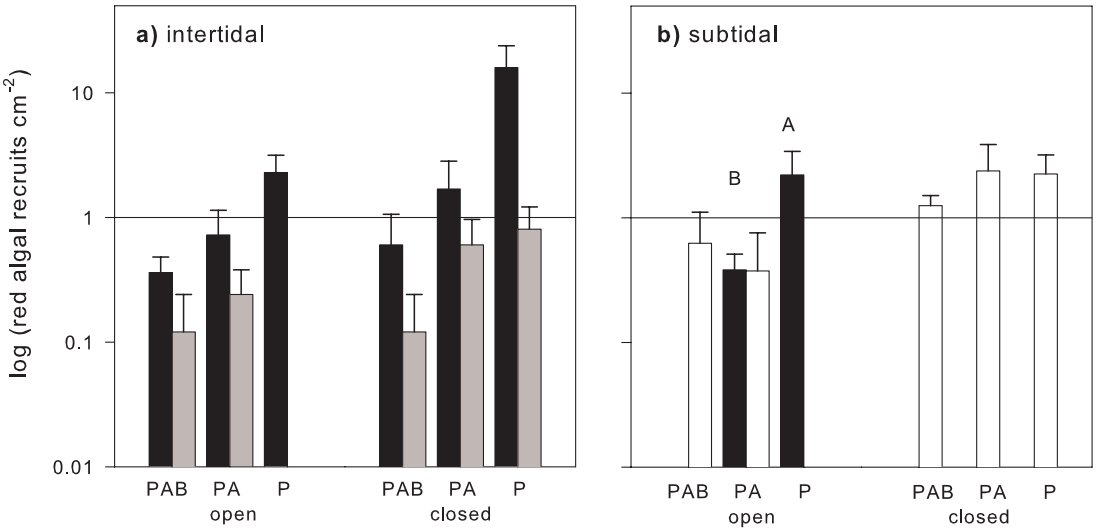

Fig. 4 Effects of UV (PAB = PAR + UV-A + UV-B, PA = PAR + UV-A, P = PAR) and grazers (open and closed cages) on red algal recruits at the two samplings sites (mean ± 1 SE, n = 4). Black bar *Palmaria decipiens*, grey bar unidentified red algae recruit in the intertidal (Red int), white bar unidentified red algae from the family Delesseriaceae in the subtidal (Red sub). Note logarhythmic scale. Different letters indicate significant differences between treatments. Kruskal-Wallis showed a significant UV effect on *P. decipiens* in the intertidal and ANOVA a significant grazer effect on Red sub, however following post-hoc test did not give any significant results.

Discussion

Main differences between the two sites were found in the species composition and in the dry weight due to general differences of the two locations. However, for this particular sampling date and year, effects of UVR and grazing on the respective assemblages followed a similar pattern at both sites. We would like to note that results presented here are only a small part of the complete experiment (one sampling out of four and only one season out of two studied).

The cages at Peñón Uno were installed at a shallower water depth than at Peñón de Pesca, therefore one might expect more pronounced UV effects at the intertidal site. However, the water body at Peñón de Pesca was clearer, due to the circulation patterns in the Potter Cove (see Richter et al. this issue). The water reaching Peñón Uno was richer in sediments from the nearby glacier, whereas Peñón de Pesca was influenced mainly by oceanic water entering from Maxwell Bay (Roese and Drabble 1998). Therefore, the UV doses actually reaching the respective algal assemblages were relatively equal (Richter et al. this issue) and responses to UV did not show pronounced differences at both sites. Nevertheless, intertidal communities are exposed to more stressors than subtidal ones, such as desiccation, temperature changes, wind, ice and higher maximal irradiance if local noon and low tide coincide.

Generally, dry weight was lower in the intertidal than in the subtidal. This was mainly due to a higher biomass of benthic diatoms in the subtidal (see also Campana et al. this issue). A higher exposure to mechanical forces at the intertidal site, (ice abrasion, strong wave action), among other stressors (e.g. desiccation) may have impeded growth of colony-forming diatoms in the intertidal

which were dominant at the more sheltered Peñón de Pesca. We don't ascribe this to UV effects as the diatom assemblages were not affected by UVR (Campana et al this issue). Nevertheless, both assemblages were top-down controlled by grazing. The main effects were caused by gastropods, like *Nacella concinna* and *Laevilacunaria antarctica* which were shown to feed effectively on macroalgae and microphytobenthos (Iken 1996; Kim 2001), as well as amphipods in the subtidal. Grazing effects in the subtidal resulted stronger in UV shielded assemblages (open P treatment). A reduction of the algal palatability of UV exposed assemblages, the algal composition or even a direct UV effect on grazers might explain this trend (Bothwell et al. 1994; see also Campana et al. 2005).

UV and grazing effects on green algal recruits were species-specific as shown by different results for the intertidal (mainly microscopically *Monostroma hariotii*) and the subtidal (mainly *Urospora penicilliformis* filaments) green algae. However, green algal recruits at an earlier successional stage were also shown to be UV sensitive in the subtidal (Campana et al. 2005). Generally early stages of succession were shown to be more vulnerable to UV stress than adults of the same species (Coelho et al. 2000) but have the possibility to adapt as they mature (Lotze et al. 2002).

At both sites the red algal recruit *Palmaria decipiens* was UV sensitive, especially to the UV-B part of the spectrum. A total lack of *P. decipiens* recruits in the closed cages in the subtidal was probably due to the dense settlement of colony-forming diatoms (not being present in the intertidal) which limited growth of *Palmaria* due to interspecific competition (see Huang & Boney 1985). Moreover, the density of *Palmaria* recruits has been shown to be favoured by grazer presence at longer times scales (Campana et al. 2006; Zacher et al. in press).

Overall, UVR and grazers significantly shaped both, the intertidal and the subtidal site in Antarctica. Especially red and green algal recruits showed species-specific sensitivity to UVR and grazing, resulting in interactions of these two factors. An increase in UV-B radiation may therefore affect the species composition (affecting some species more than others), with a consequent alteration of the trophic interactions in the benthic system in the Potter Cove.

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Short-term UV effects on the photosynthesis of Antarctic benthic diatoms

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Introduction

The seasonal depletion of the stratospheric ozone layer and the resulting increase in solar ultraviolet-B radiation (UV-B; 280-315 nm) reaching the Earth's surface, particularly over the Antarctic region, is a potential threat to all organisms including marine microalgae. In the area studied (Potter Cove, King George Island), UV-B could penetrate down to 16 m and UV-A (315-400 nm) down to 19 m water depth (1% of the air measurements, see also Richter et al. this issue), thus, also benthic subtidal diatoms may be affected by ultraviolet radiation (UVR).

Marine microalgae constitute the basis of the marine food web and are responsible for 40% of the global primary productivity (Field et al. 1998). In Potter Cove, benthic macro- and microalgae are important primary producers. On the soft-bottoms, the microalgae are of particular interest because the phytoplankton biomass is not sufficient to explain the benthic consumer abundance and it has been hypothesized that microbenthic algae account for the nutrition of the local fauna (Schloss et al. 1998).

In some studies on benthic microalgae, UV radiation has been shown to damage the photosynthetic apparatus, the photosynthetic pigments, the DNA and, in addition, to decrease growth and primary productivity (reviewed in Villafañe et al. 2003). Ultraviolet-A radiation (UV-A; 315-400 nm) and photosynthetically active radiation (PAR; 400-700 nm) are involved in photoreactivation and photorepair of the DNA (Karentz 1994 and references therein). And therefore of particular concern is that ozone depletion results in increased harmful UV-B radiation without a proportional increase in UV-A and PAR.

The study was motivated by the fact that there are very few studies dealing with the response of Antarctic benthic microalgae to UVR, particularly marine benthic microalgae. The objective of this study was to estimate the short-term impact of UV-B and UV-A radiation, respectively, on the photosynthetic efficiency of a benthic microalgal community.

Materials and methods

The study was carried out in December 2003 at Dallmann Laboratory, Potter Cove, King George Island, Antarctica (62° 15´S, 58° 41´W). Fine grained sandy sediment was collected from 5-7 m water depth. The top layer (1 cm) was scraped off and the sediment was brought to the laboratory, gently shaken and

sieved (mesh size 500 µm) using filtered surface seawater. The sediment was stirred and the overlying water containing suspended microalgae (diatoms) was left to grow and develop a diatom mat under dim white radiation (ca 10 mol photons m^{-2} s^{-1}).

Experimental set-up
Four short-term experiments (B1, B2, C1 and C2) took place in a temperature (2-4 °C) and radiation controlled chamber. Different radiation treatments applied were PAR+UV-B+UV-A (PAB), PAR+UV-A (PA), and PAR (P). The PA treatments were covered by 0.13 mm transparent polyester film (Folanorm-SF/AS, Folex GmbH, Cologne, Germany). The P treatment was covered by Ultraphan URUV Farblos (Digefra GmbH, Munich, Germany). The experimental treatments are shown in Table 1 and 2.

Prior to experiment B1 and B2, 24 pieces (ca 1 cm^2) of the mat dominated by the benthic diatoms *Cylindrotheca closterium* and *Gyrosigma fasciola* were transferred to a 24 well microtiter plate. For B2, the semi-natural community was kept in darkness (4°C) ca 48 h before the experiment started. The cell number in the respective wells varied between 0.7 and 1.2 $*10^6$ cells L^{-1} (no significant differences between cell numbers in the wells, P = 0.88). The microalgae were left under PAR for 5 min before the treatments started. The diatoms were exposed to UV radiation for 6 h followed by recovery radiation for 20 h and a period of darkness (<1 µmol photons m^{-2} s^{-1}) for another 20 h (Table 1).

Table 1. Treatments and exposure time of the two short term experiment with microalgal mats (n = 12). PAB = PAR + UV-A + UV-B, PA = PAR + UV-A, P = PAR
PAR = 212 (180-226) µmol m^{-2} s^{-1}, UV-A = 6.85 (5.5-8.2) W m^{-2}, UV-B = 0.575 (0.5-0.7) W m^{-2}.

expt	treatments	exposure time	"recovery"	***darkness <1 µmol s^{-1} m^{-2}***
			without UVB	
B1	PAB and PA	0, 2, 4, 6 h	1, 3, 5, 10, 20 h	4, 7, 10, 20 h
			without UV	
B2	PAB and P	0, 2, 4, 6 h	1, 3, 5, 10, 20 h	4, 7, 10, 20 h

Table 2. Treatments and exposure time of the two short term experiments with microalgal suspensions (n = 3). PAB = PAR + UV-A + UV-B, PA = PAR + UV-A, P = PAR.
PAR = 107 (80-136) µmol m^{-2} s^{-1}, UV-A = 4.0 (3.5-4.5) W m^{-2}, UV-B = 0.4 (0.32-0.46) W m^{-2}.

expt	treatments	exposure time	"recovery"	***darkness <1 µmol s^{-1} m^{-2}***
			without UVB	
C1	PAB and PA	0, 6 h	6 h	12 h
			without UV	
C2	PAB and P	0, 6 h	6 h	12 h

Because benthic diatoms can migrate down into the sediment to reach more favourable radiation conditions, another set of experiments were run but instead of using the intact mat diatom suspensions were used (C1, C2). The diatom mat was diluted in seawater and 2 ml of the suspension was put in each of the 24

wells. The cell numbers in the wells varied between 0.4 * 10^5 and 2.1 * 10^5 cells L^{-1}. No significant differences between cell numbers in the wells were found (*P* = 0.88). The microalgae were exposed for PAR for 15 min before the treatments started. The diatom suspensions were exposed to UV radiation for 6 h followed by recovery radiation for 6 h and a period of darkness (<1 μmol photons m^{-2} s^{-1}) for another 12 h (Table 2). In experiment C2, after 3 h radiation treatment the lamp had to be changed and the microalgae were left in the chamber for 25 min before the radiation treatment could continue.

Radiation treatments
Radiation was provided by a 400 W Metallogen lamp (Philips MSR 400 HR). Irradiation in the chamber was measured using a Solar Light PMA 2100 radiometer (Solar Light, Philadelphia, USA) equipped with a UV-A (PMA 2110) and a UV-B broad-band sensor (PMA 2106; Solar Light, Philadelphia, USA). PAR was measured using a flat-head LICOR 190 SA quantum sensor (cosine corrected) connected to a LICOR LI-1000 datalogger (LI-COR Bioscience, Lincoln, USA, Table 1 and 2).

Diatom photosynthesis
The effects of UV radiation on photosynthetic efficiency of the diatom mat and suspension, respectively, were determined by measuring the emission of variable chlorophyll fluorescence of PS II by use of a pulse-amplitude modulated fluorometer (B1 and B2 = PAM 2000, C1 and C2 = Water-PAM, connected to a PC with WIN CONTROL Software, Walz GmbH, Effeltrich, Germany). For B1 and B2, the whole mat was used for repeated (non-destructive) measurements of effective quantum yield of photosynthesis $\Delta F/F_m'$ directly under the respective treatment (n = 12, Table 1). For C1 and C2, the content of the well was sampled immediately after the radiation treatment (n = 3, Table 2). The cell suspension was filled into a 5 ml Quartz cuvette equipped with an automatic stirrer. The sample was stirred during the last minute of a 5 min dark adaptation. The last 10 seconds of the dark adaptation the stirrer was turned off to let larger grains settle. Optimum quantum yield of photosynthesis (F_v/F_m) was measured. Prior to the dark adaptation, the samples were exposed for 5 s of far-red light.

Results

In the experiments with the intact microalgal mats (B1 and B2), initial values of effective quantum yield were 0.601 to 0.585 and 0.472 to 0.478, respectively (Fig. 1a-b). In both experiments, effective quantum yield decreased significantly after 2, 4 and 6 hours exposure to UVR or UV-A, respectively (Fig. 1a-b).

During “recovery” under radiation, in experiment B1, the PAB treatment differed significantly from the PA treatment after 1, 3, 5 and 10 hours of exposure to PAR + UV-A (no UV-B). The non-significant difference after 20 hours was the result of a steady decline of the effective quantum yield of the PA treatment rather than a recovery of the PAB treatment (Fig. 1a). Effective quantum yield of both treatments (PAB and PA) was reduced to 70 and 77 % (after 20 h recovery) in comparison with the initial values, respectively. In the following exposure to “darkness”, both treatments recovered to initial values.

During “recovery” under radiation, in experiment B2, the PAB treatment differed significantly from the P treatment after 1, 3 and 5 hours of exposure to PAR (no UVR). The non-significant difference after 10 and 20 hours resulted in a steady decline of the effective quantum yield of the P treatment rather than in a recovery of the PAB treatment (Fig. 1b). Effective quantum yield of both treatments (PAB and P) was reduced to 69 and 77 % (after 20 h recovery) in comparison with the initial values, respectively. A partly recovery was observed after exposure to “darkness”, being faster in the P treatment than in the PAB treatment (Fig. 1b). However, the effective quantum yield didn’t recover completely to initial values.

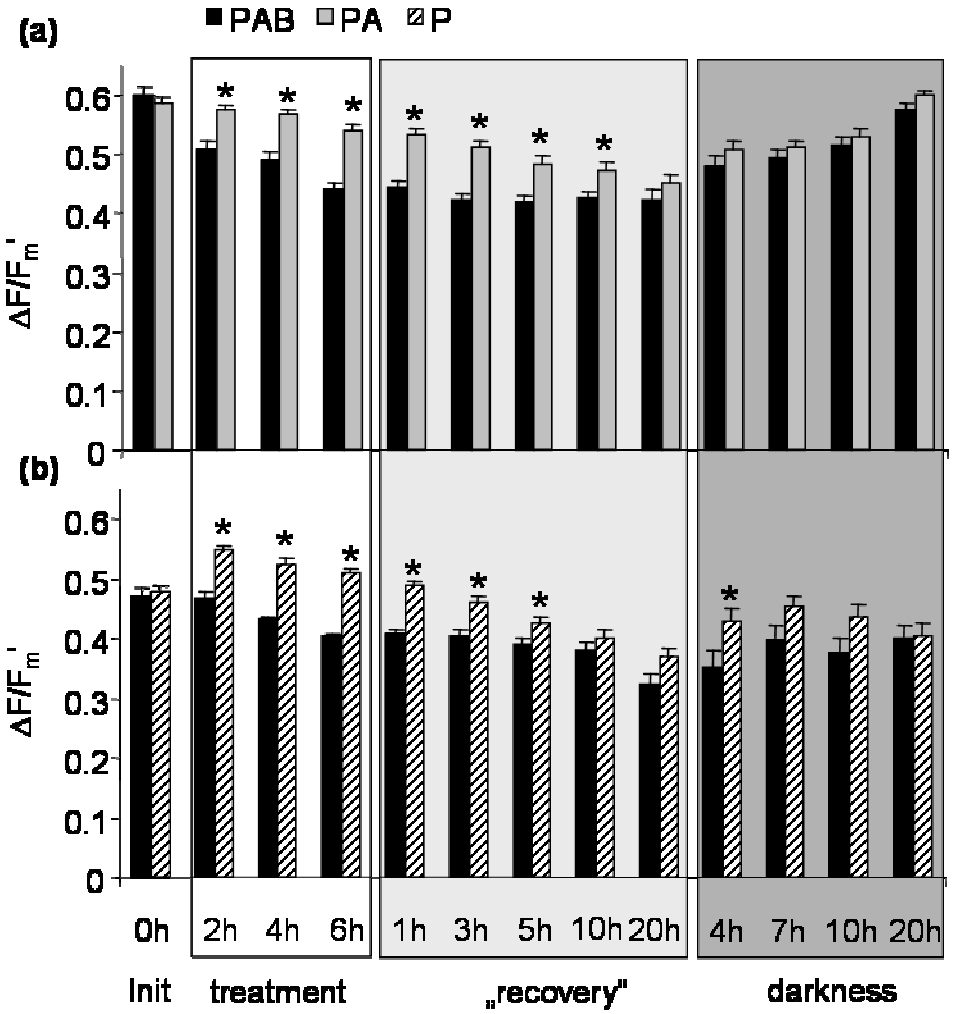

Fig. 1a-b. Experiment B1 **(a)** and B2 **(b)** (n = 12). Effective quantum yield of microalgal mats after exposure to different radiation treatments (± SE), without UV-B or UVR, respectively (“recovery”) and in darkness (<1 μmol photons m^{-2} s^{-1}). (*) marks significant differences within the respective radiation treatments (one-way ANOVA). PAB = PAR + UV-A + UV-B, PA = PAR + UV-A, P = PAR

In the experiments with the microalgal suspension (C1 and C2), initial values of optimum quantum yield (F_v/F_m) were 0.570 to 0.557 and 0.530 to 0.573 (Fig 2a-b), respectively. In both experiments, exposure to UVR or UV-A didn’t lead to significant differences in optimum quantum yield (Fig. 2a-b). However, the PAB

values were always lower than F_v/F_m from PA or P treatments, although not statistically significant.
In experiment C1, both treatments recovered completely after 12 hours in "darkness" (Fig. 2a). In experiment C2, the PAB treatment did not recover to initial values, resulting in a significant difference after 12 hours of exposure to darkness (Fig. 2b).

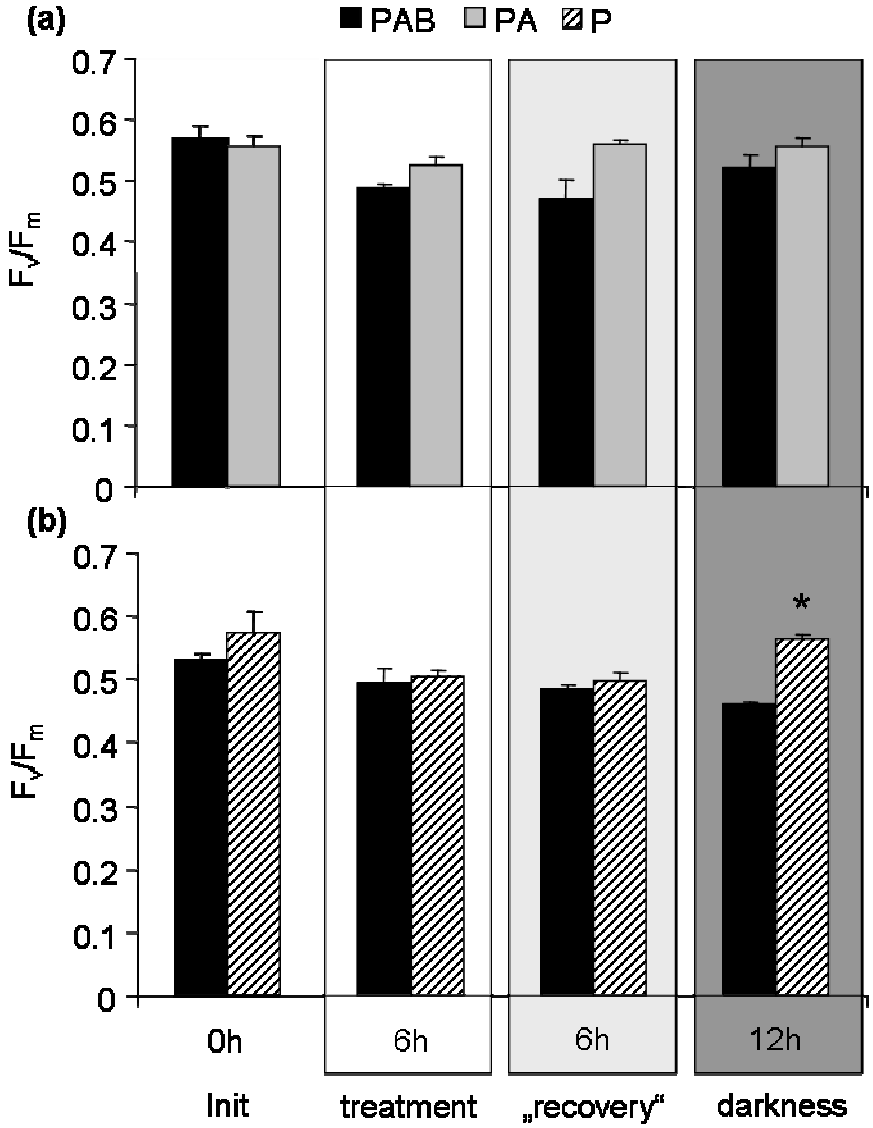

Fig. 2a-b. Experiment C1 **(a)** and C2 **(b)** (n = 3). Optimum quantum yield of microalgal suspension after exposure to different radiation treatments (± SE), without UV-B or UVR, respectively ("recovery") and in darkness (<1 μmol photons m^{-2} s^{-1}). (*) marks significant differences within the respective radiation treatments (one-way ANOVA). PAB = PAR + UV-A + UV-B, PA = PAR + UV-A, P = PAR

Discussion

Decreased photosynthetic rate appear to be the most frequently observed short-term effect for benthic microalgae (Villafañe et al. 2003). The results are, however, ecologically relevant only when realistic, moderate increases of UV

levels are used (Villafañe et al. 2003). In our experiments, the diatoms were exposed to a UV-B intensity of ca 0.4-0.6 W m^{-2}, an intensity they will probably never experience at 5 m water depth at the study site. We can thus conclude that due to the high UV-B intensities applied, the effectiveness of UV-B was overestimated compared with field conditions and our experimental approach was thus mechanistic. Nevertheless, in all our experiments the diatom photosynthesis recovered from the UV treatment effects.

The reduced photosynthetic capacity observed in the first set of experiments (B1, B2) was due to the UV-B part of the spectrum because there was no apparent difference between the P and PA treatments.

Vertical migration has been suggested to be a key mechanism for epipelic benthic diatoms to avoid UV-B radiation (Underwood et al. 1999). To detect a possible impact of a downward migration we used two different experimental approaches. First we used an intact diatom mat and a non-destructive probe for measuring effective quantum yield of photosynthesis (expts B1, B2). We thus measured the upper layer of the most exposed cells. Lower $\Delta F/F_m'$ in the UV-B treatment could be caused by photoinhibition and/or photodamage of PSII (Hanelt et al. 1997) but it could also be due to downward migration of the cells (Underwood et al. 1999). In the study by Underwood et al (1999), the epipelic diatom *Gyrosigma balticum* responded to UV-B by vertical migration, but a significant damage to PSII was still apparent after 5 days of repeated UV-B exposure. Although UV-B has been shown to penetrate down to 0.6 mm sediment depth (Wulff et al. 1999), we cannot exclude that the diatoms in our experiment escaped the UV-B radiation. In the second set of experiments (C1, C2) we used a diatom suspension and we measured the optimum quantum yield of photosynthesis (dark adapted cells). Experiment C1 and C2 confirmed the hypothesis of vertical migration because no UV-B (or UV) effects were observed although *all* cells and not only cells of the upper layer were measured. We want to point out, however, that the UV-B intensity differed between the first and second experimental set-ups (0.6 and 0.4 W m^{-2}).

If only the UV-B or UV part of the radiation spectrum was removed, no recovery was observed. Under “darkness”, however, a recovery in all treatments formerly exposed to PAR and UV-A could be observed (B1, C2). When shielded from UVR, no increase in photosynthetic capacity was detected (B2, C2). UV-A has been suggested to counteract UV-B effects in phytoplankton (Smith et al. 1992) and cyanobacteria (Quesada et al. 1995). The reduction in UV-B damage has been attributed to a UV-A and blue-light mediated repair of the DNA and a stimulation of photosynthesis (Franklin and Forster 1997 and references therein). In experiment B2, however, the P treatment did not recover to initial values and no treatment effects could be observed. The diatom assemblage in experiment B2 seemed to be stressed (photoinhibited) already at the beginning of the experiment possibly influencing the outcome of the experiment because no recovery to initial values was found over time.

Self shading has been proposed as a mechanism to avoid harmful UVR (Garcia-Pichel et al. 1996). However, due to the low cell numbers this was not the

case in our experiment. If all cells lie flat on the sediment they could still not cover the sediment surface

In earlier field experiments regarding UV effects on marine microbenthic communities the benthic diatoms generally seemed to be very tolerant to UV-B radiation (Wulff 1999 and references therein). Our study confirms these earlier results. However, we believe that determinations of UV-B effects on natural microphytobenthos require *in situ* measurements of the photosynthetic activity and productivity.

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Impacts of UV radiation and grazing on the structure of a subtidal benthic diatom assemblage in Antarctica

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Introduction

Benthic microalgal communities are of vital importance for the ecological function in marine habitats. They constitute the local basis of the food webs in shallow areas (Villafañe et al. 2003) and they play a key role in the marine successional process in hard bottom systems, particularly at the early stages (Neushul et al. 1976; Huang and Boney 1985).

Stratospheric ozone depletion has resulted in enhanced ultraviolet-B radiation (UV-B, 280-320 nm) reaching the Earth's surface, Antarctica being one of the most seriously affected regions. The susceptibility of microphytobenthos towards ultraviolet radiation (UVR, 280-400 nm) has mainly been investigated in studies on soft bottom communities. Ambient UV-B was proven to be a stress factor for sand-living microbenthic communities and a selective force during early growth and succession (Wulff et al. 2000). Important functional factors such as primary productivity and carbon allocation were strongly affected by ambient (Wulff et al. 1999) and enhanced levels of UV-B (Sundbäck et al., 1997; Wulff et al. 2000). Effects on structural variables such as biomass and species composition were not affected by ambient or enhanced levels of UV-B (Sundbäck et al. 1997; Wulff et al. 1999). Vertical migration was suggested to be a key mechanism for sand living benthic diatoms to avoid UV-B (Underwood et al. 1999). Thus, UVR is a stress factor that should be considered in colonization studies, particularly on hard bottom substrata.

Besides environmental factors, the colonization process is determined by several mechanisms that include intraspecific and interspecific interactions grazing being an important structuring force in the succession of primary producers (Connel 1987). Depending on their feeding preferences, grazers can accelerate or stall succession and induce a shift in the climax state (Souza and Connel 1992).

In Antarctic coastal ecosystems, poor development of pelagic microalgae (Hapter et al. 1983, Schloss et al. 1998) but an important contribution of resuspended benthic diatoms to the phytoplankton have been suggested and/or observed (e.g. Ahn et al. 1994, Gilbert 1991a, Gilbert 1991b). In the Arctic, a detailed study of carbon cycling in a fjord system showed that the primary production of phytoplankton and ice algae could not account for the carbon input

required by the benthic community (Glud et al. 2000; Rysgaard et al. 2001). Thus, benthic microalgae are likely to provide an important food source for both benthic and pelagic heterotrophs.

Except for a few studies, there is a general lack of ecological information on Antarctic marine benthic diatoms (Wahl et al. 2004, Zacher et al. in press). To our knowledge, information on subtidal marine Antarctic benthic microalgal assemblages is lacking. In the present study we report the first results of a long term field study addressed to analyze the effects of UVR and grazing on a subtidal hard bottom diatom assemblage in Antarctica.

Materials and methods

Study site. The study was carried out in Potter Cove, 25 de Mayo/King George Island, South Shetland Islands, Antarctica. The field experiment was performed in a sheltered subtidal area situated on the north-western coast in the mouth of the cove at *Peñón de Pesca* (62° 14´S, 58° 40´W). For details of the experimental site location see Zacher & Campana (this issue). The upper subtidal slopes in this area are weakly inclined and characterized by hard bottom substrata, comprised by solid rock and boulders covered by macroalgal vegetation with clearings of increasing frequency in the upper 10 m (Klöser et al. 1996, Quartino et al., 2005). Diatom assemblages are found colonizing the rock bed and growing as epiphytes on macroalgae (Klöser 1998). The experimental set up was placed at 2 m depth, at approximately 50 m from the coastline. During the experimental period, the maximal tidal range was around 2 m. On average, 30% of incident UV-B (305nm); 49% (340nm) and 60% (380nm) of UV-A and 65% of PAR penetrated down to 2 m during the field season 2003/2004 (PUV 510, Biospherical Instruments, USA) (Campana et al. this issue).

Experimental design and set up. A factorial design was performed to test the effects of UVR (three levels: photosynthetically active radiation (PAR, 400-700 nm), PAR + UV-A and PAR + UV-A + UV-B) and grazing (two levels: open and closed cages) on the structure of the diatom assemblage. Two control treatments were performed in order to test for cage (half cage treatment) or filter (full sunlight treatment) artifacts. Ceramic tiles (5 x 5 cm) were placed in each cage and served as settlement substrata. For every treatment four replicates were used. For details of the design see Campana et al. (this issue). The field experiment started November 27th 2004 and samplings were performed after 32, 54 and 89 days of colonization.

Microalgal species analyses. The microalgae were removed by scraping and rinsing the tiles. The cells were concentrated and preserved in glutaraldehyde (final concentration 2.5%). After shaking each sample a 20 µl subsample was put on an object slide and cell numbers were counted (Olympus microscope, 400x) until a total of 300 cells under a known number of light fields in the microscope (various subsamples were used for each sample). Empty frustules or frustules with rudimentary chloroplasts were referred to as dead cells. Total cell number was calculated as the number of living plus dead cells, per square centimetre. The diversity (Shannon-Weaver index), the evenness (Pielou's Index), the richness (S) and the species relative abundance were estimated (Margalef

1983). When possible, cells were identified to species level, and otherwise allocated to size and shape groups.

Microalgal species identification. Naphrax mounted slides were prepared for diatom species identification. Samples were washed with distilled water to remove the salts and then boiled with 30% H_2O_2 to remove organic matter. 1-2 drops of 50% HCl were added to remove carbonates and to eliminate H_2O_2. After washing, diatom suspensions were allowed to settle on a cover slip and left to dry before mounted. For species identification, differential interference contrast and phase contrast microscopy at 1000 X magnification were used (Zeiss Axiovert 135). Diatoms were identified following Hustedt (1961-1966), Krammer and Lange-Bertalot (1986, 1988), Hendey (1952, 1964) and Witkowski et al. (2000). The nomenclature was updated with the help of Round et al. (1990).

Statistical analyses. Treatment effects were tested with MANOVA/ANCOVA, using irradiance and grazing as independent variables and time as covariable at α=0.05 significance level. *Post-hoc* multiple means comparisons were performed using Tuckey test (α=0.05 significance level). Homogeneity of variances was checked using Cochran's Test (Zar, 1996). *Statistica 6.0* software was used for the statistical analyses and *PRIMER* software for diversity and evenness calculations.

Results

Cell numbers. The total cell numbers were not affected by the irradiance treatment, but were significantly reduced by grazing (MANOVA/ANCOVA $p<0.05$) (Fig. 1). The same result was observed for the number of living cells: light treatments did not affect the number of living cells, but was significantly affected by grazers (MANOVA/ANCOVA $p<0.05$) (Fig. 1).

Species relative abundance. Diversity was reduced by grazing in all the irradiance treatments (MANOVA/ANCOVA $p<0.05$). A similar pattern was observed for evenness (Fig. 2). The number of species (richness) was not affected by grazing or irradiance (data not shown). The closed cages were covered by a dense mat, consisting mostly of an overstory of the colony-forming species *Fragilaria striatula* Lyngbye, with a varied mixture of other diatom species. Open cages had notable less biomass than closed cages. The dominant species were *Fragilaria striatula*, *Navicula* cf *perminuta* Grunow, and *Navicula* cf *hanseni* Möller. *Navicula* cf *perminuta* is presented in two different size groups: 8µm x 3.2µm and 11.2µm x 4.8µm (valve view) and they were analyzed separately.

The relative abundance of the three dominant species was affected by grazing but were unaffected by UVR. The colony-forming *Fragilaria striatula* was significantly reduced by grazing (MANOVA/ANCOVA $p<0.05$), whereas the small sized *Navicula* cf *perminuta* showed the opposite pattern: the relative abundance in the open cages was significantly higher than in closed cages (MANOVA/ANCOVA $p<0.05$) (Fig. 3). The relative abundance of the larger *Navicula* cf *perminuta* and *Navicula* cf *hanseni* was not affected by grazing within the same light treatment.

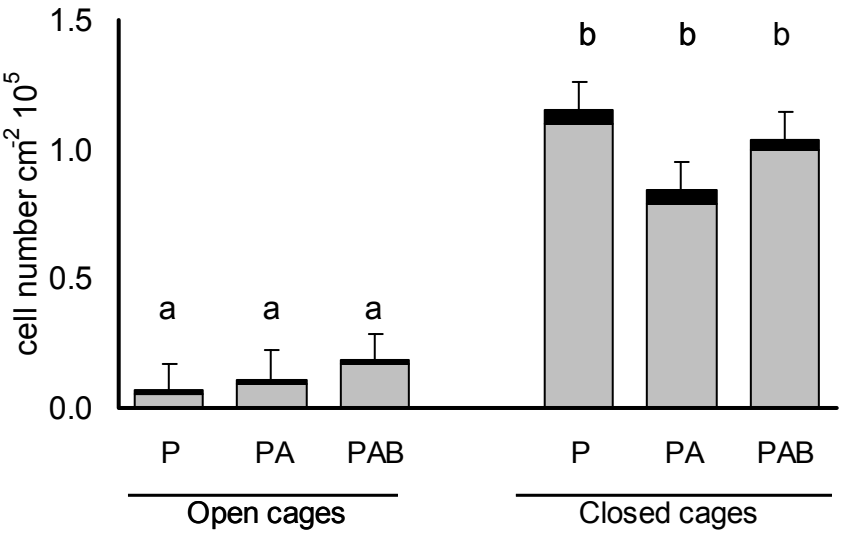

Fig.1. Effects of UVR (PAR = P, PAR + UV-A = PA and PAR + UV-A + UV-B = PAB) and grazing (open and closed cages) on cell numbers. The number of living cells is represented as a part of the number of total cells (gray bars). Shown are means + standard error, n=12). Letters show significant differences (Tuckey Test, p<0.05).

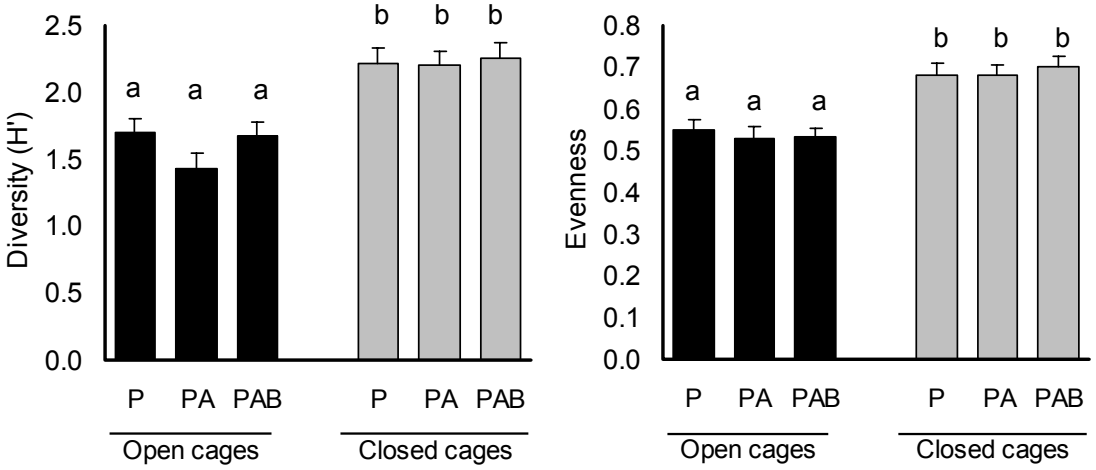

Fig. 2. Effects of UVR (PAR = P, PAR + UV-A = PA and PAR + UV-A + UV-B = PAB) and grazing (open and closed cages) on species diversity and evenness. Shown are means + standard error, n=12). Letters show significant differences (Tuckey Test, p<0.05).

Discussion and conclusions

The effects of UVR on plant-herbivore interactions have been studied in freshwater systems (Bothwell et al. 1994) and in intertidal marine environments (Lotze et al. 2002, Roux et al. 2002). Strong interactive effects were found, UVR and grazing being identified as significant structuring forces in the marine suc-

cession. In the present study, the structure of the community of benthic subtidal diatoms was not affected by ambient UVR whereas grazing caused a stronger effect on the structure of the diatom assemblage, being the main factor controlling the community.

UVR effects. Benthic subtidal diatoms were not affected by UVR in this study. Although functional attributes of benthic diatom mats can be affected by ambient UVR (Wulff et al. 1999), no main structural changes have been reported in the studied community. UVR was not identified as a mortality factor, as the number of living cells was not affected in UVR-treated communities. These results are comparable to others performed with benthic diatoms, in which a very high resistance to UVR was detected (Peletier et al. 1996; Sundbäck et al. 1997, Wulff et al. 1999, 2000). As vertical migration cannot be considered to be a possible mechanism to avoid harmful UVR in hard bottom assemblages, the studied community must rely on other defense mechanisms. Mycosporine-like amino acids (MAAs) production has been found to be a protective mechanism

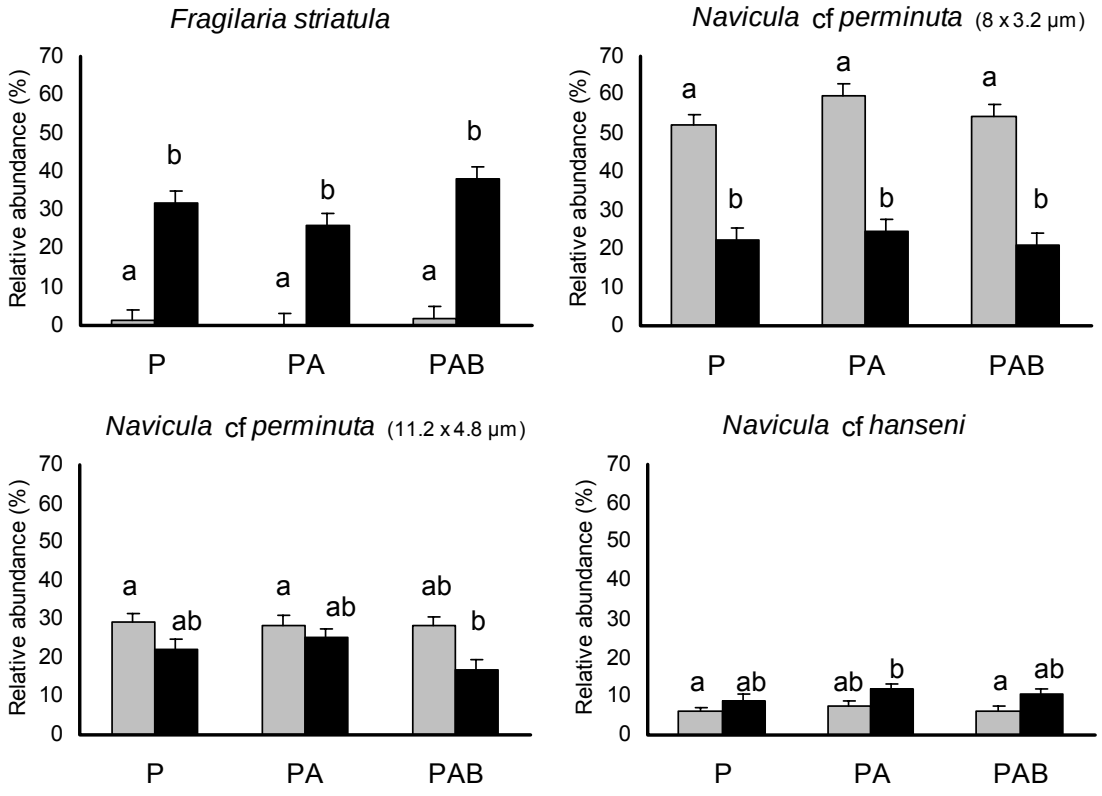

Fig. 3. Effects of UVR (PAR = P, PAR + UV-A = PA and PAR + UV-A + UV-B = PAB) and grazing (open and closed cages) on dominant species relative abundance. Shown are means + standard errors, n=12. Letters show significant differences, each single letter representing a homogeneous group (Tuckey Test, $p<0.05$).

in planktonic central Antarctic diatoms (Hernando et al. 2002). Although no studies have been performed in subtidal benthic Antarctic diatoms, we must consider that these communities are comprised mostly by pennate diatoms that have been shown to produce very low, if any, concentrations of UV absorbing

compounds (Helbling et al. 1996, Wulff et al. 1999, Roux et al. 2002). The benthic diatoms in the present study were analyzed for the presence of MAAs. However, no MAAs were found (Campana and Wulff, unpublished).

Efficient UV induced damage repair mechanisms must be considered and further studied (Peletier et al. 1996, Buma et al. 2006).

Grazing effects. The macroconsumers found in the open cages were examined by SCUBA diving and were comprised by amphipods, (mostly *Gondogeneia antarctica*) and gastropods (the limpet *Nacella concinna* and the snail *Laevilacunaria antarctica*). Grazers had a significant impact on diatom species composition, and caused major reductions in the biomass (total cell numbers). A strong reduction in the diversity index was caused by a reduction of the community evenness, the total number of species being unaffected. Overstory colony forming species were conspicuously reduced when grazers were present. *Fragilaria striatula* forms long ribbon-shaped filaments of moderate-sized cells (Kuylenstierna 1991) and constitute a thick overstory on ungrazed areas. The morphology of the colony and the location of the chains in the outer part of the diatom mat may facilitate its ingestion, making it readily available to grazers (Nicotri 1977; Hillebrand et al. 2000).

Diatoms present in grazed treatments were mostly the small sized *Navicula* cf *perminuta* (> 50 %). Its small dimensions and tight attachment to the substrata (Holland et al. 2004) may be the reasons for not being consumed. The lower relative abundance in closed cages may also be due to light limitation caused by the canopy. The increased relative abundance of this understory species due to grazing is consistent with previous studies (Steinman, 1996).

The grazer preference for large chain-forming species has been reported to cause strong effects on the physiognomy of microalgal communities in freshwater (Steinman 1996) and marine environments (Nicotri 1977, Hillebrand et al. 2000). Grazers are capable of causing a drastic reduction of the overstory species and thus, increasing the relative abundance of the understory species (mainly small, prostrate forms) (Nicotri 1977, Hillebrand et al. 2000). Therefore, the further settlement and growth of macroalgal recruits might be altered in marine habitats.

The shifts in the relative abundance of the dominant taxa observed in our study could be due to a different susceptibility of diatoms towards grazing. The reasons could be size, morphology, strength of attachment or grazer accessibility. These results agree with others performed in marine (Nicotri 1977, Hillebrand et al. 2000) and freshwater environments (Steinman 1996 and references therein). The asymmetric relation between the dominant grazers and the benthic algae (this study) shown by a small prey:grazer ratio (Steinman, 1996) suggests that a true selection by grazers is not likely (Nicotri 1977; Steinman 1996). As in other studies, a passive selection, resulting from differences in diatom growth forms and accessibility, explain the observed tendencies.

Interaction effects of UV and grazing. The interactive effects of UVR and grazing on benthic diatoms may play a key role in the succession in rocky marine benthos, being two possible coexisting mechanisms. On the one hand, UV resistant diatom mats may provide an environment that protects UV sensitive early developmental stages of macroalgae from UVR exposure (Wiencke et al. 2000, among others). On the other hand, grazing on diatoms can decrease

competition and thus favor the establishment of macroalgal recruits (Campana et al. 2006; Zacher and Campana, this issue).

Benthic diatoms seem to be adapted to UV stress by having developed protective or repair mechanisms that have to be further studied. Thus, the role of benthic diatoms in marine hard bottom substrata may be critical to determine marine colonization processes in the subtidal in an ozone depletion scenario.

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Impact of ultraviolet radiation on two isolated Antarctic marine bacteria

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Introduction

The annual depletion of the ozone layer selectively reduces absorption of ultraviolet-B radiation (UVB, 280-320 nm), resulting in higher UVB irradiance on the Antarctic surface (Staehelin et al. 2001). It was reported that the UVB is responsible of significant detrimental biological effects on aquatic environments (Vincent and Neale 2000, Booth et al. 2001a, Biggs and Moody 2003). Ultraviolet A radiation (UVA, 320-400 nm) may also be responsible for certain types of photobiological damage (Kim & Watanabe 1994, Sommaruga et al. 1997). UVA can affect bacterial viability and activity (Helbling et al. 1995), either directly or indirectly via production of oxidant compounds from dissolved organic matter. However, the major part of the studies dealing with the effect of UV radiation (UVR) on aquatic organisms has been focused on phytoplankton assemblages, the effect on the aquatic bacteria being less investigated. Bacteria play a key role in mineralization of nutrients and provide a trophic link to higher organisms (Azam et al. 1983; Ducklow et al.1992). This role is crucial in the Southern Oceans, where phytoplankton stocks are low and the spring algal blooms is scarce or do not develop (Karl 1993). Marine bacteria seem to be more susceptible than other planktonic organisms to the effects of UVR (Jeffrey et al. 1996). The lack of UV-protective compounds in marine bacteria other than cyanobacteria could be one of the causes of this susceptibility (Cockell and Knowland 1999). Reports about changes in species composition of marine bacterial communities under UVB stress (Arrieta et al. 2000) reflect the different sensitivity that different members of these communities has to the UVR stress. Solar radiation has not only detrimental effects on bacterioplankton. Crucial roles in DNA-damage repair has been found for UVA and PAR (Kaiser and Herndl 1997) as activators of the photoenzymatic repair mechanisms. This fact determines a complex relationship between positive and negative effects of solar radiation on marine bacteria which largely depends on the characteristics of the environments under study. In this work, two Antarctic marine bacteria were isolated from surface water at Potter Cove, Antarctica and used as biological models in different experimental designs in order to analyse their response to solar radiation exposure. In a first step, the effects of PAR, UVA and UVB on the isolated strains were evaluated at different time periods in days with different irradiance regimes. In a second step, the role of the water column as attenuating factor was analysed by exposure of the bacterial strains at three depths in the water column: surface, 1 m and 3 m. Finally, the effect of a simulated vertical mixing of 4 m h^{-1} on the bacterial survival was evaluated.

Materials and methods

Bacterial strain and study area.
Bacterial strains were isolated from surface marine water at Potter Cove, King George Island (Isla 25 de Mayo), South Shetland Islands, Antarctica (62°14'S, 58°40'W). Strains were psychrotolerants, with a growth temperature range of 0-30ºC. One of the strains (UVvi) belong to the genus *Arthrobacter* and the other (UVps) was related to *Flavobacterium-Cytophaga-Bacteroides* (FCB) group, which is a complex group into the *Bacteroidetes* division (Hernández *et al*. 2004). 16S rDNA sequences of UVps and UVvi were deposited in the Gene-Bank under accession numbers AY220353 and AY220354 respectively.Land experiments were carried out on a beach of Potter Peninsula, whereas water column experiments were performed in the first four meters at Potter Cove coastal waters. Laboratory assays were conducted in the Argentinean-German Dallmann Laboratory (Jubany Station, Argentina).

Experimental design.
This work summarises results from a number of experiments carried out during several Antarctic summer expeditions (1999 to 2005). From cultures on marine agar plates (Difco 2216) bacterial suspensions of the strains were prepared in filtered natural seawater. The first experimental design was used for the land experiments and consisted in one set of quartz bottles containing 50 ml of the mixed bacterial suspension placed into an incubation chamber. This chamber was immersed in a continuous water circulation bath to minimise temperature fluctuations in the flasks (average temperature was 5.5ºC ± 1.0ºC). Interferential quartz filters (Schott R) were used to cover the flasks determining different irradiance treatments (triplicates). Treatments were: (a) DARK, control system with a black filter; (b) PAR, covered with a 420 nm cut-off filter; (c) UVA360, (PAR + UV-A >360 nm) covered with a 360 nm cut-off filter; (d) UVA320, (PAR + UV-A) covered with a 320 nm cut-off filter; (e) UVB305, (PAR + UV-A + UV-B >305 nm) covered with a 305 nm cut-off filter; (f) UVB280, (PAR + UV-A + UV-B) (without filter). Samples from treatments were taken at times differing among experiments due to the different solar radiation regimes existing during each one. Serial dilutions of the samples were plated on marine agar and incubated in dark for 7 d at 20ºC. This temperature was chosen because in previous analysis it proved to shorten the incubation period of these psychrotolerant bacteria and yielded the same results as those obtained using lower temperatures (data not shown). Bacterial colonies (ranged between 30 and 300) were counted and results were expressed as colony forming units per ml (CFU ml^{-1}). In the second experimental design, carried out in the water column, quartz flasks were filled with the bacterial suspensions and exposed to solar radiation at surface, 1 m and 3 m depth. At the surface the quartz bottles were placed on a metal frame maintained on place by means of buoys attached to each side. This structure supported other two metal frames located at 1 and 3 m depth. Finally, in the third experimental design a vertical mixing of 4 m h^{-1} was simulated. For this purpose, in addition to the surface frame described above, a second frame supported by a mechanical arm was used. This device permitted us to vary the depth at which the frame was placed. Attached to this mobile frame, quartz flasks were initially maintained during 15 min at 4 m depth. After that, the mobile frame was progressively moved to the surface at 1 m intervals, main-

taining the flasks during 15 m at each depth. When flasks reached the surface a first sampling was made. After that, the cycle was continued by moving the frame 1 m steps until 4 m-depth level and returning to the surface by a second time. At this stage a second sampled was taken and the assay was stopped. Samples obtained at different times during the water column experiments were processed as described for the first experimental design.

Solar radiation measurements.

Incident UVR was measured continuously with a multichannel UV spectroradiometer (Isitec Bremerhaven, Germany) having a multichannel detector system. UVB data were recorded every second from 290 nm to 320 nm range with a data point every 1.35 nm by means of the 32 detection channels. Data were stored as 1-min average. Sub aquatic solar radiation data were recorded using a PUV 500 (Biospherical instruments inc.). This radiometer obtains data every 5 seconds in the range of PAR and at 380, 360, 320 and 305 UV wavelengths. Attenuation coefficient was calculated according to Kirk (1983) as follow:

$$Kd\ (\lambda) = 1/z * \ln(I_0/Iz)$$

Where I_0 represent irradiance at surface level, I_z represent irradiance at depth z and λ is the wavelength at which the irradiance values were obtained.

Statistical analysis.

Comparison of bacterial counts data obtained from different treatments were analysed by Repeated Measures ANOVA and Tukey's Multiple Comparison Test. Individual comparisons of pairs of values were made using Unpaired T-test. Viability values obtained at different sampling times from 10 independent land experiments were analysed by Non-linear Regression Analysis, considering survival (expressed as percentage of the dark treatments) as the dependent variable and UV-B integrated dose as the independent one (n = 29 for each strain).

Results

Fig. 1 illustrates experiment 1, which was performed using the first experimental design. This experiment was carried out a sunny day and was considered as a "high dose" situation because it showed the highest UVB radiation levels among the 10 land assays performed. The UVB dose rate during this assay was 3.81 kJ m^{-2} h^{-1} and the dose was 30.45 kJ m^{-2}. This dose was accumulated in 8 h, and represents a high UVB radiation level for Potter Peninsula, where a maximum UVB dose of 52.4 kJ m^{-2} was reported by Hoyer et al (2001) for December 1997 for a daily period of 20 h. After 2 h of exposure (UVB dose 15.0 kJ m^{-2} and UVA dose 169.5 kJ m^{-2}), UVvi and UVps showed 0.01% of survival under UVB280 treatment. During this initial period both strains showed differences in viability between the UVR treatments and PAR and DARK ($p<0.01$). Both UVB treatments (UVB280 and UVB305) showed similar effects on UVps and differed ($p<0.05$) from UVA320 and UVA360. For UVvi, even though UVB treatments caused a greater reduction in viability than the UVA treatments, the effect of UVB280 was more pronounced ($p<0.05$) than that induced by the UVB305 and

UVA treatments. Viability observed under PAR was significantly lower than those observed under DARK ($p<0.05$). This difference was more evident at the end of the assay, when a sharp decrease in viability under PAR occurs. At the end of this assay, PAR dose was 3520 kJ m^{-2}. Other similar experiments, performed under low PAR doses (1580 kJ m^{-2}) showed no differences in viability between PAR and DARK treatments (data not shown).

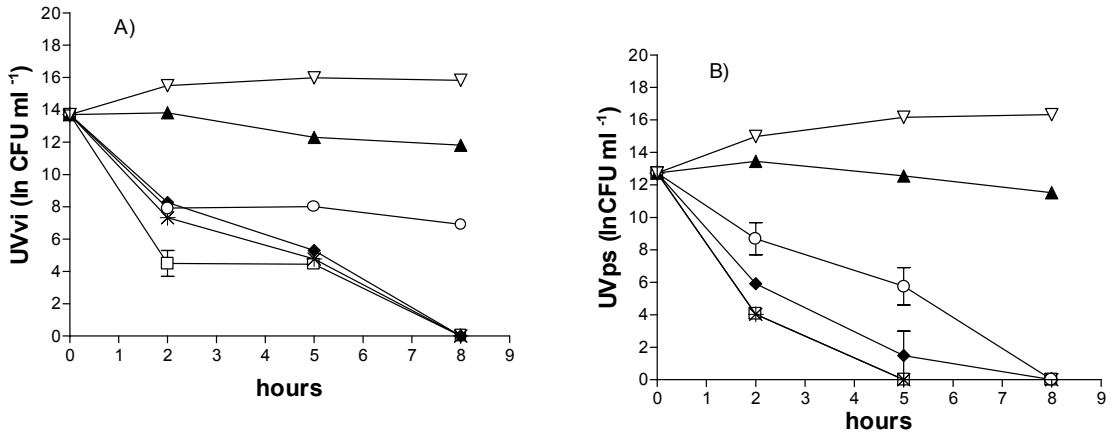

Figure 1. Effect of solar radiation on viability of **A.** *Arthrobacter* UVvi strain and **B.** UVps strain, registered on Experiment 1, carried out on 27 December 2001 (11:30 h AM to 7:30 h PM) in Jubany Station. Treatments: DARK (▽), PAR (▲), UVA360 (O), UVA320 (♦), UVB305 (∗), UVB280 (□). Error bars indicate standard deviation (SD) of triplicates. When bars of the SD are not visible then the SD is smaller than the symbol.

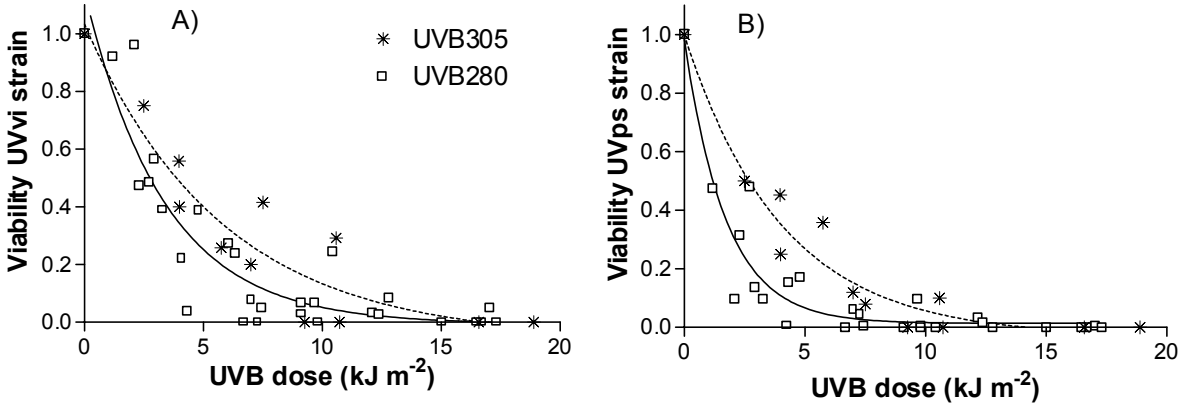

Figure 2. Significant logistic regression ($p < 0.05$) plotted between bacterial viability values (expressed as the ratio between UVB280 or UVB305 and DARK treatments) and UV-B dose for **A.** *Arthrobacter* UVvi strain and **B.** UVps strain. Data were obtained from 10 experiments performed under different radiation regimes.

Significant logistic regressions were obtained ($p<0.05$) for both strains (Fig. 2) between bacteria viability (survival) and UVB dose. UVps showed significant regression under both UVB280 ($r^2=0.88$) and UVB305 ($r^2=0.94$) treatments (Fig. 2B). Lethal dose 50 (LD 50) for UVB280 (1.2 kJ m^{-2}) and UVB305 (2.8 kJ m^{-2}) were significant different ($p < 0.05$) for this strain. Similar logistic regressions (Fig. 2A) were observed for UVvi ($r^2=0.84$ under UVB280 and $r^2=0.88$ under

UVB305). In this case the LD50 were 2.3 kJ m^{-2} for UVB280 and 4.0 kJ m^{-2} for UVB305 ($p<0.05$). When the strains were compared using the LD 50, UVps proved to be more sensitive to UVB radiation than UVvi and significant differences ($p<0.05$) were observed under both UVB280 and UVB305 treatments. In addition, under UVB280, UVps showed 10% of remaining viability at 4.0 kJ m^{-2} and UVvi showed the same remaining viability at 7.5 kJ m-^{2}. Under UVB305 UVps and UVvi showed 10% of initial viability at 8.4 kJ m^{-2} and 11.1 kJ m^{-2} respectively.

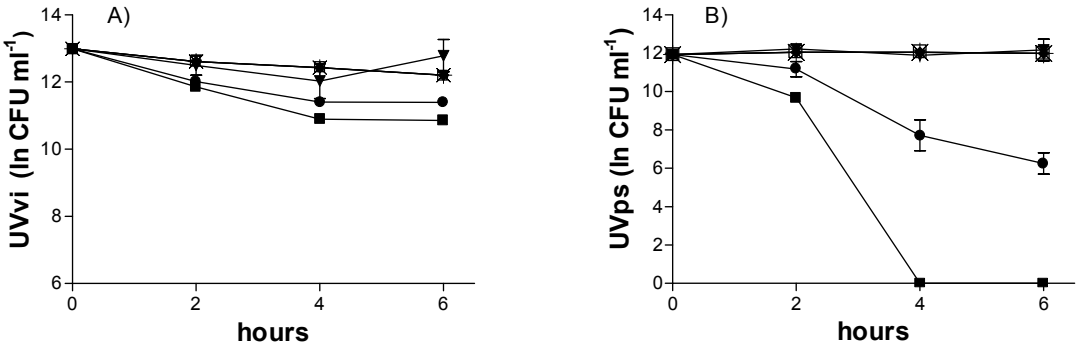

Figure 3. Effect of solar radiation on viability of **A**. *Arthrobacter* UVvi strain and **B.** UVps strain maintained at 0, 1 and 3 m in the water column. (■): 0 m, (●): 1 m, (▼): 3 m. Dark controls: (♦) 0 m, (✳) 1 m, (□) 3 m.

Studied strains were also exposed to solar radiation at different depths in the water column (second experimental design, Fig. 3). After 2 h at surface (UVB dose 8.4 kJ m^{-2}) both strains showed a significant decrease in viability ($p<0,05$). Loss of viability was lesser for UVvi (50 %) than for Uvps (90 %). At the end of the assay (6 h), when UVB dose at the surface was 20.1 kJ m^{-2}, less than 1% of the initial counts were detected for both strains. In this experiment $Kd_{(305)}$ was 2,38 m^{-1}. This fact determined that at 1 m depth UVB dose after 2 h was 2.1 kJ m^{-2}. Under this condition, 55% of mortality was observed for both strains. However, at 6 h mortality level for UVps (>99 %) was significantly higher ($p<0.01$) than those observed for UVvi (75%). Finally, at 3 m depth UVps and UVvi showed viability values similar to the dark control, possibly due to the high attenuation degree of UVR (UVB dose 0.0084 kJ m^{-2}). In a set of experiments where flasks were covered with cut-off filters, a similar negative effect was observed under both UV treatments at 0 and 1 m depth. In this case dark control and PAR treatment maintained a similar bacterial counts value throughout the assay and showed no significant differences between them (data not shown). Using the third experimental design (a simulated vertical mixing of 4 m h^{-1}), two experiments were made under different UVR regimes (Fig. 4). When the experiment performed under the highest solar radiation was carried out, a $K_{d(305)}$ of 1,95 m^{-1} was measured in the water column. Under this condition a significant decrease in viability was observed for both strains when maintained in the surface metal frame. After 1 h of exposure treatments under simulated vertical mixing showed bacterial counts values similar to those measured in the dark control ($p>0,05$). After 3 h (UVB dose at surface was 7.7 kJ m^{-2}) mortality values were as high as those observed in the flasks attached to the surface metal frame (Exp. 3, Fig. 4A and 4B). The second experiment (Exp. 4) was

made under lower radiation doses (4,8 kJ m^{-2}) and with a $K_{d(305)}$ of 1,24 m^{-1}. Under these conditions vertical mixing significantly attenuated ($p<0,05$) the deleterious effects of RUV. However, although UVps showed significant differences in viability when the system under vertical mixing was compared with the corresponding dark control, mortality was lower than the observed in the surface metal frame. On the contrary, no differences were observed for UVvi under the same experimental condition (Fig 4C and D).

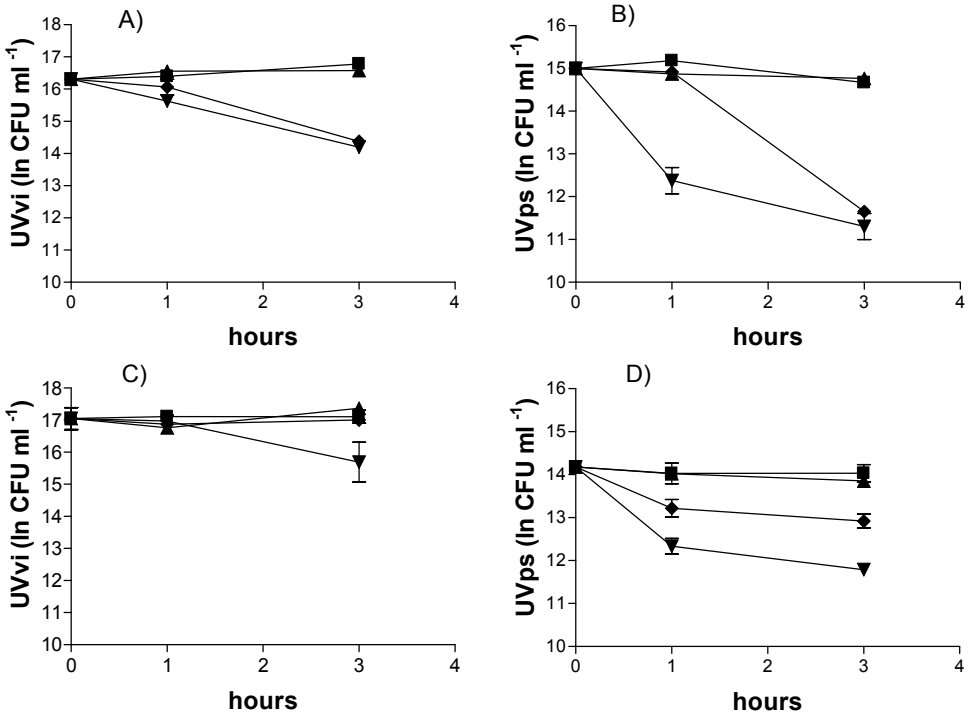

Figure 4. Bacterial counts observed for the studied strains exposed to solar radiation in the water column under a simulated vertical mixing of 4m h^{-1}. **A.** *Arthrobacter* UVvi under the higher radiation dose (Experiment 3) **B.** UVps under the higher radiation dose (Experiment 3) **C**. *Arthrobacter* UVvi under the lower radiation dose (Experiment 4). **D**. UVps under the lower radiation dose (Experiment 4) Treatments: Dark surface (■), Dark vertical mixing (▲), Solar radiation surface (▼), Solar radiation vertical mixing (♦).

Discussion

Studies on the effect of solar radiation on bacterial isolates and communities have been carried out continuously at Potter Cove during the period 1999-2005. In this paper, which represents a brief summary of our work, we included some of such studies. In a general view, our results confirmed the deleterious effect that the UVR reaching Antarctica exerts on bacterial strains inhabiting coastal Antarctic waters. This effect has been previously observed by other authors (Helbling et al.1995, Davidson and van der Heijden 2000). In this sense, in a previous paper working with UVvi and UVps strains exposed to UVR (Hernández et al 2002), we reported mortality levels greater that those reported by Davidson and van der Heijden (2000). This fact showed that despite the origin of these strains (surface Antarctic marine waters) no effective UV-protection mechanism seems to be acting when they were exposed to natural UVR. In addition, we observed that one of the strains (UVps), even failed to recover from the UV-induced damage after 24 h in the dark (Hernández et al 2004). We

found also a significant negative effect of UVA, which agree with other reports working with marine bacteria (Kim and Watanabe 1994, Sommaruga et al. 1997, Booth et al. 2001b). When different wavelength ranges in the UVB and UVA bands were analysed in relation to their effect on the Antarctic bacteria, a complex interaction among λ, irradiance and the individual responses was observed. It was found that even the UVA wavelengths closest to PAR caused deleterious effect on the strains. As is known that these UVA wavelengths are involved in the photoenzymatic DNA repair systems, our results suggests that under the assayed conditions such repair mechanisms were inefficient. In addition, although UVA320 treatment also showed high mortality, it was lower than those caused by the UVB(305 and 280) treatments. Analysis of the response of the strains to these UVB wavelengths (which are directly affected by changes in the ozone layer) is an important point to infer the effects that the presence of the ozone hole, and the subsequent increase in the incident UVB radiation, could exert on the Antarctic bacterioplankton. The regression analysis where survival data from 10 experiments were plotted against UVB dose showed a different sensitivity of the strains that was evidenced by differences in their LD50. Other authors also reported interspecific differences in UVB sensitivity of marine bacteria (Joux et al. 1999, Arrieta et al. 2000). Although in experiment 1 we did not observe significant differences between the effect of UVB280 and UVB305, when data from 10 experiments were plotted together, regression analysis showed that when the UVB band between 280 and 305 nm was present, mortality values were significantly higher than those observed when only UVB λ<305 reached the bacterial cells. For planktonic organisms, vertical mixing regulates the intensity and length of solar exposure, which represent two important aspects of the cumulative impact of UVR.(Karentz and Bosch 2001). As diurnal stratification of marine surface layers is a common phenomenon (Doney et al. 1995), microorganisms confined to these stratified layers are therefore under high UVR levels. In our experiments, the water column of Potter Cove strongly attenuates UVR, mainly in the UVB range. Under conditions predominating in summer at Potter Cove (high level of suspended particulate matter carried by the seasonal fresh-water streams) less than 1% of the UVB reach 3 m depth. Under these conditions, the negative effect of the UVR would be relevant only above the 3 m depth level. Vertical mixing is one of the main mechanisms proposed to explain the survival of microorganisms in the surface layer of marine waters. It moves away the cells from the surface allowing the repair mechanisms to act far from the highly irradiated zone (Boelen et al. 2001). In our assays, the simulated vertical mixing proved to enhance bacterial survival, mainly when irradiance level was moderate or low. However, at high irradiance levels (as in Exp. 3) the mixing regime was not efficient to attenuate the effect of solar radiation on bacterial cells and showed no differences compared with those observed in surface.

In conclusion, the studied Antarctic bacteria showed a differential response to UVR wavelength ranges. However, for both strains, UVA wavelengths were harmful enough to significantly reduce bacterial viability, principally for λ<360. When exposed to the UVB bacterial strains lost viability exponentially with dose. The UVB-filtering action of the water column was only significant in the first meters of the water column at Potter cove. Finally, an attenuating effect of the vertical mixing was observed. This effect seems to be dependent of the extent and speed of the mixing but further studies using the same biological model will

be necessary for a deeper understanding of the role of this key attenuating factor.

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Response of blood parameters of the Antarctic fish *Notothenia coriiceps* to warming and hypoxia

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Introduction

There are at least 274 species of fish that are regarded as “endogenous Antarctic”; 95 of these are notothenioids, which constitute a major proportion of Southern Ocean fish biomass (Eastman 1993). All extant notothenioids are closely related and believed to have evolved from a benthic ancestor trapped beneath the ice cover over times of intense glaciation (Eastman 1993). Isolated for more than 25 million years as the Southern Ocean progressively cooled, Antarctic fish have evolved into a highly cold-adapted phenotype (Peck et al. 2005) including antifreeze proteins (Chen et al. 1997) and plasma osmolality, twice as high as that of temperate marine fish (Gonzalez-Cabrera et al. 1995). Moreover, these fish have apparently lost the inducible heat shock response (Hofmann et al. 2000).

Constant low temperatures and high oxygen concentration in Antarctic waters, have, moreover, led to special adaptations of the haematological characteristics of Antarctic fish, such as reduced haematocrits, low haemoglobin concentrations, and haemoglobin multiplicity with low haemoglobin oxygen affinity (Feller & Gerday 1997, Cocca et al. 1997). The decrease in haematocrit counteracts high blood viscosity at low temperature and, thereby, reduces cardiac work load. The notothenioid heart generates a large stroke volume, but only at low pressures and at very low vascular resistance (Davison et al. 1997).

An increase in the concentration of circulating catecholamines in order to maintain cardiovascular and respiratory function, and thus, adequate levels of oxygen in the blood, is the prime stress response of fishes. Interestingly, Antarctic teleosts lack this important stress response (Reid et al. 1998). Only under extreme heat stress on exposure to 10°C for 10 min, catecholamines release to the blood occurred in two notothenioid species, the pelagic *Pagothenia borchgrevincki* and the benthic *Trematomus bernacchii* (Forster et al. 1998). Also, handling stress did elevate heart rate and ventral aortic pressure in *T. bernacchii* (Davison et al. 1997). In *P. borchgrevincki* an extreme increase to over 110% in haematocrit was observed after exercise, which could be mainly attributed to the release of erythrocytes from the spleen (Franklin et al. 1993). Together, these observations suggest that Antarctic fish display the capacity to adapt their oxygen transport system to varying demands. Although environmental hypoxia is rarely occurring in pelagic Antarctic regions, Antarctic fish may suffer from functional oxygen limitation during exhaustive exercise and have obviously conserved a basic response to hypoxia.

To test this hypothesis, we studied the response of several blood parameters to different time periods of experimental warming to 5°C and to hypoxia (20% air saturation, representing critical PO_2 conditions in the Antarctic fish *Notothenia*

coriiceps). Below this PO_2 the fish oxygen uptake decreases, and presumably anaerobic energy conservation starts and involves lactate production.

Material & Methods

Animal collection and maintenance

Notothenia coriiceps were fished with baited traps in 15 m water depth in Potter Cove, King George Island, Antarctic Peninsula (62° 14`S; 58° 40`W) in the Antarctic campaign November 2005 to March 2006. The animals were transported to the station and kept in an aquarium system with natural seawater, constant aeration and a natural day-light cycle at 0.7°C. Fishes were fed pieces of frozen fish for the first time five days after capture and afterwards every ten days. They were maintained at least for seven days (ten days in case of determination of critical PO_2) in the aquaria, before experimentation started. Mean fish length was 31.5 ± 3.0cm at a mean weight of 427 ± 129g.

Warm acclimation (5°C)

For the warm acclimation animals were directly transferred (two at a time) to a separated aquarium with natural seawater, thermostated to 5°C, and incubated for 12, 24, 48, 96 (= 4 days), 168 (= 7 days), 336 (= 14 days) hours. The water was kept fully aerated. For sampling, the fish were narcotized by a blow on the head, weighed and killed by cutting through the spine. Blood was immediately taken from the *vena cava* with heparinised syringes and stored on ice. Subsequently, various tissues were sampled removed and stored at -80°C. Sex and length of each fish were determined after sampling. Haematocrit values and the concentration of monomeric haemoglobin were measured directly after finalizing the sampling. Blood lactate content was measured in frozen (-80°C) and re-thawed blood samples.

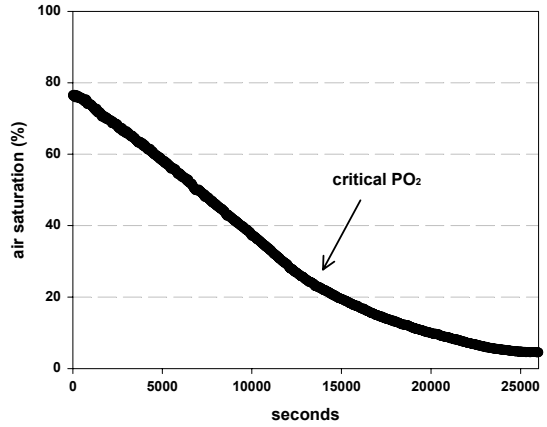

Figure 1
depicts a typical respiration measure (air saturation *versus* time) and demonstrates that at 20% air saturation *N. coriiceps* cannot longer keep constant the oxygen consumption with decreasing oxygen content, i.e. the critical PO_2.

Determination of critical PO_2

Prior to experimentation, the fishes were kept without food for ten days in order to eliminate impact of specific dynamic action (increase of respiration rate due

to food digestion) on respiration rates. The critical PO_2 was determined in a pre-experiment, in which fishes were transferred to a respiration chamber in which the air saturation was recorded using oxygen microoptodes connected to a Microx TX 2-array (PreSens GmbH). The respiration chambers were Perspex cylinders of different volumes (9 to 10l), adjustable to the size of the animals. Water temperature was kept at 0.7°C. Microoptodes were calibrated to 100% and 0% air saturation at that temperature. Fishes were allowed to accommodate to the chambers over night in the open system at 100% air saturation. For the actual measurement of the respiration rate of the fish, the system was closed and the decrease air saturation, respectively PO_2 in the chamber recorded. Fig. 1 depicts a typical respiration curve with a break at 20% air saturation, the PO_2 where *N. coriiceps* cannot longer keep its respiration rate constant.

Hypoxia exposure (20% air saturation)
For the hypoxia exposure, fish were transferred to a separate incubation basin with natural seawater, thermostatted to 0.8°C (= control temperature) and adjusted to 20% air saturation, corresponding to the critical PO_2. Air saturation was maintained constant by bubbling a gas mixture from a gas mixing pump (Wösthoff), into the aquarium. The fishes were incubated (three at a time) for 12, 24 or 48 hours before sampling was carried out as described above. Longer hypoxia incubations could not be carried out due to the limited amount of gas bottles that could be shipped to Antarctica.

Determination of blood parameters
Haematocrit values were determined in capillaries using a haematocrit centrifuge. The monomeric haemoglobin concentration was determined spectro-photometrically using the absorbances at 540 and 570 nm according to Antonini & Brunori (1972). Blood lactate levels were determined using an Accutrend® Lactate test (Roche Diagnostics, Mannheim, Germany) following the instruction manual.

Statistics
Differences between experimental groups were analysed by ANOVA and Bonferroni/Dunn PostHoc test, using StatView 5.0 with a p-level of 5%.

Results
No differences were found between male or female individuals, with respect to neither length nor weight or the hepatosomatic index so that data of both sexes were pooled. Further, neither body mass indices nor condition factors varied between different captures throughout the campaign, or with the time of maintenance in the aquaria.

Warm acclimation of Notothenia coriiceps
Upon acclimation of *N. coriiceps* to 5°C, blood lactate content increased significantly during the first 48 hours and returned to control level during the following 5 days. (fig. 2; p-values: 0.0269 / 12 h, 0.0011 / 24 h, 0.0004 / 48 h, 0.0028 / 4 days, 0.2517 / 7 days). After 14 days of warm acclimation blood

lactate had increased again (p = 0.0002). By contrast, the haematocrit remained unchanged within the first 4 days of warm acclimation (p-values ≥ 0.2444) and was elevated after 7 days (p < 0.0001), when blood lactate was already back to control level (fig. 3). After 14 days, also the haematocrit was back to control level (p = 0.5045). Thus, blood lactate and haematocrit demonstrated an alternating response to warm acclimation. However, plotting lactate content against the haematocrit in different fish revealed no significant correlation between both parameters (StatView 5.0, R^2 = 0.034, p = 0.3176). The concentration of monomeric haemoglobin showed the same pattern as the haematocrit values, however, with higher variation between individuals in several experimental groups (fig. 4). There was no significant change of the ratio of haemoglobin / haematocrit (p-values ≥ 0.4306) indicating that the amount of haemoglobin per blood cell remained constant within two weeks of warm acclimation (fig. 5).

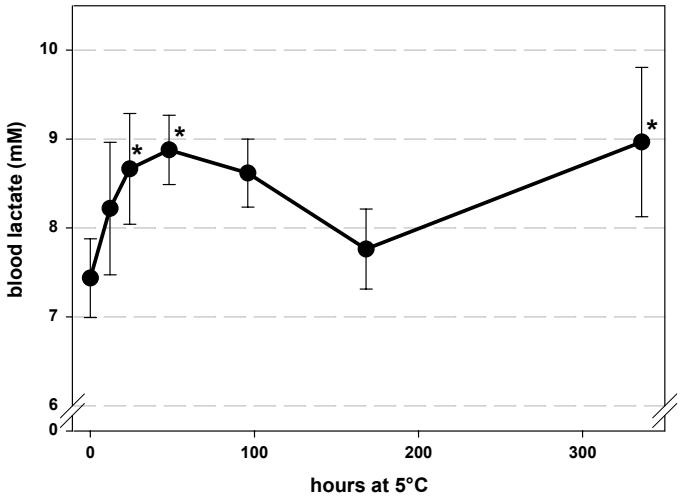

Figure 2
Depicts the blood lactate content of *N. coriiceps* upon two weeks of warm acclimation to 5°C. n = 4-8, p-level 5%, * indicates significant difference to unstressed controls (0 hours).

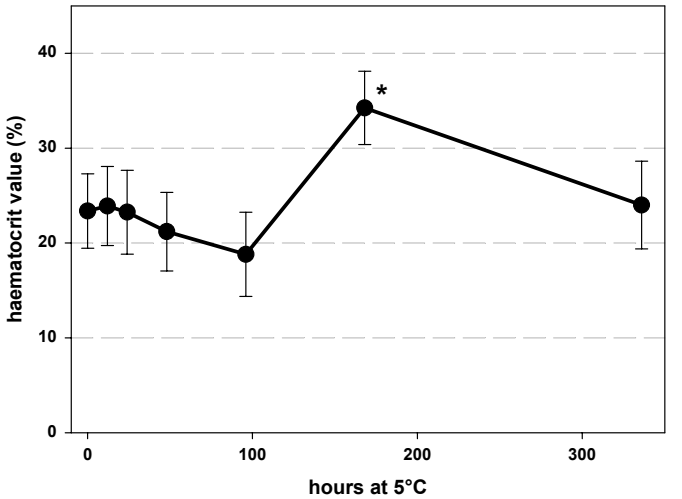

Figure 3
Depicts the haematocrit value of *N. coriiceps* upon two weeks of warm acclimation to 5°C. n = 4-8, p-level 5%, * indicates significant difference to unstressed controls (0 hours).

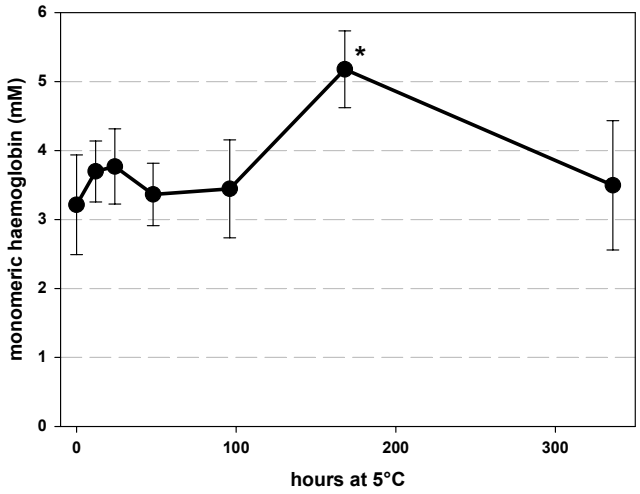

Figure 4
Depicts the content of monomeric haemoglobin of *N. coriiceps* upon two weeks of warm acclimation to 5°C. n = 4-8, p-level 5%, * indicates significant difference to unstressed controls (0 hours).

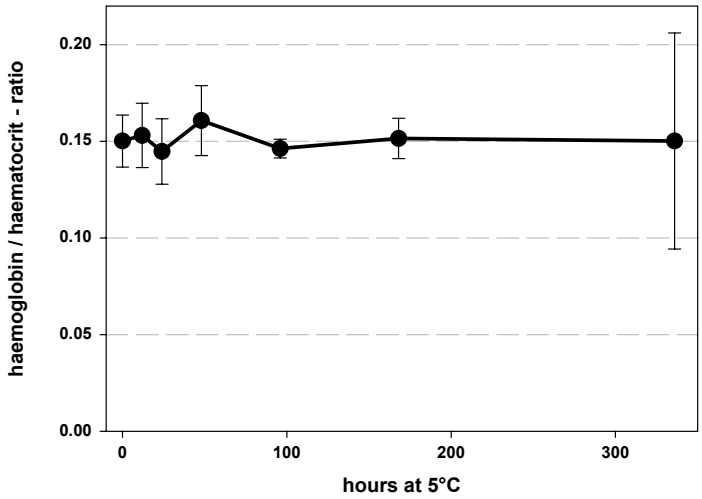

Figure 5
Depicts the ratio of haemoglobin / haematocrit, indicating the amount of respiratory pigment per red blood cell of *N. coriiceps* upon two weeks of warm acclimation to 5°C. n = 4-7, p-level 5%, * indicates significant difference to unstressed controls (0 hours).

Hypoxia exposure

Hypoxia exposure to critical PO_2 conditions (20 % air saturation) led to a significant increase in blood lactate within the first 12 hours. Subsequently, the blood lactate content started to decrease steadily, without reaching control level again within 48 hours (fig. 6; $p < 0.0001$ / 12 h, < 0.0001 / 24 h, 0.0059 / 48 h). The haematocrit increased significantly during the first 12 h of hypoxic exposure and remained elevated until the end of the experiment after 48 h hypoxia (fig. 7; $p \leq 0.0002$). The haemoglobin content followed the same pattern as the haematocrit, however the changes did not reach significance ($p \geq 0.0194$). The ratio of haemoglobin / haematocrit was lower in all hypoxia exposed groups

compared to control fish (fig. 9; p ≤0.0034) indicating lower amount of haemoglobin per red blood cell in hypoxia exposed than in unstressed fish.

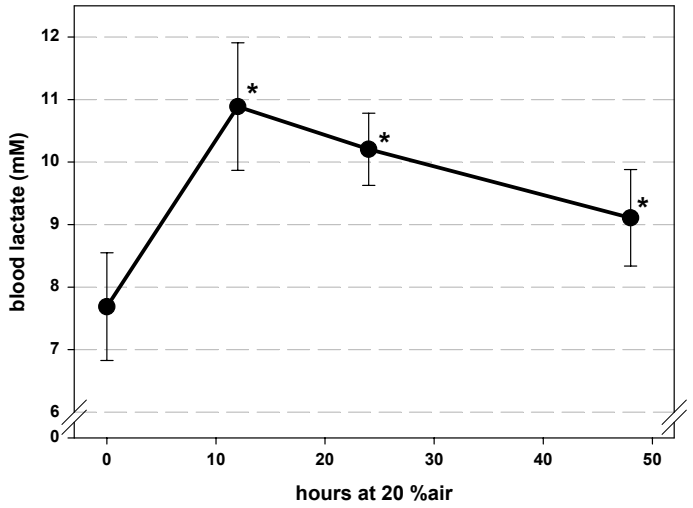

Figure 6
Depicts the blood lactate content of *N. coriiceps* upon 48 hours of hypoxia exposure to 20% air saturation (= critical PO_2 conditions). n = 3-8, p-level 5%, * indicates significant difference to unstressed controls (0 hours).

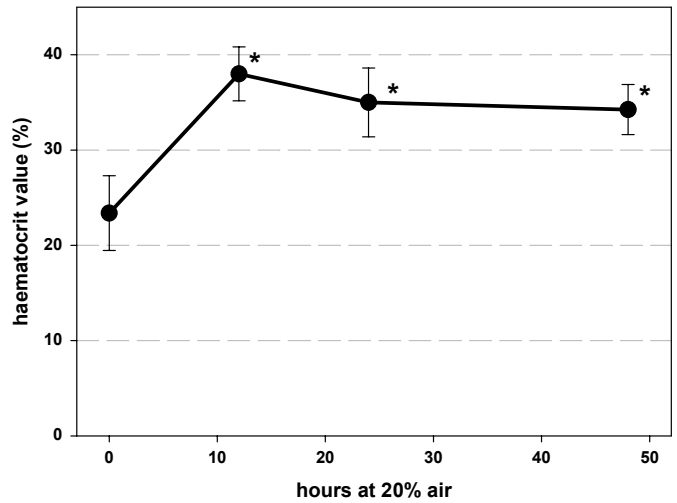

Figure 7
Depicts the haematocrit value of *N. coriiceps* upon 48 hours of hypoxia exposure to 20% air saturation (= critical PO_2 conditions). n = 3-8, p-level 5%, * indicates significant difference to unstressed controls (0 hours).

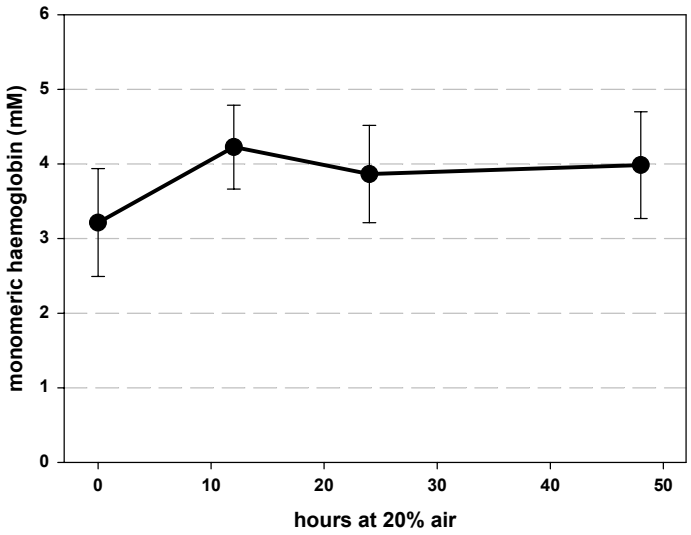

Figure 8
Depicts the content of monomeric haemoglobin of *N. coriiceps* upon 48 hours of hypoxia exposure to 20% air saturation (= critical PO_2 conditions). n = 3-8, p-level 5%, * indicates significant difference to unstressed controls (0 hours).

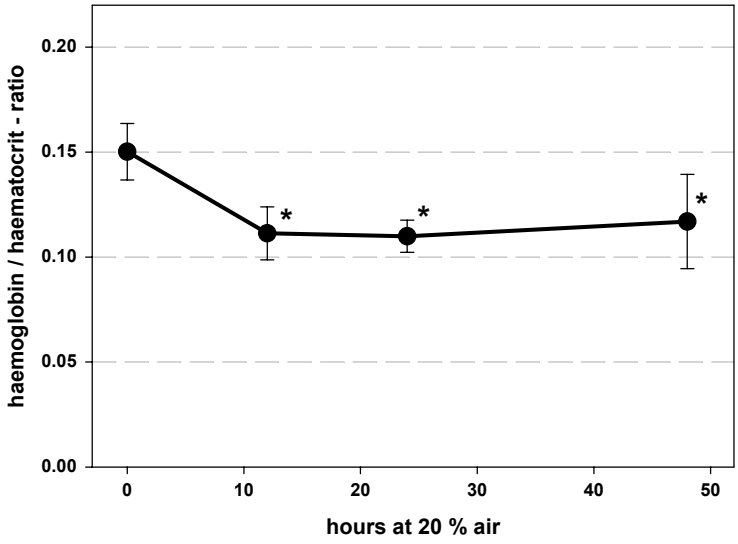

Figure 9
Depicts the ratio of haemoglobin / haematocrit, indicating the amount of respiratory pigment per red blood cell of *N. coriiceps* upon 48 hours of hypoxia exposure to 20% air saturation (= critical PO_2 conditions). n = 3-8, p-level 5%, * indicates significant difference to unstressed controls (0 hours).

Discussion

Not only hypoxia but also the initial phase of warm acclimation to temperatures outside the optimum temperature range is considered to cause oxygen shortage in central organs when the temperature-dependent increase in metabolic rate exceeds oxygen transport capacity (for review see Pörtner 2002). One of the characteristic responses of fishes to oxygen limitation is an increase in

haematocrit value (Jensen et al. 1993), which can result from erythrocyte swelling, release of stored red cells, changes in plasma volume, plasma skimming, and new red cell formation (Gallaugher & Farrell 1998, Nikinmaa 1990, Nikinmaa & Tervonen 2004). Additionally, anaerobic metabolism is induced by an upregulation of lactate dehydrogenase isoform expression (Gracey et al. 2001).

We were interested to see whether in spite of their special haematological adaptations and the absence of environmental hypoxia in their natural habitat, Antarctic red-blooded nototheniids have conserved the basic responses to oxygen limitation known of fish from lower latitudes. Whereas for many Antarctic fish species low haematocrit values between 10 and 18% have been reported (Mark et al. 2002), we found haematocrits of 23±4% in control fish, which is well within the range reported from non-polar fishes (18-38%, Petri et al. 2006, Ribeiro et al. 2005, Jain & Farrell 2003, Moraes et al. 2002, Madison & Wang 2006) and aligns with the comparatively high swimming activity of the investigated species. Wells et al. (1980) have also found higher haematocrit values and haemoglobin concentrations in more actively swimming Antarctic fish compared to slow moving species.

At first sight, 5°C appears as a quite high temperature for an Antarctic fish, especially when taking into account the reports by Weinstein & Somero (1998) that benthic notothenioids survive only few weeks at 4°C. However, more recently, Lowe et al. (2005) have shown an unexpected capacity to warm acclimate in the more active pelagic *Pagothenia borchgrevincki* and Lannig et al. (2005) maintained Antarctic eelpout *Pachycara brachycephalum* at 5°C for as long as nine months without apparent loss of condition factors. We were therefore not too surprised to see that a coastal Antarctic fish like *N. coriiceps* acclimates at 5°C. Especially, as in the Antarctic summer season 2005/2006 water temperatures as high as 2.5°C were reached in 10 m depth in Potter Cove (see also Schloss et al this volume).

The up-regulation of anaerobic lactate formation in the initial 48 hrs of experimental warming can be viewed as a first aid response to bridge energetic deficits (see Hochachka & Lutz 2001), until, following 7 days of warm acclimation, the haematocrit and also the haemoglobin content were elevated, such that higher oxygen transport capacity was reached in the blood. It remains to be shown whether this is due to release of pre-formed blood cells from the spleen or to new synthesis of red blood cells. In contrast to experimental warm acclimation, under hypoxia the blood lactate content increased on much shorter time scale. This indicates that oxygen shortage was more dramatic when exposing the fish to 20% air saturation than to acute warming stress. Also, the decline in blood lactate content after the initial increase during hypoxia occurred much faster than during warm acclimation, which can be explained by the simultaneous rapid increase of the haematocrit within the first 12 hours of hypoxia. Taken that the response in haematocrit was delayed by 7 days during acute warming stress compared to the fast response under hypoxia, the release of pre-formed blood cells or erythrocyte swelling appear more likely than erythrocyte new synthesis. This shows that exposure to 20% hypoxia represents a much more severe stress for these fish than acclimation to 5°C.

Taken together, the different time scale of change upon hypoxia exposure and warm acclimation in blood parameters may relate to different defence

mechanisms: A fast one to ensure survival during critical oxygen shortage in the environmental, but also on exhaustive exercise, and a slower response possibly requiring gene expression and new synthesis of red blood cells, occurring upon warm acclimation. However, as the hypoxia experiment lasted only 48 hours (because of limited amounts of gas bottles; see material & methods) it remains unknown whether this treatment would induce gene expression as well upon longer time scales of several days.
Our future goal is to investigate the molecular mechanisms behind the observed differences in the haematological responses to warm acclimation and to hypoxia exposure.

Acknowledgements
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Benthic community shifts: A possible linkage to climate change?

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Introduction

The Earth mean temperature has increased around 0.6º C in the last 50 years and there are critical areas such as the Antarctic Peninsula and the Arctic Ocean where the increase has reached up to 2.5 º C (Vaughan et al. 2003). As a consequence the majority of the glaciers within the Antarctic Peninsula region have shown a significant retreat (Cook et al. 2005). Ecological impacts arising from these processes are difficult to asses since several variables can be affected, showing unpredictable responses. However, based on our knowledge of community patterns and the natural history of key species it is possible to develop potential scenarios. In the case of coastal marine ecosystems the most important processes will probably be: (1) The increased input of terrigenous materials transported by increased glacier runoff. Sediments are not only affecting local primary production due to the reduced light penetration on the water column (Schloss et al. 2002), but also cause stress to shallow benthic ecosystems, especially filter-feeders, by diluting potential food items, clogging respiration and filtration apparatus and finally hindering the settlement of larvae. (2) A higher ice abrasion could be expected due to the increase of brush ice and growlers calved from retreating glaciers. This disturbance would have a greater impact at the start of the process, showing a decrease as the glacier retreats in land. (3) Another important factor would be water temperature increase; however, no clear signal of a steady water temperature increment has been recorded in Antarctic waters (Barnes et al. 2006). But on a smaller scale, a slight trend has been measured at Potter Cove (Schloss et al. this volume).

Changes in macrobenthic community structure in a temporal scale have long been used as important tools for assessing both short- and long term changes in marine ecosystems following both, anthropogenic and natural disturbances (Ruhl and Smith 2004; Blanchet et al. 2005; Grebmeier et al. 2006 and many others). It is clear that for accurate analysis and interpretation of forcing factors and ecosystem processes it is fundamental to observe and fully understand community patterns (Underwood et al. 2000). Although much work has been carried out on Antarctic benthic community patterns, both at shallow and shelf areas (see a comprehensive list in Clarke and Johnston 2003 and Gili et al. 2006), most of the work was devoted to spatial arrangements and studies dealing with temporal dynamics of benthic communities are almost entirely lacking with the exception of Dayton (1989). The present study aimed to

analize benthic community patterns at Potter Cove over a three year time period inferring causal processes in the frame of global warming scenario.

Materials and Methods

Sampling was carried out at three stations E_1, E_2 and E_3 in Potter Cove (Fig. 1). Each station was characterized by a different substrate type and sedimentation rate. Station E_1 is predominantly composed of silt and clay deposited by glacier discharge and is the most affected by sediment runoff. Station E_2 is rock dominated with cobble, pebbles and boulders and the least influenced by sediments. Finally station E_3 represents a transition between the previous stations. Photographic surveys were performed during the summer seasons 1994/95 and 1997/98 along transects using a Nikonos V camera, a 15-mm lens and a Nikonos SB-104 strobe, mounted on an aluminium frame (50 x 50cm). Particular depth profiles 15, 20, 25 and 30 m were sampled in 1994/95 and, on the basis of prospective divings, just 20 and 30 m were sampled in 1997/98 (for more details see Sahade *et al*. 1998). At each station photographs were taken following the respective isobath and 50 samples were obtained per transect.

Thereafter images were projected on a white screen and densities and percentage cover (superimposing a transparent film with 100 dots randomly distributed) of the main taxonomic groups were obtained. In order to compare spatial and temporal patterns multivariate analysis as classification (clustering) and ordination MDS (non-metric multidimensional analysis) were carried out using the Bray-Curtis similarity matrix on square root transformed data in order to reduce the effect of dominant species.

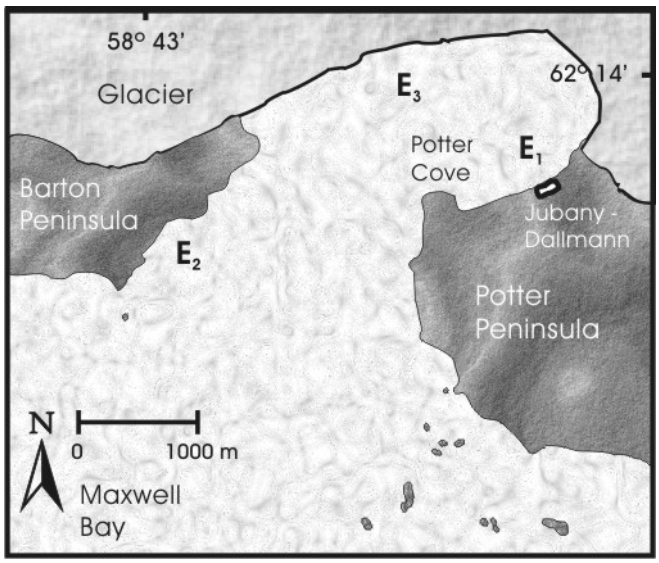

Fig. 1: Map of Potter Cove, sampling stations are highlighted.

Results and Discussion

The benthic communities of Potter Cove exhibited unexpected shifts in the three year period between samplings (94/95 to 97/98). The changes were especially marked at station E_1 where ascidians almost disappeared at 20 m and, together

with sponges showed a great retraction at 30 m depth (Table 1, Figs. 2 and 3). The ascidian *Molgula pedunculata*, dominant in 94/95 was the most affected species. While abundances of other ascidian species like *Ascidia challengeri* and *Corella eumyota* increased their percentage cover decreased, suggesting a decline in size. On the other hand, the bivalve *Laternula elliptica* and pennatulids dominated at 15 m depth in 94/95 (Sahade et al. 1998) and became dominant as well at 20 m in 97/98, extending their dominance to deeper waters probably due to the ascidian retraction.

Table 1: List of taxa and abbreviations used in figures.

Taxa		Abreviation
Phaeophyta	*Desmarestia* spp.	D. spp.
	Himantothallus	Hg
	Unidentified spp.	Phae.
Rhodophyta	Unidentified spp.	Rod.
Porifera	Unidentified spp.	Por.
Bryozoa	Unidentified spp.	Brio.
Ctenophora		Cte.
Cnidaria		
Actinaria		Act.
Hydrozoa		Hidro
Pennatulacea		Pen.
Nemertina	*Parborlasia corrugatus*	Pc
Polychaeta	Unidentified spp.	Pol.
Mollusca		
Gastropoda	*Neobuccinum eatoni*	Ne
	Nacella concinna	Nc
Nudibranchia	Unidentified spp.	Nud
Bivalvia	*Laternula elliptica*	Le
Crustacea		
Isopoda	*Glyptonotus antarcticus*	Iso.
Picnogonida	Unidentified spp.	Pic.
Echinodermata		
Asteroidea	*Diplasterias brucei*	Db
	Unidentified spp.	Ast.
Echinoidea	*Sterechinus neumayeri*	Sn
Ophiuroidea	*Ophionotus victoriae*	Ov
Crinoidea	Unidentified spp.	Crin.
Ascidiacea	*Molgula pedunculata*	Mp
	M.enodis	Me
	Aplidium radiatum	Ar
	Ascidia challengeri	Ac
	Corella eumyota	Ce
	Cnemidocarpa	Cv
	Pyura setosa	Ps
	P.obesa	Po
	Tilobranchion	Ts
	Sicozoa gaimardi	Sg
	Dicarpa insinuosa	Di
	Distaplia cilindrica	Dc
	Sinoycum adareanum	Sa
	Styela wandely	Sw

The shift was not so marked at station E_2, but again the most affected species was *M. pedunculata*. *L. elliptica* showed an important increase in density but not in percentage cover at both 20 and 30 m depths, possibly indicating a recent recruitment. Gastropods and the ophiurid *Ophinotus victoriae* also showed an important numerical increase. Macroalgae, especially *Desmarestia* spp. and *Himantothallus grandifolius* also raised their percentage cover. Station E_3, the

transition station, both because of the substrate type and the sediment load in the water column, showed an important increase in macroalgal cover while the

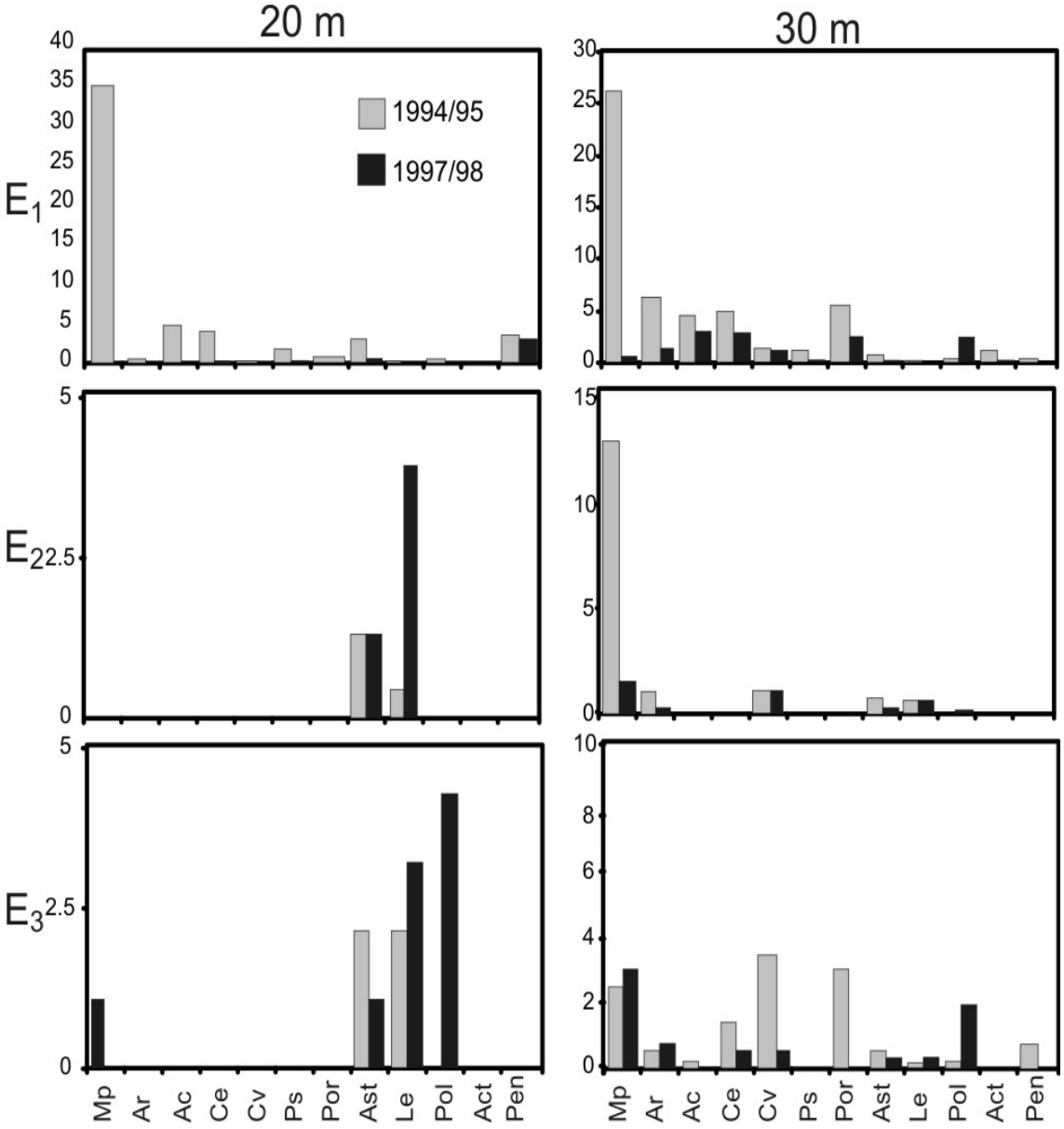

Fig. 2: Percentage cover of taxa that showed major differences at each depth and station during both sampling seasons. Note the different scale.

sponges almost disappeared and ascidians decreased in general. As in station E_2 abundances of *L. elliptica* and gastropods were higher in 97/98 while poliquetes numerically increased significantly at stations E_3 and E_1.

Multivariate analyses (Fig. 4) showed a clear separation of stations and also depths indicating that substrate type and depth are important factors shaping these communities. But samples were also grouped according to the sampling year especially those of station E_1, indicating a marked difference between 94/95-97/98. In stations E_2 and E_3 the samples were closer but were still showing the shift in community structure.

These results are remarkable for the short time period between the samplings and the previous assumption that Antarctic benthic communities are very stable

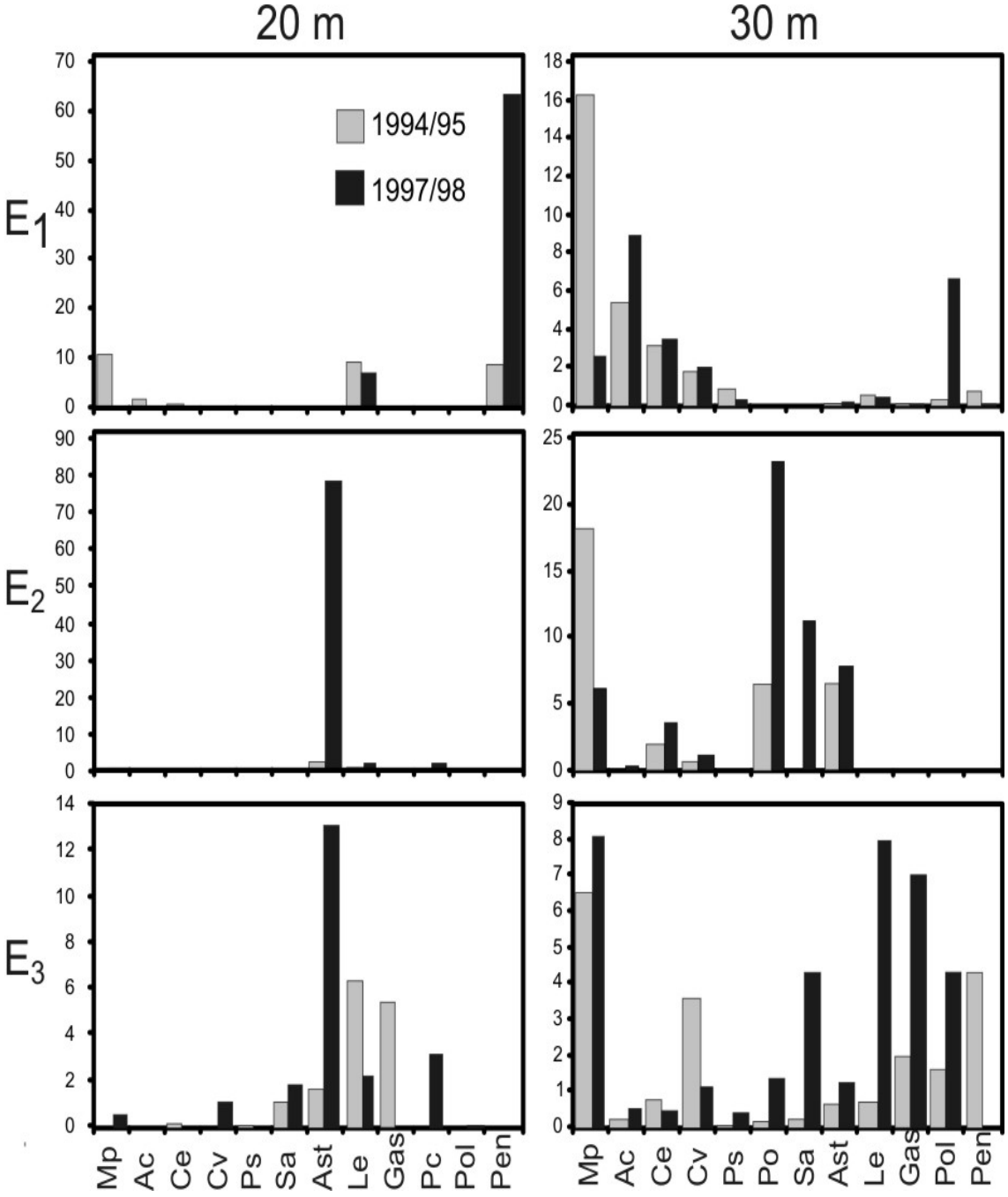

Fig. 3: Densities (Ind*m^{-2}) of taxa that showed major differences at each depth and station during both sampling seasons.

especially below the more ice affected depths. Shifts were reported in other Antarctic areas (Dayton 1989) and a possible linkage with large scale phenomena like the ENSO was suggested, however detailed studies in a temporal scale are still lacking.

To our knowledge this study provides the first possibility to analyse a community shift, especially at E_1, that can be analogized to a small scale "ecosystem displacement" like process, where the axis of displacement is depth rather the more typically used latitudinal gradient. In this model depth would be acting as buffer for the major causal factors.

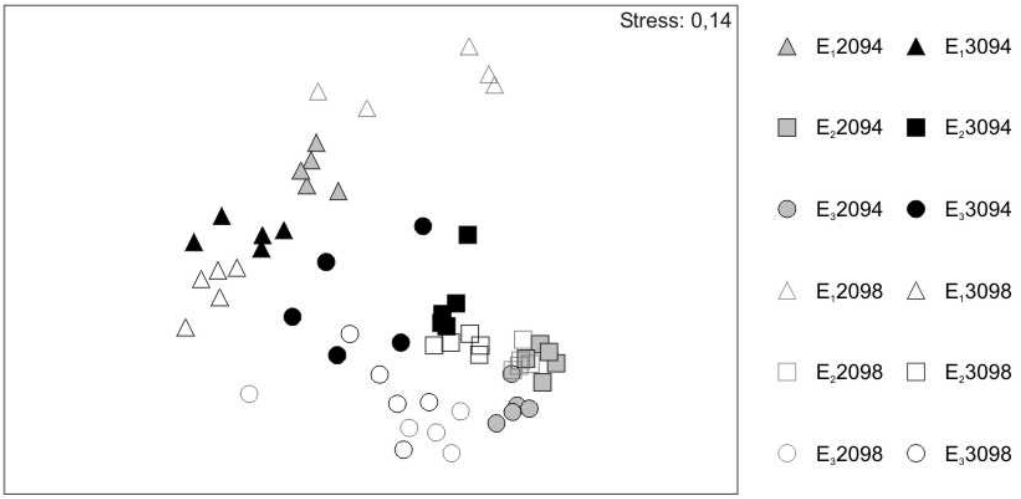

Fig 4: MDS analyses of the samples taken in each season 94/95 and 97/98. Bray-Curtis Similarity distance on square root transformed data was used

The observed changes could be related to natural local extinctions, recruitment events or interannual or interdecadal population variations. But it is also feasible to link the observed patterns to the increase of sediment load in the water column (Schloss et al. this volume) and the possible increase of ice impact. The density and coverage decreases of *M. pedunculata* and sponges together with the increase of the ascidians *A. challengeri* and *C. eumyota*, the bivalve *L. elliptica* and other groups that are non filter feeding like the gastropods and the ophiurid *O.victoriae* support the idea of a shift due to disturbance by increased sedimentation. Sponges and *M. pedunculata* would be less tolerant to this disturbance than the other ascidians species which are flat and are living at the bottom-water interface like *L.elliptica* (Torre and Sahade unpub. data) Therefore, it is possible to think that they are more capable of coping with higher amounts of sediment load in the water column than *M. pedunculata* and the sponges. On the other hand, the near disappearance of all the species except *L. elliptica* and pennatulids at 20 in E_1 suggests an increase in ice disturbances as well, since these are the organisms more resistant to such disturbance (Sahade et al. 1998). The discussed processes can be linked to the observed retreat of the Fourcade glacier, which in turn can be caused by the temperature increase of the Antarctic Peninsula. Thus, this study may be the first evidence of a direct effect of the global warming in an Antarctic benthic ecosystem.

Study of the processes and experimental assessments of the involved factors are complicated, however mathematical models provide powerful tools to analyse the ecosystem responses to different causal factors. A model run by Momo et al. (this volume) supports our hypothesis.
In the actual scenario of global change the main challenge is probably to identify the proxies that will affect ecosystems. In this context, these results suggest that in the near future the increased sediment discharge and iceberg and growlers occurrence from retreating glaciers would play a fundamental role in modulating shallow coastal ecosystem dynamics. This shows the urgent necessity of a temporal assessment of benthic community dynamics which is an important part of the ClicOpen program in the frame of the IPY 2007/08.

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5. EFFECTS OF ANTHROPOGENIC CHANGES ON ORGANISMS AND ON THE ECOSYSTEM

Small-scale studies on biodegradation of hydrocarbons in acutely contaminated Antarctic soils

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Introduction

During the last century, the use of petroleum hydrocarbons as energy source has increased dramatically. This fact has been accompanied by a concomitant increase in the number of pollution events in which different quantities of hydrocarbons have been spilled into the environment, either accidentally or deliberately. Antarctic regions are no exception. Near the scientific stations petroleum derivatives, mainly aliphatic hydrocarbons from fuel spills (diesel oil, gasoline, etc.) are the main contaminants found in soils. (Aislabie et al. 2001, Snape et al. 2005). However, polycyclic aromatic hydrocarbons (PAHs), although present in lower concentrations in Antarctic soils, are also a significant environmental contamination problem. PAHs are formed during combustion of fuels and other organic materials and are widespread pollutants in the environment possessing known toxic, mutagenic and carcinogenic properties (IARC, 1983). Pollution caused by PAHs have been reported all around the world (Muñiz et al. 2004; Tolosa et al., 2005 Qiao et al. 2006), including Antarctica (Cripps 1992, Mazzera et al 1999, Martins et al 2004).

At the present time, bioremediation has emerged as a cost-effective and environmentally friendly tool to eliminate *in situ* organic pollutants from the environment. Chronically contaminated environments (with a long history of hydrocarbon pollution) have a great number of bacteria adapted to metabolize the hydrocarbons. This fact was observed not only for fuel contaminated soils (Miethe et al. 1994, Margesin & Schinner 2001, Ruberto et al. 2003) but also for those contaminated with PAHs. (Cerniglia & Heitkamp 1989; Cerniglia 1992).

In these cases, if the level of inorganic nutrients (N and P) in soil is adequate and the C:N:P ratio is adjusted, bioaugmentation (inoculation of hydrocarbon-degrading microorganisms previously cultured in the laboratory) seems to be unnecessary and an adequate biodegradation rate can be reached applying biostimulation techniques (Margesin & Schinner 1997). On the contrary, when the spill occurs on a pristine soil (where hydrocarbon-degrading bacteria are absent or present in small numbers) it was reported that bioaugmentation improves the bioremediation processes shortening the time period required for hydrocarbon metabolization. This “shortening effect” could be crucial in Antarctic areas, where only during summer does the thawing of soils and temperatures above 0°C permit to operate bioremediation processes.

Soils from areas near fuel storage tanks in Antarctic stations are contaminated with diesel oil and other light fuels. In addition, low but significant levels of PAHs have been detected in some areas (see Vodopivez et al., this issue) These conditions make Antarctic stations potential candidates for developing low-temperature bioremediation processes for reducing aliphatic and aromatics hydrocarbons.
In this work we evaluate the effect of biostimulation and bioaugmentation on the removal of fuels and PAHs from an acutely contaminated Antarctic soil. For this purpose, experiments were carried out on soil microcosms, using a bacterial Antarctic consortium (M10) and a mixture of hydrocarbon-degrading strains (ADH, M10dp, DM1-41 and MP2-4).

Material and Methods

Study area

The experiment took place over 40 days (February-March 2002) at Jubany station (62 14'S, 58 40'W), King George Island (25 de Mayo Island), South Shetlands Islands. Soil used in this study was obtained from Marambio (64°14'S, 56°37'W), a station that is an important airport facility in Antarctica, with high fuel demand and vehicle transit. Due to this condition it is one of the stations more affected by hydrocarbon contamination. Soil samples were collected in January 2002 at 0.3 m depth from a site far from station buildings in an area without history of significant fuel spills. Approximately 45 kg of frozen soil were collected and stored at –20 ºC until its utilization at Jubany station.

Soil preparation and analysis

Large stones (1cm diameter) were manually removed from the soil. Water content was determined gravimetrically by heating sub-samples at 105 ºC for 24 h. Soil pH was measured by duplicate after mixing 10 g of soil with 100 ml deionised water. After shaking for 1 min, pH was measured with a Beckman I pHmeter. The remaining soil fraction after extraction of stones was analyzed for texture, carbon and nitrogen content.

Source of microorganisms and Inoculum preparation

A hydrocarbon-degrading bacterial consortium and a mixture of several hydrocarbon-degrading bacterial strains were used. Bacterial consortium M10 and *Pseudomonas* M10dp strain were isolated from enrichment cultures inoculated with soils from Marambio and using a mixture of PAHs as sole carbon source. *Stenotrophomonas* DM 1-41 and *Stenotrophomonas* MP 2-4 strains were also isolated from Marambio soil samples by culturing but using diesel oil and phenanthrene respectively as carbon sources. *Rhodococcus* ADH strain was isolated from Jubany station soil samples with crude oil as carbon source. From frozen (in 20% glycerol at -40ºC) stocks of consortium M10 and other strains, inocula were prepared in 250 ml Erlenmeyer flasks containing 50 ml of sterile saline basal medium (SBM) as described by Espeche et al (1994). Sterile diesel oil (2% v/v) was the carbon source for preparation of ADH and DM1-41 inocula, as well as for growth of the M10 consortium further inoculated in a system containing fuel-contaminated soil (system D). A mixture of sterile phenanthrene, anthracene, fluorene and dibenzotiophene (10:1:1:1) was used as carbon source (1 g of PAHs/l) for growth of M10dp and MP2-4 strains as well as for

preparation of M10 inoculum applied to a system containing PAHs-contaminated soil (system E).
Flasks were incubated at 15ºC and 250 rpm (New Brunswick rotary shaker) for 8 d. Cells were harvested by centrifugation and resuspended in 20 ml of phosphate buffer. Final cell density in suspensions were $2.5x10^8$ CFU/ml for M10 in diesel oil, $1.9x10^7$ CFU/ml for M10 in PAHs and $2.3x10^9$ CFU/ml for the mixture of strains.

Experimental Design
Uncontaminated soil was spread out in steel trays (40 cm length, 32 cm wide, 5 cm height) coated with an inert enamel. The trays were filled with 2.5 kg of soil resulting in a 5 cm-depth layer. Acute diesel oil contamination was simulated by adding 69 ml (19000 ppm) of sterile diesel oil to B, D, F and H systems. PAHs acute contamination was simulated by adding 1385 ml (3600 ppm) of a PAHs stock solution (phenanthrene 5 g/l, anthracene 0.5 g/l, fluorene 0.5 g/l and dibenzothiophene 0.5 g/l in hexane) to C, E, G and I systems.
Systems B, D, F and H were biostimulated by adding NH_4Cl (250g/l) and Na_2HPO_4 (33 g/l) to reach a C:N:P ratio of 100:10:1. Table 1 summarizes the conditions present in each performed system. An undisturbed control (system A) and two different abiotic controls (systems B and C) were included.

Table 1. Characteristics of the nine microcosms systems performed at Jubany Station. Pristine soil was exposed to an acute contamination with PAHs or diesel oil.

System	$HgCl_2$ (1 g/kg)	PAHs (ppm)	Diesel oil	biostimulation (C:N:P=100:10:1)	bioaugmentation
A	-	-	-	-	-
B	+	-	19000	-	-
C	+	3600	-	-	-
D	-	-	19000	+	M10
E	-	3600	-	+	M10
F	-	-	19000	+	Mixture of strains
G	-	3600	-	+	Mixture of strains
H	-	-	19000	+	-
I	-	3600	-	+	-

Hydrocarbon analysis
Total hydrocarbon concentration (TH) of Gas Oil contaminated systems was measured following the EPA 418.1 method. Briefly, 1 g of soil was accurately weighed and placed into 20 ml glass-flasks. HPLC grade CCl_4 (10 ml) and a spatula tip of anhydrous Na_2SO_4 were added to each flask. Flasks were placed into an ultrasound bath and treated overnight. Samples were then filtered, transferred to a quartz-cell and analyzed (Buck Model HC 404 Hydrocarbon analyzer IR spectrometer. Results were compared by ANOVA and Tukey`s multiple comparison test. GC analysis of PAHs was carried out with a Shimadzu GC-9A gas chromatograph using a FID detector and Helium as carrier gas (31 cm/s). Typically, oven temperature was held at 100°C for 1 min and then raised (10°C/min) to 250°C and held at that temperature for 5 min. The injector temperature was 280°C. Separation was accomplished with a fused-silica capillary column (crosslinked 5% PH ME siloxane) with a film thickness of 0.25 µm, 30 m length and 0.25-mm i.d. (Hewlett Packard). Data were collected using a PC-Chrom® software.

Microbiological analysis

Soil samples (1 g) were suspended in 10 ml of sterile saline solution (0.9% NaCl) and vigorously shaken for 5 min. Serial dilutions of samples were plated (four replicates) either on casein-peptone-starch (CPS) agar, as was suggested by Wynn-Williams (1992) for evaluation of Antarctic heterotrophic aerobic bacteria (HAB) from soils, or on solidified saline basal medium (SBM) supplemented with 2% diesel for counting of hydrocarbon degrading bacteria (HDB). For both HAB and HDB counts, plates were incubated 30 d at 15°C and results were expressed as colony forming units per gram of dry weight (CFU/g). PAHs-degrading bacteria were estimated using the most probable number method (Wrenn & Venosa 1996) in 96-wells plates incubated at 15ºC for 30 d. Serial dilution of samples (20 µl) were placed in wells containing 180 µl of Bushnell & Haas (1941) medium and a mix of phenanthrene, anthracene, fluorene and dibenzothiophene (10:1:1:1) as carbon source (1 g/l). Bacterial growth was detected by the change in colour (colorless to yellow-brown) of the culture. Bacterial counts data from the different microcosms obtained at 0, 10, 20, 30, 40 d were analysed by repeated measured ANOVA and Tukey`s multiple comparison test.

Results and Discussion

HAB and HDB are shown in Fig 1. System A, where autochthonous bacterial microbiota was neither under biostimulation nor bioaugmentation, showed an increase of one order of magnitude in HAB from day 0 to day 40. This increase, that is not related to the addition of nutrients could be due to the more favourable environmental conditions existing in soil after mixing of soil particles (causing the aeration of soil) as well as to the maintaining of an adequate water content throughout the assay. This effect was only evident during the first 20 days (Fig. 1 A and B). Under both, diesel oil or PAHs acute contamination only near 20% of the initial autochthonous HAB counts were detected at day 10^{th} (from $2.8x10^5$ to $6.1x10^4$ UFC/g under diesel oil and from $4.8x10^5$ to $7.5x10^4$ UFC/g under PAHs contamination). This showed that the natural microbiota of this pristine soil were sensitive to the presence of high levels of hydrocarbons and that under this acute stress only a fraction of the bacterial cells survive the sudden addition of pollutants. Levels of contaminants added to soil were high, and an acute toxic effect could be causing the loss of viability observed in the natural bacterial assemblage when contaminated with diesel oil and PAHs In a recent paper (Ruberto et al 2006) we did not observe similar decreases in HAB counts when 1744 ppm of phenanthrene were added to a soil from a depth of 75 cm near the former garbage-burning site of Jubany station. The low but significant presence of PAHs detected in this Jubany soil sample (1.2 ppm) (which could have act as a selecting factor of PAHs-tolerant bacteria) as well as the higher amount of PAHs used for simulation of the acute contamination in the present study could be the cause of the different results observed. Levels of total petroleum hydrocarbons similar to the diesel oil concentration added to soil in the present work has been reported previously for some highly polluted sites in Antarctica (Aislabie et al. 2004). However, such high level of diesel fuels also could have caused the loss of viability observed in system H. An acute toxic effect of the aliphatic hydrocarbons (Sikkema et al. 1995) that inhibited cellular activity of the natural bacterial community could be the cause of this observa-

tion. Many of the most toxic components of the diesel oil are the most volatile ones, which comprise an important fraction of this fuel, and that were not detected in the soil even one day after addition (day 0, Fig. 2A).
It is important to note that all the systems into which the M10 consortium or the mixture of strains were inoculated also showed a sharp decrease in HAB at day 10^{th}, reaching at this time values similar to system A (control system). At the end of the experiment, HAB counts in the system where diesel oil contaminated soil was bioaugmented with M10 (system D), showed significantly higher ($p<0.01$) values than all the rest of the systems (Fig. 1A). This result is in accordance with the high HDB counts observed for system D (Fig. 1C). However, also the system which was inoculated with the mixture of strains showed high HDB counts evaluated in diesel oil agar plates at d 40 (Fig. 1C). This showed that, whereas HDB at d 40 in system inoculated with M10 represented less than 5% of the total HAB, in system where the mix of strains was added HDB represented 18% of the HAB.

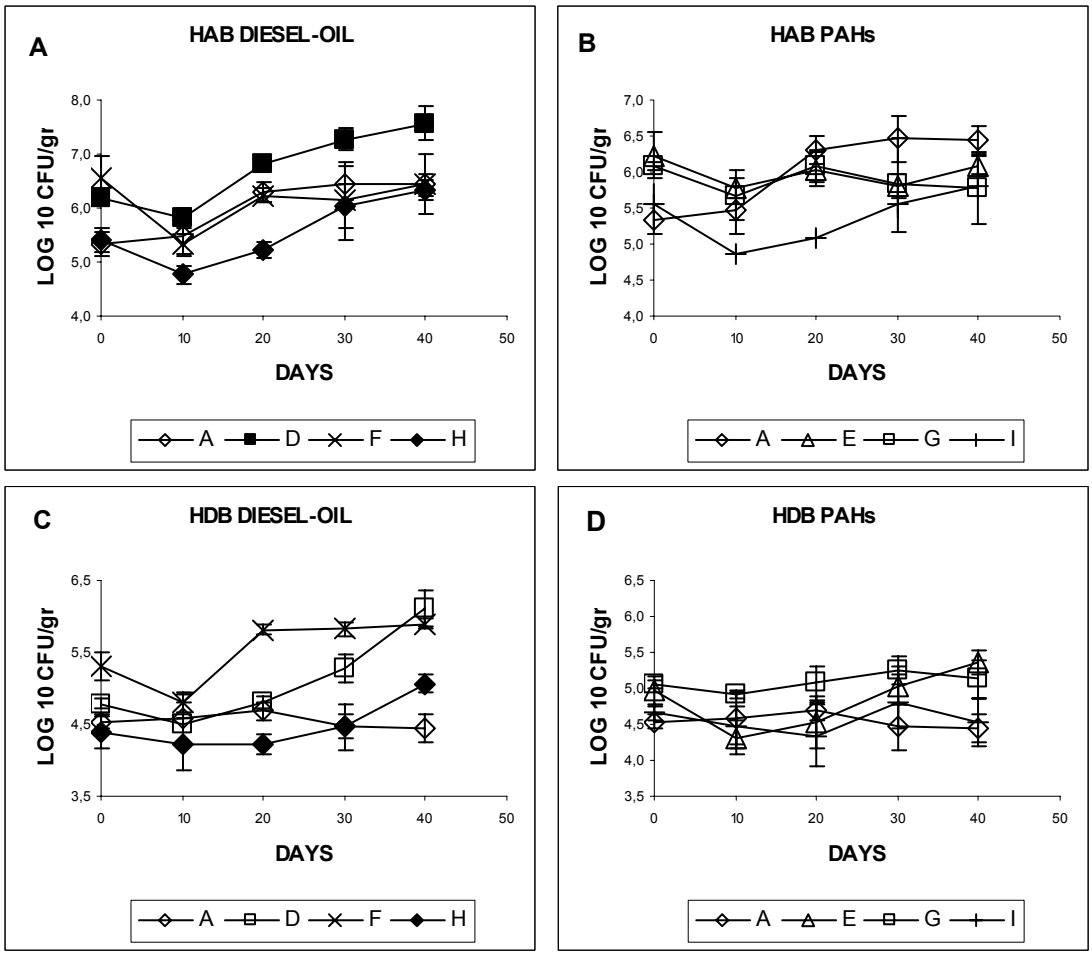

Figure 1. Heterotrophic aerobic bacteria (HAB) and hydrocarbon degrading bacteria (HDB) from the different microcosms. **A.** HAB from systems contaminated with diesel oil. **B**. HAB from systems contaminated with PAHs. **C.** HDB from systems contaminated with diesel oil. **D**. HDB from systems contaminated with PAHs. See table 1 for characteristics of the systems. Bars indicate SD from four replicates.

Changes in the counts of the natural microbiota when contaminated with diesel oil, although differing from the M10 inoculated system by one order of magnitude, showed a similar shape and at day 40th presented a HDB/HAB percentage ratio of 5%. However, despite the differences in HAB and HDB counts values, all systems contaminated with diesel oil (D, F and H) showed similar loss of total hydrocarbons (Fig. 2A) and no significant differences were detected between them. Significant differences in hydrocarbon concentration ($p<0.001$) were observed between all these systems and the abiotic control (system B).

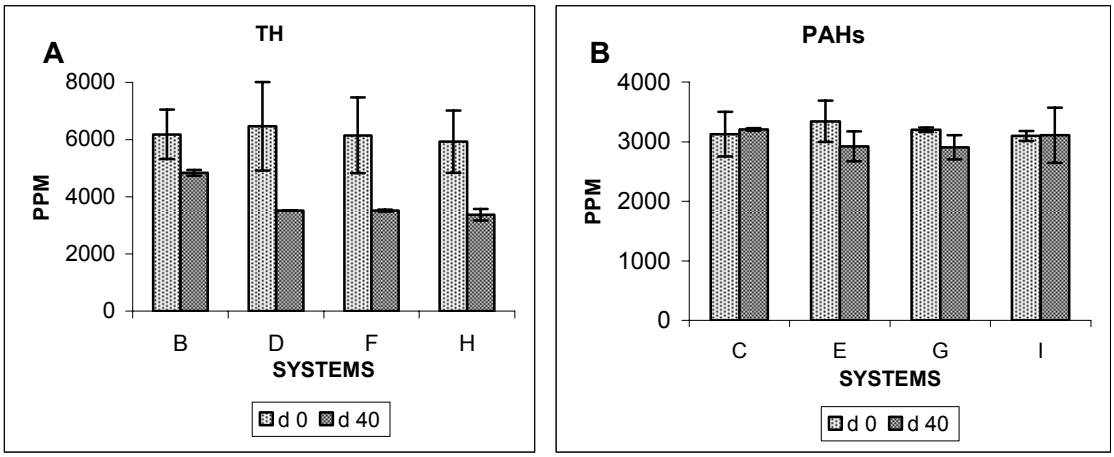

Figure 2. A. Total hydrocarbons concentration at 0 (initial) and 40 (final) days from systems contaminated with diesel oil. B. Total hydrocarbons concentration from microcosms contaminated with PAHs. Bars indicate SD from triplicates.

These results showed that under the experimental conditions, bioaugmentation with two different biological systems caused no improvements in diesel oil removal compared with the activity of the autochthonous microbiota. This contaminant-degrading activity of the natural microbiota from non-polluted sites has been reported by us and other authors for different soils (Mac Cormack et al. 1998, Solano-Serena et al. 2000). However, this study did not confirm previous results obtained using soils from Jubany station, where a positive effect of bioaugmentation was found for the removal of diesel oil from non polluted soils (Ruberto et al 2003). All this comments confirm the complexity of the biological interactions emerging when an external inoculum is added to a natural environment, bringing about different results depending on a great number of factors, as the inoculum composition, the soil origin and physicochemical characteristics, climate conditions during the processes and many others.

The addition of high amounts of PAHs to the soil did not result in any evident degradation activity either in biostimulated or in bioaugmented systems. Despite the initial decrease in HAB counts mentioned above in system I (PAHs contaminated autochthonous bacterial flora), counts in this system increased from day 10th to day 40th, reaching similar values to those showed by the bioaugmented systems (E and G). Also the control system (system A) showed similar HAB counts. HDB on PAHs showed a significantly ($p<0.05$) higher value for the M10 bioaugmented system (Fig. 1D) compared with the non-bioaugmented ones (I and A). The system bioaugmented with the mixture of strains (system G) also showed a higher (but non significant) number of HDB compared with systems I and A. However, despite these differences in final HDB values, no difference was observed in PAHs concentration at the end of the experiment (Fig.

2B). In addition, comparison with the abiotic control (system C) showed that no loss of PAHs took place during the experiment. In this case, the greater stability of PAHs compared with the components of diesel oil could have been the cause of the absence of detectable degrading activity. It is possible that long-term experiments (several months) would be necessary to reveal the capacity of the inoculated systems to degrade fuel components, mainly when acutely contaminated pristine soils are being studied. However, other factors could be playing a relevant role in the absence of PAHs removal. One of these factors is the nutrient addition. It is known that the source and concentration of nutrients have a crucial role in the success of bioremediation (Walworth and Reynolds 1995, Coulon and Delille 2003). Breeveld and Sparrevik (2000) have reported that addition of inorganic nitrogen and phosphorous enhanced degradation of 4-rings PAHs in creosote-contaminated soils from Norway but no improvement was observed for 3-rings PAHs (as phenanthrene). Based on these observations it is possible that a combination of the chemical structure of PAHs and the type and concentration of the N and P could have limited the biodegradation under the conditions of the experiment. Another factor to consider is the nature of the soil under study. Marambio soil has a high fraction of small particles (silt and clay) and adsorption on such particles could significantly reduce the bioavailability of PAHs used in this study, extending the time required for detect significant levels of degradation as was reported by Krauss and Wilcke (2002). This fact also could explain why we found a significant phenanthrene biodegradation in another Antarctic soil bioaugmented with the M10 (Ruberto et al. 2006). In that study soil was obtained from Jubany station and had low levels of small particles and a high content of sand, which could have reduced the adsorption processes allowing M10 to carry out a significant degradation of the hydrocarbons activity in a time period (day 56^{th}) similar to those used in the present study.

Conclusions

In conclusion, this work has shown that the previously uncontaminated Antarctic soil studied here has a significant diesel-degrading activity. This property renders unnecessary the bioaugmentation procedure, even with Antarctic bacterial strains or consortia having a high hydrocarbon-degrading activity under laboratory conditions. In addition, the length of the assay probably combined with unfavourable characteristics of soil and a non-optimum source of nutrients avoided any biological removal of PAHs from the studied soil.

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Distribution pattern of polycyclic aromatic hydrocarbons in soil, surface marine sediments and suspended particulate matter in the seawater column near Jubany Station (Antarctica)

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are among the most common organic pollutants in aquatic sediments and soils. Although PAHs are naturally occurring compounds in fossil fuels and are produced during combustion of wood and other organic materials, anthropogenic activities (as fuel spills, petroleum refinery activities, coal combustion and vehicles powered by gasoline or diesel fuels) are the main sources of PAHs contamination reported in many areas of the world (Naes et al. 1995; Gocht et al. 2001; Qiao et al. 1999). At the present time Antarctica is considered one of the few pristine regions in the planet. However, logistic and scientific activities at the stations, as well as the tourist activity have caused local but significant pollution events including PAHs contamination (Mazzera et al. 1999; Martins et al. 2004).

Because of the chemical structure of PAHs they have a tendency to accumulate in the food chain (UNEP 2002), and considerable damage could be caused on the biota by the long-term release in the environment of low quantities of these compounds. In addition, factors such as low temperatures and low nutrient availability observed in many Antarctic soils and sediments significantly reduce the activity of PAH-degrading microorganisms and could contribute to the accumulation of significant quantities of PAHs. In recent years, phenomena associated to the global warming like melting of glaciers, downward migration of the upper surface of the permafrost and increasing precipitation are causing an increase in the movement of particles from coastal areas to the marine sediments. As PAHs tend to adsorb on soil particles due to their hydrophobicity, they could be transported in association with these particles causing an increase in PAHs concentration in coastal marine sediments. At the present time no studies have been published reporting PAHs concentration in suspended particulate matter (SPM) for Antarctic marine waters except for the paper of Cripps (1994) who analyzed PAHs associated with particulate matter in the Bransfield Strait. In this work, values reported ranged between not detected and 427 ng/g dw. Although Cripps suggested a possible bioaccumulation process in krill from the phytoplankton biomass, the distribution patterns of the individual PAHs found in these two biological systems were different showing that further studies will be necessary to prove this assumption.

Jubany scientific station is located near the Fourcade glacier (which is showing increased ablation), on the coast of Potter Cove, 25 de Mayo Island (King

George Island) and has had a considerable and permanent human influence since 1952. For this reason, PAHs produced by human activities in Jubany could be accumulating either in soils or in the surface sediment of the adjacent Potter Cove. The aim of this work was to analyse the levels of 16 priority PAHs (USEPA 1985) and several derivatives present in the area during the years 2004 and 2005. Sampling was carried out on sediment and SPM (at two different depths) from nine sites at Potter Cove. In addition, PAHs were analysed in surface soil and different levels in the active layer and the permafrost to evaluate a possible effect of soil and groundwater contamination to marine environment.

Materials and Methods

Sampling procedure

During the first sampling period (January 2004), soil from 9 sites in the surface active layer was collected Samples were taken at three different depths (surface, 25 and 75 cm). Surface marine sediments were obtained from 3 sites of the inner part of Potter Cove (Fig. 1). Results obtained during this first year lead us to design a new sampling scheme for the next year (February 2005). The same nine sites were sampled at different depths (surface, 75, 100, 150 and 200 cm). Two of these sites (D and I) were also sampled at active layer/permafrost interface and at 20, 30, 50 and 60 cm depth below the interface. Surface sediment was collected in nine different sites of Potter Cove including those already sampled one year previously At the same sites SPM samples were obtained from surface and two meters above the bottom waters. This zone, which was not sampled in January 2004, was included to investigate the contribution of the SPM to the quantity and quality of PAHs found in surface sediments. Soil samples were taken manually using a stainless steel corer (8-cm diameter). Marine sediments were obtained with a stainless steel grab. SPM samples were obtained by filtering (GF/F fibreglass filters, 14 cm diameter) 100 l of seawater (collected with Niskin bottles). All samples were placed in acid-cleaned amber-glass flasks, freeze-dried and stored at –20 °C. Soil, permafrost and sediment samples were sieved (1 mm mesh) and placed in glass vials (20 ml). Extraction and quantification techniques were conducted at ISMER Laboratories (Canada).

Analysis of PAHs

Twenty five different PAHs were analysed: 1: Naphtalene, 2: 2-methylnaphthalene, 3: Acenaphthylene, 4: Acenaphthene, 5: 2,3,5-trimethylnaphthalene, 6: Fluorene, 7: Phenanthrene, 8: Anthracene, 9: 2-methylanthracene, 10: Fluoranthene, 11: Pyrene, 12: 9,10-dimethylanthracene, 13: Benzo(c)phenanthrene, 14: Benzo(a)anthracene, 15: Chrysene, 16: Benzofluoranthene, 17: 7,12-dimethylbenzo(a)anthracene, 18: Benzo(a)pyrene 19: 3-methylchloranthrene, 20: Indeno(1,2,3-cd)pyrene, 21: Dibenzo(a,h)anthracene , 22: Dibenzo(g,h,i)perylene, 23: Dibenzo(a,l)pyrene, 24: Dibenzo(a,i)pyrene, 25 :Dibenzo(a,i)pyrene.

Either 1 g of dry soil, permafrost or marine surface sediment or the filter containing the SPM sample were placed into 50 ml Teflon tubes filled with 10 ml of dichloromethane (DCM) and sonicated (ultrasound bath Branson 5210) for 30 min. Samples were then shaken overnight (Burrell 75 shaker) and finally

sonicated again for 30 min. Samples were centrifuged and the supernatants were transferred to conical glass tubes for evaporation under a stream of nitrogen to a final volume of 0.5 ml. Two ml of hexane were added and the solvent mix evaporated to a volume of 1.0 ml. Extracts were purified using Solid Phase Extraction column Supelclean Envi-18 (Supelco). Elution was made with 5 ml of hexane:DCM (9:1) and the volume adjusted to 8 ml. A new evaporation step was performed under nitrogen stream to a final volume of 100 µl. An internal standard consisting in 40 µl of a solution of deuterated naphthalene, anthracene and perylene (0.5 µg/g) was added and the mix diluted to 200 µl with hexane:DCM (9:1). Quantification was made using gas chromatography-mass spectrometry (Thermo Finigan GC-MS Trace DSQ AS 2000). Accuracy of the method was tested using standard reference materials consisting of PAHs in marine sediment (SRM 1941b).

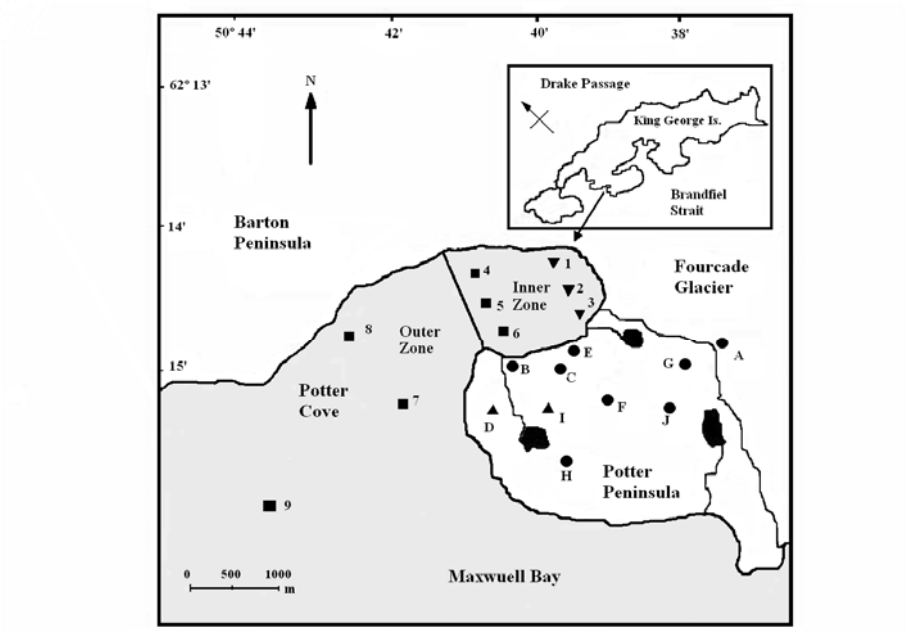

Figure 1. Sampling sites at the sorroundings of Jubany station ●: soil 2004/05. ▲: soil 2004/05 and permafrost 2005. ▼: sediment 2004/05 and SPM 2005. ■: sediment and SPM 2005.

Statistical Analyses

Comparisons of PAHs concentration between pairs of data were made using T-student test. When more than two data sets were compared one way ANOVA and Tukey's multiple comparison test was used.

Results

Surface sediments sampled in January 2004 showed the presence of low levels of PAHs, with values ranging from 28 to 312 ng/g (see Table 1). Pattern of compounds showed the presence of PAHs with low and medium molecular weight, phenanthrene being the dominant one (66-85%, Fig. 2A). Sediment samples obtained in February 2005 presented values ranged between 36 and 1908 ng/g (Table 1) and evidenced a significant increase in PAHs level from sites 1 and 2, in the inner basin of the Cove. These points are located near the

north coast of the inner Potter Cove (Fig. 1) and previous works have reported this area as possessing the higher proportion of finest sediment particles (Mercuri et al 1998, Veit-Köhler 1998).

Table 1. Total PAHs concentration (ng/g dw) in sediment samples obtained in January 2004 and February 2005. Values represent mean ± standard deviation of triplicates. ns: not sampled.

	Sampling sites								
year	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
2004	312±24	276±25	28±	ns	ns	ns	ns	ns	ns
2005	1762±139	1908±114	36±	251±17	63±	112±12	42±	85±	90±

Table 2. Total PAHs concentration (ng/g dw) in suspended particulated matter (SPM) obtained in February 2005. Values represent mean ± standard deviation of triplicates.

	Sampling sites								
Depth	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Surf.	116±2	49±12	58±13	405	129	374±4	107	133	95
Botto	67±13	533±1	128±2	500±4	169±2	526±6	90±14	143±2	118±2

PAHs were detected on SPM at both analyzed depths (surface and y 2 m from the bottom) with values ranging between 49 and 526 ng/g dw. Level of PAHs was not clearly related to any source of contamination, no gradients were detected and phenanthrene and anthracene were also the dominant compounds (78-96%).

The change in PAHs levels observed in sediments between 2004 and 2005 was coincident with an inverse change in PAHs concentration in coastal soils. In soil samples obtained in January 2004, a concentration gradient (with values increasing from surface to depth) was observed (Table 3). At 75 cm depth, concentration values ranged between 162 and 1182 ng/g dw. One year later (February 2005, Table 4), PAHs concentrations in surface soils were similar to those observed one year earlier, with the exception of sampling site C, where concentration of PAHs (552 ng/g dw) was considerably higher than one year ago. However, this increase was associated with a diesel spillage which occurred a few months earlier in the fuel storage tanks located close to site C.

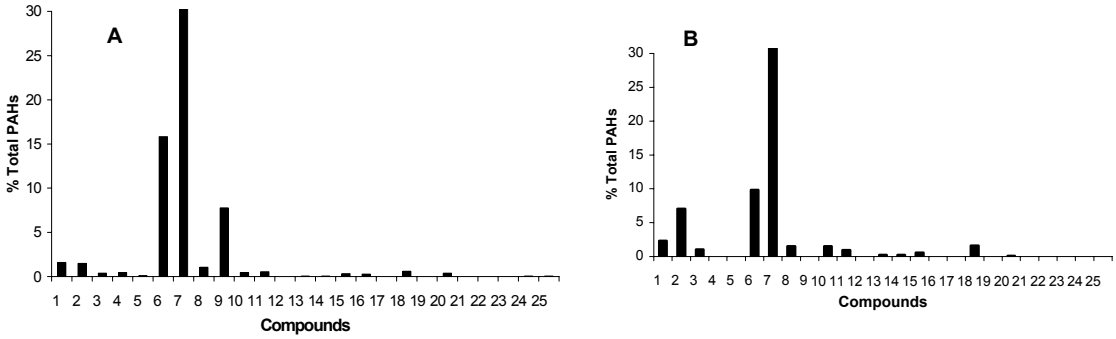

Figure 2. Representative patterns of total PAHs in surface marine sediments (A) and 75 cm depth soil samples (B). Numbers in X-axis represent the individual compounds as were numbered in Material and Methods section.

Table 3. Total PAH concentrations (ng/g dw.) found in soil samples collected during the austral summer 2004 in Jubany station.. Values represent mean ± standard deviation of triplicates. Site H: control value.

Depth	Sampling site								
	B	C	D	E	F	G	H	I	J
0-5	21±2	23±3	19±2	42±3	32±3	19±3	13±2	35±3	24±2
25-30	15±1	32±3	13±2	11±1	104±14	20±3	14±2	20±3	10±1
70-75	1016±111	264±19	1182±113	857±73	1052±111	681±43	187±16	191±23	162±15

Table 4. Total PAH concentrations (ng/g dw.) found in soil samples collected during the austral summer 2005. Values represent mean ± standard deviation of triplicates. Site A: control value.

Depth (cm)	Sampling site								
	A	B	C	D	E	F	G	H	I
0-5	16 ± 1	19 ± 2	552 ± 36	12 ± 1	22 ± 3	19 ± 3	14 ± 2	16 ± 3	22 ± 3
70-75	-	43 ± 3	153 ± 22	21 ± 1	31 ± 6	32 ± 3	12 ± 1	17 ± 3	31 ± 3
100-105	-	66 ± 4	22 ± 5	18 ± 3	48 ± 6	44 ± 3	26 ± 3	18 ± 3	32 ± 3
150-155	-	80 ± 6	85 ± 6	31 ± 3	126 ± 7	64 ± 4	30 ± 4	19 ± 2	60 ± 5
200-205	-	99 ± 9	76 ± 6	47 ± 4	156 ± 9	90 ± 5	34 ± 2	24 ± 2	73 ± 7
210-215	-	-	-	69 ± 4	-	-	-	-	114 ± 10
220-225	-	-	-	71 ± 7	-	-	-	-	100 ± 8
230-235	-	-	-	62 ± 4	-	-	-	-	59 ± 4
240-245	-	-	-	33 ± 3	-	-	-	-	39 ± 3
250-255	-	-	-	22 ± 2	-	-	-	-	31 ± 3

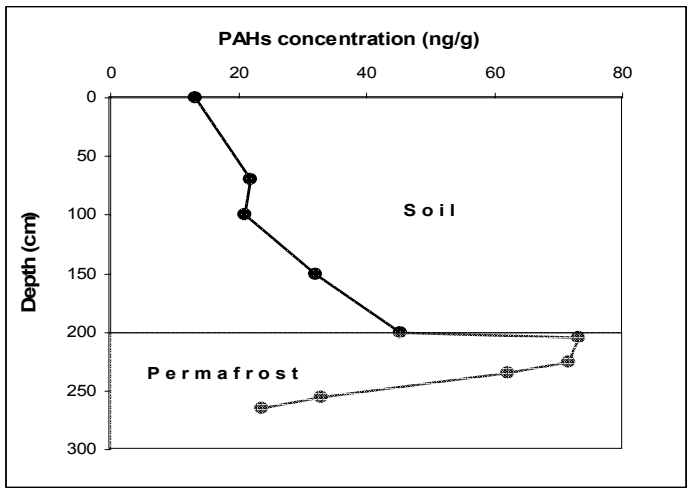

Figure 3. Total PAHs concentrations in the active layer and permafrost from site D sampled in February 2005.

As had been observed in 2004, total PAHs concentrations increased progressively with depth into the active layer, showing the highest values at 200-205 cm depth layer, corresponding to the layer just above permafrost. However, values of total PAHs found at 75 cm depth during February 2005 were significantly lower ($p<0.01$) than those registered during the first sampling year (Table 4). Finally, pattern of compounds found in soil samples taken at depth proved to be very similar to those found at the surface level (Fig. 2). In sites where the permafrost layer was analysed (sites D and I, Table 4), a decrease in total PAH concentration was observed from the active layer/permafrost interface to the 60-65 cm-depth level into the permafrost. Figure 3 shows the level of PAHs in the active layer and permafrost for site D. Again, phenanthrene was predominant in permafrost at both sampling sites.

Discussion

This study suggests that surface sediments from Potter Cove are affected by activity at Jubany Station. However, PAHs concentrations detected in this small bay (28-1908 ng/g dw) are comparable to those reported for other Antarctic Stations (Cripps 1992; Kennicutt 1992a; Bicego et al.1998; Martins 2004), except for the high levels found by Kennicut (1992b) in sediments close to Palmer Station.

One point to highlight is the sharp increase in total PAH concentration in surface sediments measured in 2004 and 2005 at sites 1 and 2. This could have been caused by episodic washout processes of soils toward the nearby marine ecosystem. This hypothesis is supported by the changes observed in PAHs concentration in soil samples from a depth of 75 cm where a decrease of one order of magnitude between 2004 and 2005 was detected.

Why PAHs accumulation was not similar for all the sediment samples sites could be related to a combined effect of the bottom topography, predominant winds and water circulation. As was indicated in Fig. 1, Potter Cove is divided in outer and inner basins by a transverse sill. The inner basin is enriched in silt and clay, reaching in some areas values as high as 13% and 80%, respectively (Veit-Köhler 1998). Most of the water draining from the Potter Peninsula flows into the inner basin (Varela 1998) and surface sediments may be acting as a trap strongly retaining hydrophobic contaminants and preventing their fast transport to the outer basin of the cove by spring flood currents. A similar mechanism was postulated by Mazzera et al. (1999) to explain the accumulation of PAHs in sediments from McMurdo Sound. PAHs concentration observed in all sampling sites located in the outer cove (sites7-9) were significantly lower ($p<0.001$) than those from sites 1 and 2. The lower PAH levels found in site 3 seem to be related to the particular topography and hydrology of the cove (Roese and Drabble 1998). Water circulation in Potter Cove is strongly influenced by winds which cause a cyclonic current in surface. This current influences the mixing of freshwater entering to the south coast of the cove and carries an important volume of fine particles. The presence of the transverse sill forces the circulating freshwater masses to form a clockwise vortex with a small proportion of the sediment-bearing surface layer passing over the sill and flowing outside the cove. The deepest zone along the northeast coast acts as a sediment trap, as was reported by Mercuri et al. (1998).

Therefore, the combined effect of topography, winds and water circulation could be the cause of the lower PAHs content in site 3 compared with sites 1 and 2.
In the water column, levels of PAHs associated with the SPM showed no detectable gradient. The pattern of distribution of the compounds was similar to that observed in surface sediments and deep soils, with phenanthrene as the dominant compound. Highest values were found in SPM from sites near the operation area of the boats. Although is known that the outboard motor frequently spill a significant fraction of fuel, the proportion of low molecular weight PAHs derivatives which characterize this kind of pollution source was not significantly greater in water samples from this area. It could means that either pollution from outboard motors is not relevant or, most probably, that the lighter compounds from this source of pollution are quickly volatilized, degraded or transformed.
As was mentioned above, increases in PAHs concentration in surface sediments were accompanied by corresponding significant decrease in PAHs levels in coastal soils. This fact suggests that the bulk of the PAHs found in soil in January 2004 was lost during the 12-month period between the two field seasons This could be a consequence of changes in the hydrological regime of the soil and an exhaustive drainage of the water located above the permafrost layer. This process would have occurred during the summer period when above zero air temperatures induce the melting of glaciers and snow and when heavy rain occurs. Precipitation data from 2003 (one year before the first sampling) were considerably lower (431 mm) than the average of the last ten years (615 mm) whereas during the January 2004 - February 2005 interval, precipitations largely exceeded this average value (831 mm). These data support the idea that the washout phenomenon could have been particularly intense during the studied period. Further investigations will be necessary to confirm the existence of a cyclic inter-annual oscillation in soil-PAH concentrations and the relationship between precipitation regime and the permanence of these compounds in the soils studied.
Another notable feature of our results is the concentration gradient of PAHs observed in soils. The dominance of low and medium molecular weight PAHs in sub-surface samples compared to surface layer (where a large range of PAHs is present including 5-6 ring compounds) suggest a selective downward migration that could be attributed to a number of factors such as particles sieving and water solubility. In high latitudes, where soil is under a continuous process of freezing and thawing, the finest soil particles are selectively transported to deeper layers (Anderson et al. 1978). This process, which would favour downward migration of PAHs showing the highest affinity for the finest soil particles, could be one of the processes responsible of the enrichment of some PAHs in the lower part of the active layer. The reason why only intermediate molecular weight PAHs accumulated in the deeper active layer could be related to a balance between their physicochemical properties and some particular hydrogeological conditions. A water solubility of about 1-2 mg/l, a relatively low vapour pressure and a log K_{OW} around 4.2-4.5 seem to be the best combination of physical properties to favour a downward migration.
Another relevant finding of this work was the pattern of distribution of the PAHs found within the permafrost. The highest values observed just below the interface between the active layer and the permafrost and the progressive decrease detected in samples taken into the permafrost show that the

permafrost acts as a low permeability barrier for migration of PAHs in the area of Jubany station. As far as we know no other work conducted within Antarctic soils has reported a similar behaviour of PAHs distribution into permafrost.

Conclusions

This work has shown that Jubany Station and Potter Cove still have low levels of PAH contamination in soil and sediment. The pattern of PAHs was indicative of local sources of pollution from low temperature combustion of organic materials. A large inter-annual change in PAH concentrations in soils is attributed to a rapid drainage of porous soils by rain waters and melting snow and ice during summer, and was correlated to an increase of PAHs in surface sediment. The active layer/permafrost transition zone contained the highest level of PAHs and permafrost acted as a low permeability barrier to downward migration of these compounds. This behaviour highlights the risk for coastal marine environments near Antarctic stations exhibiting PAH contamination in their soils. As global warming clearly affects the Antarctic Peninsula at the present days, melting of permafrost and enhance in leaching would result in a massive flow of PAHs with unpredictable ecological consequences.

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Evidence of pollution with hydrocarbons and heavy metals in the surroundings of Jubany Station

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Introduction

In the last century the natural equilibrium of the environment has been seriously affected by man in many places of the planet. However, there exists a global consensus that the fate of Antarctica should be different and that its natural resources, scientific values and beauty must be preserved.
Antarctica is one of the largest and most pristine wilderness areas left on earth. Their relatively pristine environment has been mostly associated with the isolation from large industrial centres and the minimum human activity occurring within the area covered by the Antarctic Treaty. Although most inner areas of the Antarctic continent remain unexplored and have a minimum human presence, the coastal zone has been thoroughly navigated, with permanent human settlements since the beginning of the XX. century. Due to their closeness to South America, its less severe climate conditions and its accessibility in summer, the Antarctic Peninsula was the easiest area for the establishment of several research stations and for the commercial exploitation of the marine resources. The remnants of such human activities remain in a few places as abandoned stations, field dumps of fuel, rubbish dumps, etc.
Since the Protocol on Environmental Protection to the Antarctic Treaty was signed (Madrid, October 4, 1991), the international community showed an increasing awareness about the value of environmental monitoring in preservation of Antarctica. Human settlements (both abandoned and active stations) have been indicated as the main focus of contamination. Several studies reported the occurrence of a contamination halo around scientific station, mainly composed by hydrocarbons and trace elements (Aislabie et al. 1999; Claridge et al. 1995).

Five decades of intense scientific and logistic activity developed in the surroundings of Potter Cove could have caused a significant impact on this coastal ecosystem. Previous investigations have reported increased levels of total hydrocarbons and heavy metals in soils and sediments from the vicinity of

Jubany Station. (Mac Cormack & Fraile 1997; Vodopivez et al. 2001). The present study reports the preliminary results of the analysis of selected pollutants (hydrocarbons and heavy metals) in surface soils collected around Jubany Station.

Materials and Methods

Soil collection

Jubany Station (62º14´S, 58º40´W) is located on Potter Peninsula, 25 de Mayo Island (King George Island), South Shetlands Islands. Soils at Potter Peninsula are typical of Antarctic soils found on tills on relatively young glacial retreat surfaces. (Godagnone 1997).

Soils samples were obtained during the Summer Antarctic expedition 2002/ 2003 in the vicinity of the Jubany Scientific Station (Figure 1). Surface soil samples (0 – 5 cm depth) were collected manually using a stainless steel spoon. All samples were stored in acid-cleaned amber-glass flasks until freeze-drying and sieving (1 mm mesh) at Jubany station. Samples were placed in glass vials (20 ml) and stored at –20°C. Samples were divided into two batches, one for polycyclic aromatic hydrocarbons (PAHs) determination and the other for heavy metal measurement. PAHs extraction and quantification were conducted at the Ecotoxicology Laboratory of ISMER (Quebec University, Rimouski, Canada). Samples for heavy metal analysis were processed at the Catedra de Toxicologia y Quimica Legal, Facultad de Farmacia y Bioquimica, Universidad de Buenos Aires, Argentina, and quantification of the mineralized samples was conducted at the Comisión de Energía Atómica, Unidad de Actividad Química, Centro Atómico Constituyentes, Buenos Aires, Argentina.

Polycyclic Aromatic Hydrocarbons Determination

Twenty five different PAHs were analysed (naphtalene, 2-methylnaphthalene, acenaphthylene, acenaphthene, 2,3,5-trimethylnaphthalene, fluorene, phenanthrene, anthracene, 2-methylanthracene, fluoranthene, pyrene, 9,10-dimethylanthracene, benzo(c)phenanthrene, benzo(a)anthracene, chrysene, benzofluoranthene, 7,12-dimethylbenzo(a)anthracene, benzo(a)pyrene 3-methylchloranthrene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene, dibenzo (g,h,i)perylene, dibenzo(a,l)pyrene, dibenzo(a,i)pyrene, dibenzo(a,i)pyrene.

Soil samples (1g) were extracted with dichloromethane (DCM) using an ultrasound bath. Solvent was separated from the soil particles by centrifugation and the supernatants were transferred to graduate glass tubes for evaporation. Tubes were placed into an ice bath and evaporation was carried out under a stream of nitrogen to a final volume of 0.5 ml. Two ml of hexane were added and the solvent mix was evaporated to a final volume of 1 ml. Finally, samples were cleaned-up using solid phase extraction column Supelclean Envi-18 (Supelco). Elution was made with 5 ml of hexane:DCM (9:1) and the volume was adjusted to 8 ml. A further evaporation step was performed under a stream of nitrogen to a final volume of about 100 µl. 40 µl of a solution containing deuterated naphthalene, anthracene and perylene (0,5 µg g^{-1}) as internal standard was then added and the mix was diluted to 200 µl with hexane:DCM (9:1). The extracts were placed into 12 x 32 mm amber-glass vials (Chromatographic

Specialties Inc.). PAHs quantification was made using a GC-MS Trace DSQ AS 2000 (Thermo Finnigan) using a DB-5MS 0.32 mm x 0.25 µm x 30 m column (J&W Scientific), with helium as carrier gas.

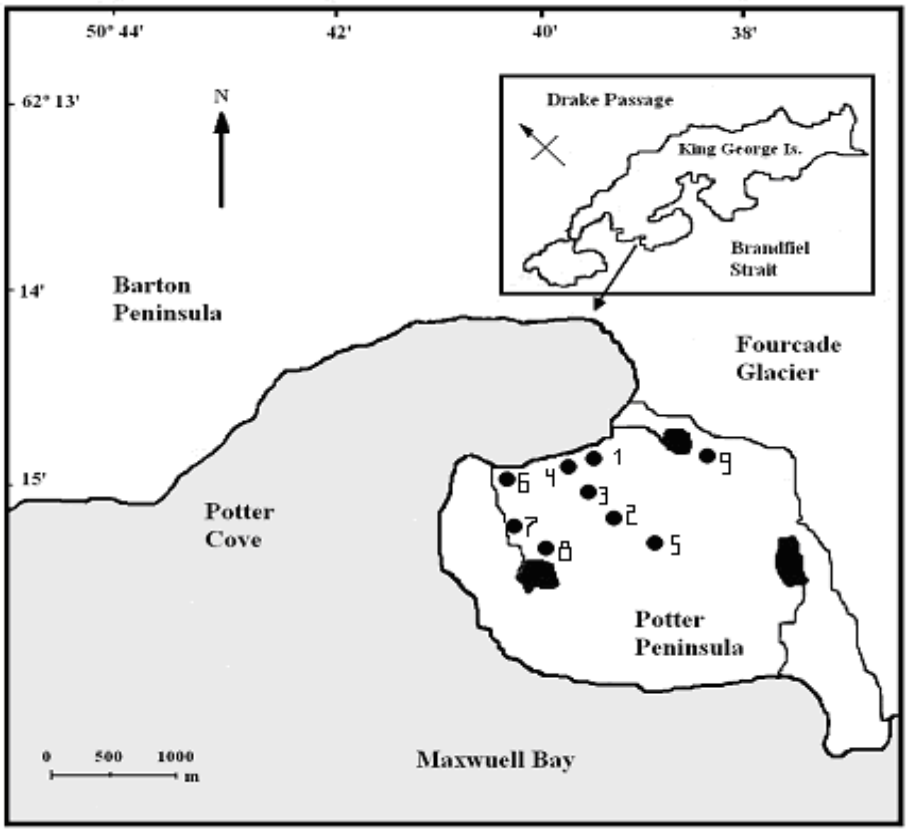

Figure 1. Soil sampling sites at Potter Peninsula. 1: main building. 2: incinerator area. 3: fuel storage tanks. 4: boat house. 5: old dumping area. 6: Mouth of Matias creek. 7: Middle part Matias creek basin. 8: High part Matias creek basin. 9: Skua lagoon (control site).

Heavy Metals Determination

The elements analyzed were Cd, Cr, Cu, Pb and Zn. Soil samples (0,5 g) were placed into Teflon vessels and 1 ml of H_2O_2 and 7 ml of HNO_3 (J T Baker Analyzed Reagent for trace metal analysis) were added. After overnight incubation samples were subjected to microwave digestion. An MLS-2000 (Milestone-FKW, Sorisole, Bergamo, Italy) MW apparatus equipped with ten Teflon PFA vessels was used to digest the samples. The residue was allowed to cool, transferred into a 50-ml volumetric flask and the solution diluted with deionised distilled water. A Perkin-Elmer (Norwalk, CT) ICP Optima 3100 XL (axial view) simultaneous inductively coupled Ar plasma optical emission spectrometer was used for trace elements determination.

In order to assess the accuracy of the methods, portions of a certified reference material, were subjected to the same dissolution procedure and included in the overall analytical process. Table 1 shows the obtained results. Certified refer-

ence material (CRM) for PAHs (Marine Sediment SRM 1941b) was supplied by National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA and CRM for heavy metals (MURST-ISS-A1, Antarctic Sediment) was provided by Instituto Superiore di Sanita, Rome, Italy.

Results and Discussion

Table 2 shows the average value and standard deviation (from triplicates) found in nine surface soil samples collected during the austral summer 2002/03.

PAH levels ranged from 18 to 45 ng g^{-1} of dried soil, and although all sites showed very low levels, higher values were detected in sites where activities are potentially PAHs producers (boats house, incinerator site). Curiously, samples collected in site 3 (oil-storage tanks), where diesel oil spills have occurred, did not show any increase of PAH level compared with the control site (Skua Lagoon).

Table 1. Results of the CRM analysis to assessment of the analytical accuracy.

PAH (ng g^{-1})	Certified	Found
Naphthalene	818± 71	848 ± 95
Fluorene	81± 16	85 ± 15
Phenanthrene	377± 33	406 ± 44
Anthracene	167± 25	184 ± 18
Fluoranthene	622±29	651 ± 50
Pyrene	574±34	581 ± 39
Benzo[a]anthracene	338±46	335 ± 25
Chrysene	306±36	291 ± 31
Benzo[b]fluoranthene	426±35	453 ± 21
Benzo[k]fluoranthene	210±20	225 ± 18
Benzo[a]pyrene	353±21	358 ± 17
Benzo[ghi]perylene	302±19	307 ± 45
Indeno[1,2,3-cd]pyrene	349±23	341 ± 57
Dibenz[a,h]anthracene	41±3	53 ± 10
Heavy Metal (μg g^{-1})	Certified	Found
Cd	0.538± 0.027	0.50 ± 0.04
Cr	42.1± 4.4	40.5 ± 3.5
Cu	6± 2	5.4 ± 0.3
Pb	21.0±2.9	19.9 ± 3.0
Zn	53.3±2.7	52.1 ± 3.1

PHA: CRM Marine Sediment SRM 1941b, Heavy metals: CRM MURST-ISS-A1, Antarctic Sediment

These results contrast with the high levels of total petroleum hydrocarbons previously reported for the same area. (Mac Cormack & Fraile 1997) and suggest that the hydrocarbon contamination in this area is represented almost exclusively by non-polyaromatic hydrocarbons.
When deposited on soil, PAHs undertake different fates including volatilisation, photooxidation, bioaccumulation, leaching and bacterial degradation. Although volatilisation is considered the major mechanism for removal of fuel oil from Antarctic soil and should not be underestimated, high rates of microbial degradation of hydrocarbons has been demonstrated in oil-contaminated soils from Potter Peninsula. (Mac Cormack & Fraile 1997; Ruberto et al. 2003).
In addition, a significant increase in precipitations has been recorded in recent years in the study area. This would favour the transfer of these compounds from the soil surface to the marine basin of Potter Cove. In addition, is known that due to their high hydrophobicity (IARC 1983), PAHs have a strong tendency to adsorb to the smallest soil particles (such as clay and silt) because of their larger surface of adsorption and their higher content in organic matter (Kan et al. 1994). In areas like Potter Peninsula, where soil is under a continuous process of freezing and thawing, the finest soil particles are selectively transported to deeper layers, the upper layers being enriched in larger-sized particles (Anderson et al. 1978). This process would favour downward migration of PAHs and could be other of the factors responsible of the low PAHs levels at the soil surface.

Table 2: Average values and standard deviation from triplicates found in surface soil samples collected during the austral summer 2002/03. $\sum$ PAHs: (ng g^{-1}), Heavy metals: (μg g^{-1})

	Sampling sites								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
$\sum$ PAHs	25±3	45±3	24±3	34±3	19±2	19±2	18±3	25±2	27±2
Cd	0.7±0.04	1.1±0.04	0.8±0.04	0.6±0.04	0.7±0.04	0.8±0.04	0.6±0.04	0.6±0.04	0.5±0.04
Cr	7.9±0.5	45.3±1.5	4.2±0.5	19.9±1.1	10.5±0.5	4.3±0.5	6.1±0.5	6.1±0.5	4.3±0.5
Cu	83±5	59±5	61±5	88±5	79±6	96±7	72±5	78±8	54±5
Pb	15.6±0.8	112±8	6.9±0.8	66±9	21±0.8	4.6±0.8	8.5±0.8	8.1±0.8	3.9±0.8
Zn	63±5	53±5	60±5	66±5	75±5	61±5	60±5	63±5	60±5

1: main building. 2: incinerator area . 3: fuel storage tanks. 4: boat house. 5: old dumping area. 6: Mouth of Matias creek. 7: Middle part Matias creek basin. 8: High part Matias creek basin. 9: Skua lagoon (control site).

PAHs average values detected in the vicinity of Jubany Station were lower than those found for other Antarctic Stations where oil spills have been reported. In fact, Table 3 illustrates the total PAHs concentration detected in soil samples from Signy Island, Palmer Station, Old Palmer Station and McMurdo Station. These results suggest that in a general view, soils around Jubany Station are not significantly contaminated by PAHs. Moreover, a constant pattern of PAHs components among the different sampling sites was not observed (data not shown). In general, phenanthrene was predominant, ranging between 8 and 27% of the total PAHs concentration, several other PAHs, were present in rele-

vant levels: naphtalene (1-16%), fluoranthene (4-16%), acenaphtylene (<1-9%) and benzo(a)pyrene (4-11%). By contrast, several authors detected higher level of naphthalene and methylnaphthalene in oil-contaminated soil from Scott Base and Palmer Station. (Aislabie et al. 1999; Kennicutt et al. 1992a).

Based on the available information and the results of the present investigation, the origin of the low levels of PAHs detected in soil might be associated with local sources of low-temperature combustion, such as those occurring during the use of diesel vehicles, electric generators and burning of organic matter. On the contrary, oil spills contribution to PAHs contamination would be negligible.

Heavy metals levels varied in a wide range. Neither Cu nor Zn showed significant differences among the 9 sampled sites. Cd concentration showed a similar trend that Cu and Zn, varying in a narrow range (0.5 0.8 $\mu g\ g^{-1}$), the exception was found in site 2 (incinerator area), where a moderate increase (1.1 $\mu g\ g^{-1}$) was found. Cr and Pb concentration varied in a wide range, but a similar trend was observed for both elements. In fact, Cr and Pb concentration showed only small differences in sites 3, 6, 7, 8 and 9 (Cr: 4.2 – 6.1 $\mu g\ g^{-1}$, Pb: 3.9 – 8.5 $\mu g\ g^{-1}$). A moderate increase for Cr (7.9 $\mu g\ g^{-1}$) and Pb: (15.6 $\mu g\ g^{-1}$) was detected in site 1 (main building), while significant concentration increases were found at sites 2 (incinerator area), 4 (boat house) and 5 (old dumping area). The Cr concentration at site 2 (45.3 $\mu g\ g^{-1}$) was one order of magnitude higher than that observed at the control site (site 9: 4.3 $\mu g\ g^{-1}$). Likewise, Pb concentration showed even a higher enrichment rate when compared respected these two sites, being Pb concentration at site 2 (112 $\mu g\ g^{-1}$) almost 30 times higher than at site 9 (3.9 $\mu g\ g^{-1}$). Increases detected for both these elements were in the following order: site 2 > site 4 > site 5.

On the other hand, if the high concentrations of Cr and Pb found in sites 2, 4 and 5 are excluded; the average values for surface soils are very similar to the metal levels reported for local rocks. In fact, the concentration found for the five elements examined is in good agreement with the geochemistry of rocks from Potter Peninsula (see Kraus & del Valle, this issue)

Our studies and the geochemical available information suggest that the heavy metals detected in soil are mainly of autochthonous origin, except Cr and Pb at sites 2, 4 and 5. Cr and Pb enrichment would be related with anthropogenic activities more than natural conditions. Several activities were proposed as potential sources of Pb in Antarctic stations (Hong et al. 1999) including diesel

Table 3: ∑PAHs (ng g^{-1}) reported for Antarctic soils.

Place	Surface soil	Source
Signy Island	71000	Cripps 1992
Palmer Station	841 -85659	Kennicutt et al. 1992b
Old Palmer Station	9273 - 345765	Kennicutt et al. 1992a
McMurdo Station	5.3 - 88452	Mazzera et al. 1999

oil combustion, use of leaded gasoline, waste incineration and pigments used in paint formulation. On the other hand, pigments containing compounds as $PbCrO_4$ have been used as colorants in paints applied to Antarctic buildings in the past. The increases by pairs of Cr and Pb would suggest that these ele-

ments could have a common origin, a source rich in both elements and able to cause the accumulation of both metals at the soil surface. In this case, the most likely source of these metals and one that should be further investigated is the residues of paint eroded from the buildings of the station.

Conclusions

Although small oil spills have been reported in Jubany Station in recent years (mainly near the oil storage tanks), no evidence of contamination from this source was found during our investigation of PAHs and heavy metals concentration. The halo of pollution by PAHs observed at the surrounding of the station could be caused by local sources of low temperature combustion such as those occurring during the use of diesel vehicles, electric generators and burning of organic matter. In fact, burning activities were frequent in the area before the adoption of the Protocol of Madrid, in 1991.

On the contrary, evidence of heavy metals (Cr and Pb) enrichment in the surface soil was observed, and this could be attributed to contamination resulting from the operation of Jubany Station, perhaps associated with the abrasion and flaking of paints and other coverings.

The adsorption of PAHs to the finest soil particles and their transport from surface to depth, as well as the significant increase in the precipitation combined with the normal summer runoff could favour the overall transfer of hydrocarbons and other contaminants from the soil surface to the marine basin of Potter Cove.

These results indicate that future monitoring and environmental impact assessments programs should recognise that the PAHs present at the soil surface may not be an adequate index of the real pollution degree of this Antarctic area. On the other hand, monitoring the soil surface level could be critical for detecting particulate material rich in heavy metals. Based on these assumptions, an environmental monitoring programme should take into account that different levels of soil (surface and subsurface) must be examined in order to obtain a complete assessment of chemical pollution in the vicinity of Jubany station and other similar Antarctic areas.

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Impact of the commercial fishery and shore-based sampling programs on inshore fish of the South Shetland Islands area

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General Introduction

The monitoring of coastal fish populations can be used to detect changes in inshore marine ecosystems. The necessity of implementing these studies in the Antarctica has been pointed out in the compass of international programs such as BIOMASS (Biological Investigations On Marine Antarctic Systems and Stocks) and CS-EASIZ (Coastal Systems-Ecology of the Antarctic Sea Ice Zone Program) (Anon. 1994). Trawling can not be used due to the lack of areas of seafloor suitable for trawling in most of the Antarctic shallow water areas.

In 1983, the Ichthyology Project of the Instituto Antartico Argentino implemented a long term monitoring program of pre-recruit demersal fish from inshore sites of the lower South Shetland Islands, mainly near Jubany Scientific Station in Potter Cove, King George Island, using trammel nets (Fig. 1). (Barrera-Oro et al. 2000). The main aim of this program was to study the impact of the offshore commercial fishery on inshore fish of the area. In addition, the local effects of an intensive sampling program on the population of the dominant fish species in the region were analysed.

In this work, we summarise the main results on this matter, from related publications of ours.

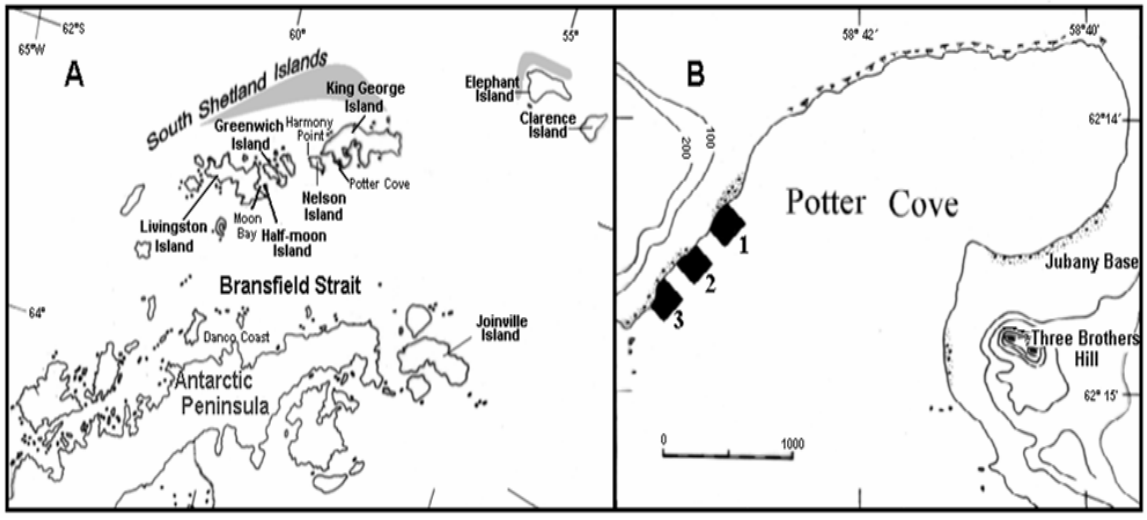

Fig. 1. A) The locations studied in the South Shetland Islands. The offshore, bottom-trawled area has been shaded; B) The sampling sites at Potter Cove, King George Island.

Effects of the offshore commercial fishery

The commercial finfish fishery in the lower South Shetland Islands (King George Island to Low Island) operated from 1977 through 1990 at 150-500 m depth, and targeted mainly *Champsocephalus gunnari* and *Notothenia rossii* (Fig. 1, A) (Kock 1992). A substantial by catch of *Gobionotothen gibberifrons* was captured along with several other species (CCAMLR 1990). Most catches of *C. gunnari* and *N. rossii* occurred in the first few years of the fishery, and by the end of the 1980s catches consisted primarily of *G. gibberifrons*. A moratorium on finfishing in the region was imposed by the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) in 1989/90 and the area has remained closed to date.

Shortly after the closure of the fishery, our monitoring program in inshore waters was intensified focusing mainly on the relative trends in abundance of *G. gibberifrons*, *N. rossii* and the ecologically similar species *Notothenia coriiceps*. A sharp decline in the inshore sub-populations of the two first species in the period 1983-1999 was reported as caused by the offshore commercial fishery in the area in the late 1970s (Barrera-Oro et al. 2000). Data before 1980, from closer localities of the South Shetland Islands (Moon Bay, Admiralty Bay and Discovery Bay), showed that the proportions of *N. rossii* and *G. gibberifrons* were well above the starting point of our series at Potter Cove, thus supporting the decline of these species (Barrera-Oro & Marschoff, 1991).

The monitoring program of fish in neighbouring sites in the lower South Shetland Islands has continued in the austral summer in years 2000-2003 at Potter Cove (PC), thus completing a continuous sampling period of 21 years and in years 2001-2003 at Harmony Cove (HC), Nelson Island. The aim was to see if the status of *G. gibberifrons* and *N. rossii* remain at low levels or if a recovery is observed (Barrera-Oro et al. 2003).

Data from 3364 specimens (2539 from PC and 815 from HC) of *N. coriiceps* (*Nc*), *N. rossii* (*Nr*) and *G. gibberifrons* (*Gg*) (this species was not recorded at HC) collected in 250 trammel nets (225 from PC and 25 from HC) were included. These were combined with data from the period 1983-99 at PC, and from split year 1995/96 at HC, and analysed with the same method (also sampling gear and design) used in Barrera-Oro et al. (2000).

Since soak time and net size depended on weather conditions and experimental design, fishing effort per haul was highly variable between years and within the same season. Therefore, the abundance of fish was studied both, as the total number of each fish species per haul and as standardised numbers of *Nr* and *Gg* relative to *Nc* according to:

$$\text{Proportion (b)} = \frac{\text{Nb}}{\text{Nc} + \text{Nb}}$$

where Nb is the number of specimens of the species considered *(Nr* or *Gg*), and Nc is the number of specimens of *Nc.*

The number of fish per haul and the relative abundances were analysed by means of an ANOVA design grouping the hauls on a split year basis (1 May to 30 April next year) using appropriate normalising transformations.

Since for all species and variables the interannual variability resulted highly significant, we fitted a non-parametric model for the relation between

abundances and time, along the methods described in Härdle (1989), using a bandwidth of 0.15 (approximately one year).

We have used *Nc* as a reference species to obtain an index of the abundances of *Nr* and *Gg* because, while these three fish species show similar ecological habits in the fjords, *Nc* was not affected by the commercial fishery.

The length composition of *Nc* (15.6-52.6 cm at PC, 11.6-51.5 cm at HC) includes immature and mature, adult specimens (Casaux et al. 1990). All *Nr* (14.2-44 cm at PC; 16.3-41 cm at HC) and *Gg* (29-36.2 cm at PC) specimens collected were juvenile (Casaux et al. 1990).

Split year means of total catches per haul are presented in Fig. 2, along with the nonparametric fit of the regression of the number of fish per haul on date. Relative abundances are presented in Fig. 3.

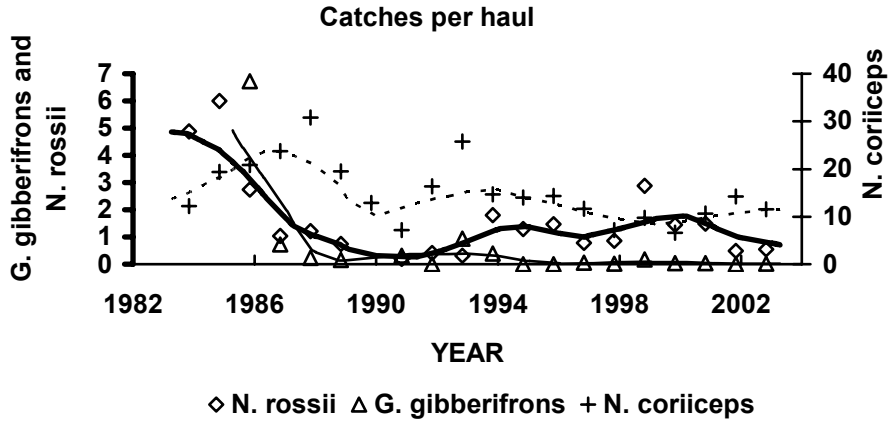

Fig. 2. Expected catches (number of fish) of *G. gibberifrons*, *N. rossii* and *N. coriiceps* as functions of catch date, together with the observed mean values.

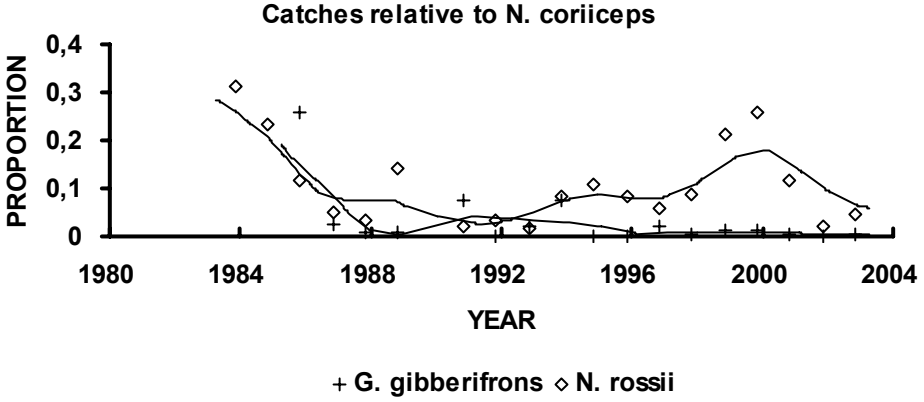

Fig. 3. Expected catches of *G. gibberifrons* and *N. rossii* relative to the catches of *N. coriiceps* as functions of catch date, together with the observed mean values.

The estimated relative abundance of *Gg* declined from 19% in 1985 to 0.2% in 1989 and remained at the same low level up to 2003 (Fig 3). Likewise, the relative abundance of *Nr* declined from 29% in March 1983 to 3% in April 1991 and then increased and remained around 8% in the period 1994-1997. It increased again to 18% in January 2000, showing a steady decline to 6% in March 2003. Although limited by the fact that the fishing effort was not kept

constant throughout the whole sampling period of 21 years, the number of specimens per haul of *Nr and Gg show* patterns similar to the relative abundances (Fig. 2).

Despite the increasing trend of *Nr* catches from 1991 to 2000, the levels of relative abundance of this species and *Gg* in PC in 2003 are well below those found in the early 1980s. At HC, the observed mean of the relative abundance of *Nr* in the split years 1995/96, 2000/01, 2001/02 and 2002/03 (0.05, 0.085, 0.203 and 0.275 respectively) show an increase, mainly in the last two years.

The results of fish monitoring from nets are supported by our knowledge on the diet of the piscivorous Antarctic shag *Phalacrocorax bransfieldensis* in the South Shetland and the Antarctic Peninsula areas since the early 1990s. Among the fish species caught inshore the first area only *Nr* and *Gg* were absent or scarcely represented in the diet of shags (Casaux & Barrera-Oro 1993, Barrera-Oro & Casaux 1996_a). In contrast, the high importance of *Gg* both as prey of shags and in trammel net catches at the Danco Coast, west Antarctic Peninsula, reflects higher availability of this fish in an area remote from the main historical fishing grounds of the South Shetland Islands (Elephant Island and north of Livingston/King George Islands) (Casaux et al. 2002_a).

Historical information from fishing vessels and scientific surveys in the area shows that, whereas the stocks of *Nr* remain at very low levels, *Gg* is still the dominant offshore demersal fish species of the whole South Shetland Island region after 1980 (reviewed in Barrera-Oro et al. 2000, Jones et al. 2001, 2003; Kock et al. 2002)). Nevertheless, the last five surveys in the northeast region off King George Island in 1986, 1998, 2001, 2002 and 2003, close to our main sampling site in PC, showed the prevalence of the non commercially exploited *Nc* over *Gg* in catches down to 100-400 m depth (Balguerias 1989; Jones et al. 1998, 2001, 2003; Kock et al. 2002). *N. coriiceps* has proliferated markedly in inshore waters of the South Shetland Islands since the early 1980s, in parallel with the decrease of *Nr* and *Gg* populations (Barrera-Oro & Casaux 1998).

In inshore waters of the South Shetland Island region a recovery was still not observed either for *Nr* or for *Gg*, more than two decades after the end of the offshore commercial fishery. However, the increase observed in the catches of *Nr* in some years since 1997 might be indicative of events of higher recruitment, not yet confirmed by the offshore scientific surveys carried out in the region.

Effect of a long term scientific sampling program

The effects of commercial exploitation of finfish on Antarctic fish populations is well known (Kock 1992, this study). However, potential effects of scientific sampling programs on fish communities are rarely documented. The advantages of using trammel nets in comparison to other inshore sampling gear (hook and lines, traps) are the capture of a larger quantity of fish in a short time, no damage to benthos, negligible by-catch of benthic organisms and easy operation from rubber boats. This sampling method has been widely used in Antarctica mainly for coastal biological studies. However, there is very little information on how a long term program using this type of gear may affect the age and size structure of local fish populations.

Notothenia coriiceps is the dominant inshore demersal fish of the South Shetland Islands area and has a high degree of site fidelity (Barrera-Oro & Casaux 1996_b). We have used time series information based on trammel net

catches of this species at different sites in PC, to analyse the effects of an intensive sampling program on the size and age structure of the population (Casaux & Barrera-Oro 2002_b).

Four hundred and ninety-three *N. coriiceps* specimens were caught from 19 December 1994 to 1 February 1995 at three specific zones of the cove (Fig. 1, B). At site 1, mark-recapture, activity and monitoring studies of this species have also been carried out intensively since December 1992 (summarised in Barrera-Oro & Casaux 1998). Fish samples were also regularly taken generally at greater depths in a radius area of about 100 m including this site since 1983. Site 2 and site 3 were two previously unsampled areas, which are respectively 200 and 700 m from site 1. The net measurements and sampling design were identical at the three sites, and are described in Casaux & Barrera-Oro (2002_b).

At site 1, the total lengths of the fish caught in 1994/1995 were compared with those obtained in the previous summer seasons (December-February) of 1992/93 and 1993/94. A decline in mean lengths of about 3 cm was observed throughout the whole period (Table 1, Fig. 4). This phenomenon could be related to: 1) a variation of the population due to a natural effect, such as a strong recruitment; 2) artificial effects attributed to human actions, such as a random error or an intensive sampling effort.

Table 1. Differences among the mean total lengths (in cm ± SD) of *N. coriiceps* caught in the 1992/93, 1993/94 and 1994/95 summer seasons in site 1.

Summer season	1992/93	1993/94	1994/95
1992/93	mean = 31.7 ± 4.8	-----	-----
1993/94	ns	mean = 30.2 ± 7.2	-----
1994/95	$P < 0.001$	$P < 0.05$	mean = 28.8 ± 6.5

The additional sampling carried out at two near but previously unsampled sites helped to clarify this question. The fish from sites 2 and 3 were significantly larger than those from site 1, all of them caught in the summer of 1994/95 (Table 2, Fig. 5). Moreover, the fish from the new sites were also markedly larger than those sampled at site 1 in the summer of 1993/94, but were similar in size to those fish sampled during the summer of 1992/93, when the intensive sampling programme started at site 1 (Table 1). It is important to recall that sampling conditions such as depth, net parameters and bottom type were the same at all three sites, hence differences in the results due to random errors in the sampling design is unlikely.

Table 2. Differences among the total lengths (in cm ± SD) of *N. coriiceps* caught in the 1994/95 summer season in the three sampling sites.

	Site 1	Site 2	Site 3
Site 1	mean = 28.8 ± 6.5	-----	-----
Site 2	$P < 0.01$	mean = 32.4 ± 6.3	-----
Site 3	$P < 0.01$	ns	mean = 31.8 ± 6.0

While the size range at site 1 does not change between years, the size range of specimens at site 3 was 3 cm larger at the minimum size and 7 cm larger at the maximum size (Casaux & Barrera-Oro 2002_b, Table I). The regular sampling of fish since 1983, generally at greater depths in a larger area which includes site 1, may have some influence on the results. Differences attributed

to sexual dimorphism in the size of *Nc* is unlikely, because the male-female ratio in this work was similar in all three years.

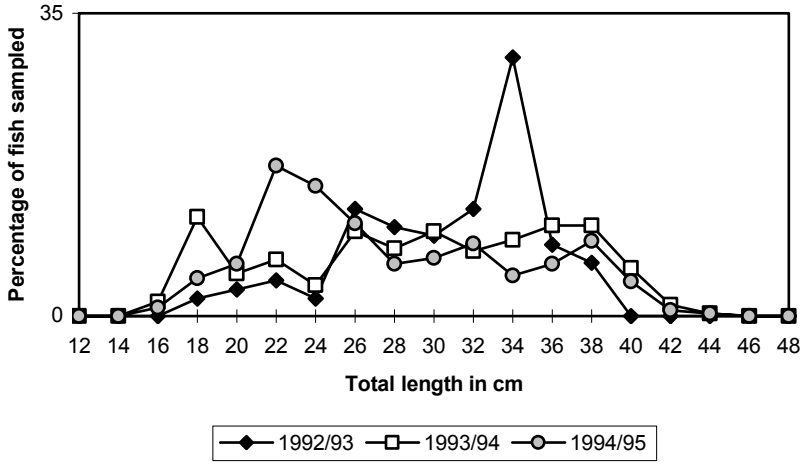

Fig. 4. Length frequency distribution of *N. coriiceps* caught in the 1992/93, 1993/94 and 1994/95 summer seasons in site 1.

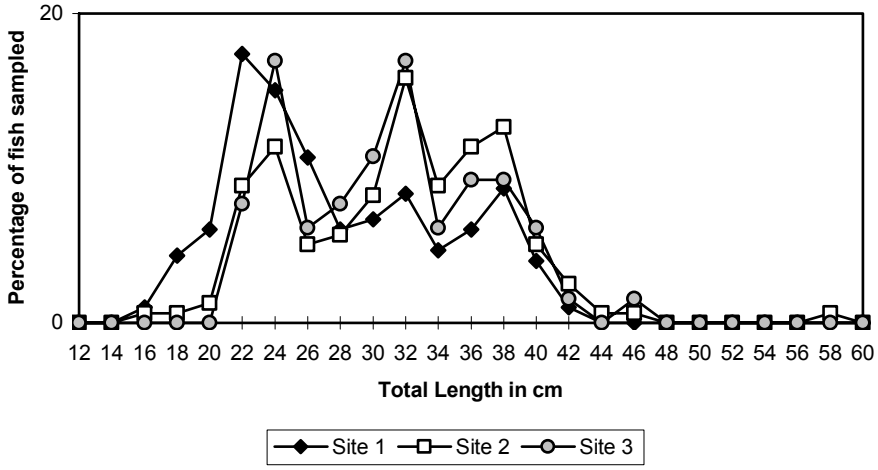

Fig. 5. Length frequency distribution of *N. coriiceps* caught in the summer season of 1994/95 in sites 1, 2 and 3.

The length frequency distribution of *Nc* in 1994/95 shows that the structure of the population in sites 2 and 3 was similar, whereas in site 1 there were higher numbers of smaller fish (from 22 cm downwards) and lower numbers of larger fish (from 32 cm upwards) (Fig. 5). The three sampling sites are located in the same cove with only short distances between them (< 700 m, Fig. 1, A); therefore the effect of a hypothetical strong recruitment (as it could be presumed in site 1) should have been uniform in the whole sampling area. According to the total length range, fish of about 3-12 years of age were represented in the whole sample (Casaux *et al.* 1990).

It is suggested that the decline in mean length of the fish sampled at site 1 is due to intensive sampling effort carried out over this study at that zone in PC.

Present results indicate that the development of long-term programs monitoring non-migratory inshore demersal fish species need to be planned carefully. The division of the sampling effort in homogeneous but different zones and the release of fish after monitoring procedures could help to avoid local

variations of the size structure/abundance of the population caused by intensive sampling in specific sites.

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Impact of human and conspecific disturbance on behaviour and heart rate of incubating Adélie penguins (*Pygoscelis adeliae*)

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Introduction

With the continuously rising numbers of scientists and tourists present in Antarctica (e.g. IAATO 2006), comprehensive guidelines for human behaviour have become increasingly important to minimise the degree of disturbance caused to Antarctica's natural inhabitants. Apart from being in the focus of a variety of scientific studies, Antarctic penguins frequently inhabit areas relevant to scientific activity not related to "penguinistic issues". As they additionally have a high "touristic value", they are subjected to potential negative impact on their welfare from all three sources of human activities.
In animal welfare research, a combination of behavioural (i.e. visible) and physiological (usually invisible) parameters is generally considered an appropriate method to examine the interplay between overt and internal reactions to potentially disturbing stimuli (see Broom & Johnson 1993 for a comprehensive overview). To gauge the level of impact caused by "unnatural" stimuli (i.e. those, the animals did not evolve alongside with), it is helpful to compare the reactions to those with reactions to "natural" stressors (e.g. conspecifics, predators).

Study aim and research objectives

It is the aim of this study to use selected behavioural as well as heart rate reactions of Adélie penguins to examine the correlation between these parameters, and their respective relationships with stress caused by conspecific and/ or human disturbance. Recommendations derived from the results should ideally enable humans to sensitively gauge their impact on the penguins and adapt their behaviour accordingly.

Animals and location

Five groups of incubating Adélie penguins (*Pygoscelis adeliae*) were studied in two consecutive breeding seasons (2000: 3 groups; 2001: 2 groups). Groups were chosen at random from the largest of the Adélie colonies (2001 census: approx. 2,750 breeding pairs). The colony was situated close to Stranger Point (Fig. 1), at the protected Antarctic Specially Protected Area, ASPA n° 132 (formerly SSSI n° 13, Potter Peninsula, 'Isla 25 de Mayo' = King George Island, South Shetland Islands, 62°15'S, 58°39'W; approximate area: 1.9 km^2). Of each of the five groups, two to four nests received artificial eggs equipped with heart

rate sensors. As some of the nest reliefs occurred within the respective study periods, heart rate data on a total of 16 penguins are presented.

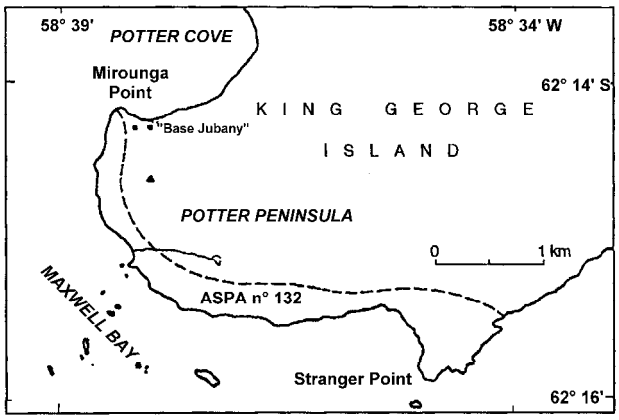

Figure 1: ASPA n° 132 (formerly SSSI n° 13). The study was conducted in the vicinity of Stranger Point. [based on: aspire.nvi.net/DocImgWeb/SSSI13_13_8.tif]

Materials and methods

To minimise bias due to observer impact, the study was designed to be predominantly hands-off. It was conducted on incubating penguins to facilitate distinction between the locomotory and the emotional component of heart rate changes (Blix et al. 1974) by minimising the former component.

Data collection

Recordings were made during the second half of two incubation periods (mid- to end of November until the beginning of December). Each day (weather permitting), data on all groups investigated in that year were obtained in the morning and/ or in the afternoon. For each group, the recording sessions in 2000 and 2001 lasted 30 min and 45 min, respectively.

For detailed behavioural evaluations, a video camera was placed out of sight of the penguins. As a non-invasive method for remotely gathering heart rate data from unrestrained animals, artificial eggs (Nimon et al. 1996) were introduced into 14 nests.

Heart rate and behaviour were examined under various conditions. Firstly, baseline data were collected from undisturbed, incubating penguins. Secondly, reactions to conspecific disturbance (other penguins passing by, penguins trying to steal nest stones) were examined. Thirdly, reactions to differing patterns of human visits were recorded. During evaluations, disturbances were categorised to compare the amounts of stress caused by differing sources of disturbance. Heart rate and behavioural data were correlated to gain an insight into the relationship of heart rate and behavioural reactions.

Behaviour was recorded on tape (Sony Hi8 video camera), and transcribed after the fieldwork periods. Therefore, it was possible for a single observer to focus in turn on each individual in a group with equal accuracy. The data presented here are based on 3-10 (median: 5) recordings per penguin (n = 16 focal animals).

Baseline records of undisturbed and records of "naturally" disturbed birds (conspecifics, predators) were obtained at regular intervals for all groups studied. These records were used to assess the range of behaviours shown in the absence of human visitation, and to determine the background level of disturbance on top of which the additional human disturbance would occur.
Human impact consisted of approaching the penguins following a previously determined schedule. Four groups (B and C in 2000; X and Y in 2001) were subjected to different "patterns" of visitor behaviour (details available on request). In both years, one group was treated in an inobtrusive manner ("Silent and Slow Approach"), while the other was approached more carelessly ("Loud and Fast Approach"). Visits lasted between 7 min and 11 min (median: 8 min 40 s, n = 25) and were immediately preceded and followed by periods of equal or greater length during which no human disturbance occurred. Group A (2000) did not experience any human visits at all.
During the first field season, impact of a single visitor was investigated, while impact of a group of 3 visitors was examined the following season. To standardise visitor conduct, two "artificial visitors" were created for the second season (Fig. 2) as it was unlikely that more than one "live" assistant would be available

Figure 2: The "Visiting Trio". To standardise visitor conduct, two dummies were attached to a mobile visitor. © Schuster 2001

at any time. The dummies were fastened to the frame of a dismantled backpack and could thus be carried by the "mobile visitor".
Heart rate was measured using artificial eggs fitted with infra-red sensors (Nimon et al. 1996). The sensor makes contact with the incubating penguin's highly vascularised brood patch and registers the pulse-varying volume of blood flow. To ensure reliable sensor contact, the egg is mounted on a platform. The

sensor is connected to a data logger via a long cable. Heart rate data were stored in a laptop for subsequent evaluation. During placement, the platform and part of the height of the egg were buried in the stones and gravel of the nest and the cable protruded through the wall. The nest was then reshaped to

its original position. This way, the artificial egg resembled a natural penguin egg in the nest. Placement of an artificial egg took on average 2.5 min. Even though the birds were not handled and were thus free to leave the nest during placement, the majority of focal animals remained seated. Those who chose to get up, stepped only a few metres away and returned while the human intruder sat quietly. To facilitate distinction between the two birds of a nest, the penguins present during egg placement were paint-marked with a long-handled brush.
Weather conditions were sampled at the beginning and end of each recording session. Temperature was measured at ground level and at 0.5 m using a standard thermometer. Wind speed (km/h) was recorded with the help of a hand-held anemometer. Cloud cover was estimated (0 %, 25 %, 50 %, 75 %, 100 %), and the precipitation was noted (none, rain, snow).

Data evaluation
Video recordings of the behaviour of a total of 25 penguins (4-6 per group; 2000: 15, 2001: 10) were transcribed on a second-to-second basis using focal-animal all-occurrences sampling (Altmann 1974; Lehner 1996). Here, results on vigilance and agonistic behaviour elements are presented for 16 of the 25 focal birds.
Conspecific behaviour was also transcribed on a second-to-second basis. For this, three zones of increasing area were designated round the focal penguin's nest. Zone "a" comprised the area around the focal penguin's nest up to the nearest neighbouring nests, zone "b" the area between one and two nests away, and zone "c" the area between two and three nests away from the focal penguin (Fig. 3). Within each zone, the number and locomotory activity (lying/ standing, walking, running) of the conspecifics was noted. Additionally, a limited number of conspecific behaviours were included in the evaluations (e.g. nest stone theft, agonistic interaction with or in the immediate vicinity of the focal animal).
Information on the human visits was similarly transcribed in that the number of visitors, their distance from the visited group of penguins, their speed and noise level were noted for each second. Heart rate data were transformed into graphs (Microsoft Excel), and heart rate was counted for 20 s-intervals from which beats-per-minute values (bpm) were extrapolated.
For a detailed comparison of the penguins' behavioural and heart rate reactions with the different types of disturbance, the percentage of time the focal animals spent performing vigilance and agonistic behaviour as well as the type and amount of conspecific and human disturbance were likewise quantified for 20 s-intervals. Owing to the range of individual reactions, calculations were performed separately for each focal animal.

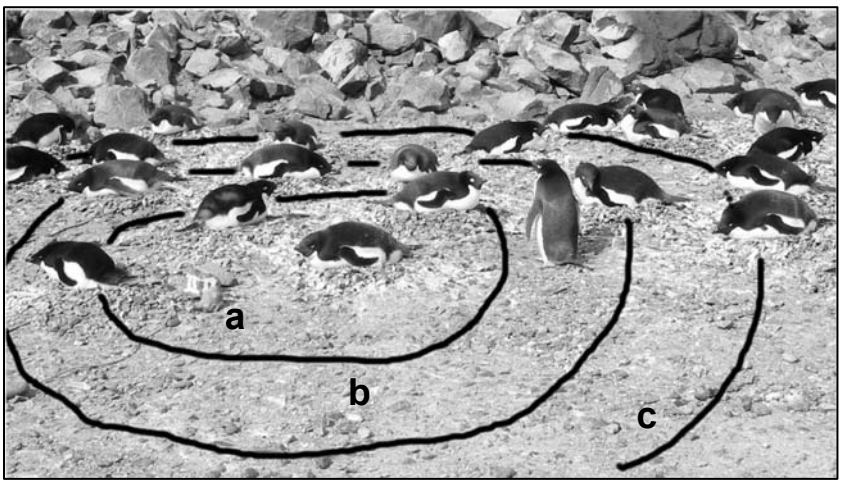

Figure 3: Nest zones. Information on conspecific disturbance was evaluated by noting the birds' presence and selected aspects of their behaviour in three zones (a, b, c) of increasing distance from the focal animal (FA).

Statistical analysis
For each focal animal, Spearman correlations (all p 2-tailed; SPSS, release 12) were calculated for three types of disturbance (total conspecific, conspecific at the nest, and human) and the three indicators presented here (heart rate, percentage of vigilance, and percentage of agonistic behavioural elements), as well as for the relationship between vigilance and heart rate (Tab. 2). Interpretation of correlations followed Sprinthall (1987, Tab. 1).

Table 1: Informal interpretation of correlations following Sprinthall (1987)

r<0.2	slight correlation, almost negligible relationship
0.2<r<0.4	low correlation, definite but small relationship
0.4<r<0.7	moderate correlation, substantial relationship
0.7<r<0.9	high correlation, marked relationship
0.9<r<1.0	very high correlation, very dependable relationship (not in this data set)

Results

While agonistic behavioural elements were more often recorded in response to conspecific disturbance, heart rate and vigilance corresponded more closely to human disturbance (Tab. 2). Heart rate responses of the majority of focal penguins did not correlate (n = 8) or were inversely (n = 3) correlated with conspecific disturbance. In contrast, heart rate of 10 of the 12 birds that were subjected to human visits was significantly positively correlated with human disturbance (max. r^2 = 0.134; Tab. 2).
Vigilance of 13 of 16 focal animals showed a significant correlation with total conspecific disturbance (max. r^2 = 0.255) and with conspecific disturbance at

the nest (max. r^2 = 0.159). Nine of 12 focal birds significantly responded to human disturbance (max. r^2 = 0.308) with increased vigilance.
Agonistic elements were significantly correlated with conspecific disturbance in all focal animals (max. r^2 = 0.642). They were found to be unrepresentative as "universal" indicators of human disturbance, since only six of 12 birds exhibited these responses (max. r^2 = 0.273). A positive correlation between heart rate and vigilance behaviour was found in seven of the 16 focal birds, in one bird, these parameters were inversely correlated, and in half of the birds studied, they did not correlate at all.

Discussion

The focal penguins examined, exhibited individual reaction patterns towards both conspecific and human disturbance. As human disturbance usually constitutes an addition to natural disturbance, further analyses are needed to partial out the effects of conspecific disturbance during human visits. The most obvious difference between conspecific and human disturbance is suggested to concern their respective predictabilities. Broom and Johnson (1993) state that "when the next stimulus is unpredictable either in time, intensity, or both, yet is certain to arrive sometime, the animal can prepare only by being constantly ready. The anticipation in such circumstances engenders a state of anxiety, and heightens the reaction when the stimulus is eventually perceived." Whereas conspecific disturbance forms a part of the penguins' natural environment, human visitors at penguin colonies normally represent an unpredictable stimulus in all respects. Therefore, habituation must be generally considered unlikely to occur.
Although heart rate and vigilance behaviour were significantly positively correlated in seven of 16 focal animals (Tab. 2), they were more often found to be complementary, thus confirming for the penguins studied the importance of examining different parameters (see Introduction). It is therefore suggested that a combination of the two indicators be used for optimum assessment of disturbance.
In the majority of human-penguin-encounters, however, scientific or touristic equipment is unlikely to consist of apparatuses related to measuring penguin heart rate. While based on scientific results, implementable guidelines must therefore do without the assessment of "invisible" indicators. Findings of this study suggest that Adélie penguin behaviour may well be able to carry the case unaided. With respect to human disturbance, vigilant and/ or agonistic behavioural reactions were observed in all but two of the birds examined. Furthermore, these two birds were situated in groups in which the other individuals did exhibit increases in these behaviours so that the overall impression of augmented vigilance and/ or agonistic behaviour remained (detailed results on overall group reactions are in preparation). While increased vigilance will commonly be encountered, elements of agonistic behaviour should not be expected, but taken seriously once they occur. Thus, learning to recognise these behaviours (an excellent overview is given in Spurr 1975) and to gauge their intensity in a given situation would enable human visitors to correctly assess their impact on the majority of the birds.

Table 2: Correlations of Adélie penguin heart rate and behavioural responses to conspecific and human disturbance, and correlation between vigilance and heart rate. Interpretation of correlations following Sprinthall's suggestions (Tab. 1). Focal animals identified by alphanumerical code, significance levels (two-tailed) of the correlations represented by asterisks: *p<0.05, **p<0.01, ***p<0.001, n.s.: not significant, inv.: inverse correlation; bpm: beats per minute; t (sampling points per penguin and parameter): 54-1053 (median: 280).

	indicator measured	r<0.2	0.2<r<0.4	0.4<r<0.7	0.7<r<0.9
total conspecific disturbance per 20 s interval	heart rate (bpm)	A5-2$^{n.s.}$, A6-1$^{n.s.}$, A6-2$^{n.s.}$, B3-1$^{n.s.}$, B3-3*, B4-1$^{n.s.}$, B4-2$^{n.s.}$, C1$^{n.s.}$, C2$^{n.s.}$, X1**, Y5-1*	A5-1***$_{(inv.)}$, C11*$_{(inv.)}$, X2-1***, X2-2***$_{(inv.)}$, Y5-2*		
	percentage of vigilance	A5-2$^{n.s.}$, A6-2$^{n.s.}$, B3-1***, B4-2**, Y5-2$^{n.s.}$	A5-1***, A6-1***, B3-3***, B4-1**, C1**, C11***, C2***, X2-1***, X2-2***, Y5-1***	X1***	
	percentage of agonistic behavioural elements		A5-1***, A5-2***, B3-1***, B3-3***, B4-1***, B4-2***, C11**, X2-2***, Y5-1***, Y5-2***	A6-2***, C1***, C2***, X1***, X2-1***	A6-1***
conspecific disturbance at the focal nest per 20 s interval	heart rate (bpm)	A5-1$^{n.s.}$, A5-2$^{n.s.}$, A6-1$^{n.s.}$, A6-2$^{n.s.}$, B3-1$^{n.s.}$, B3-3$^{n.s.}$, B4-1$^{n.s.}$, B4-2$^{n.s.}$, C1$^{n.s.}$, C11$^{n.s.}$, C2$^{n.s.}$, X1*, X2-2*$_{(inv.)}$, Y5-1***, Y5-2$^{n.s.}$	X2-1***		
	percentage of vigilance	A5-1*, A5-2$^{n.s.}$, A6-1*, A6-2$^{n.s.}$, B4-2**, C2***, Y5-1*, Y5-2$^{n.s.}$	B3-1***, B3-3***, B4-1***, C1**, C11**, X1***, X2-1***, X2-2***		
	percentage of agonistic behavioural elements	A6-2$^{n.s.}$, Y5-1***	A5-1***, A5-2***, B4-2***, C1***, C11*, C2***	A6-1**, B3-1***, B3-3***, B4-1***, X1***, X2-1***, X2-2***, Y5-2***	
total human disturbance per 20 s interval	heart rate (bpm)	B3-3**, B4-2*, C2***, X1$^{n.s.}$, X2-1***, Y5-2$^{n.s.}$	B3-1**$_{(inv.)}$, B4-1***, C1***, C11***, X2-2***, Y5-1***		
	percentage of vigilance	B3-1$^{n.s.}$, C1$^{n.s.}$, C2***, X2-2$^{n.s.}$	B3-3***, C11***, X1***, X2-1***, Y5-1***	B4-1***, B4-2***, Y5-2***	
	percentage of agonistic behavioural elements	B3-1$^{n.s.}$, B3-3$^{n.s.}$, C11$^{n.s.}$, X1***, X2-1$^{n.s.}$, X2-2$^{n.s.}$, Y5-1$^{n.s.}$	B4-2***, C1**, C2***	B4-1***, Y5-2***	
correlation between heart rate and behaviour		A5-1**$_{(inv.)}$, A5-2$^{n.s.}$, A6-2$^{n.s.}$, B3-1$^{n.s.}$, B3-3*, B4-1$^{n.s.}$, C1$^{n.s.}$, C2$^{n.s.}$, X2-1***, X2-2$^{n.s.}$, Y5-2$^{n.s.}$	A6-1**, B4-2*, C11**, X1**, Y5-1**		

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6. LONG-TERM DATA SETS

Long-term hydrographic conditions and climate trends in Potter Cove*

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* *This work is dedicated to the memory of Augusto "Alfa" Thibaud and Teófilo González, who lost their lives in a glacier crevasse near Jubany during the overwintering in 2005.*

Introduction

A marked warming of mean air temperature has been recorded over the last 50 years in the Western Antarctic Peninsula (WAP) (see Turner et al., 2005 and references therein). This rise in air temperature was mainly observed during the autumn-winter months (Kejna, 2003). In particular, in King George Island (25 de Mayo), South Shetlands air temperature rose on average by 1.1 ^{o}C between 1947 and 1995 (Ferron et al., 2004); if only the winter months are considered, temperature increase in the same period amounts to 1.9 ^{o}C. This trend was also apparent in the air temperature data from the meteorological station in Jubany station for the decade 1994-2004 (Schloss, 2003). Climate warming in Antarctic environments has been associated with glacier retreat and increased ice melting (Cook et al., 2005) which, in turn, change the vertical structure of the water column, especially in Antarctic shallow coastal environments in the WAP. Moreover, glacier runoff has been shown to transport high particle loads, affecting water column light climate and changing the optical conditions for phytoplankton photosynthesis. Light and salinity changes are, therefore, indirect consequences of regional air temperature increase. A direct effect on sea water temperature could also be expected. Although stable sea water temperatures have been recorded around Antarctica for a period of at least 10 million year (Peck, 2005), making it one of the most thermally stable environments on Earth there is already some evidence on surface water temperature warming (Meredith and King, 2005).

Since the beginning of the Argentinean – German cooperation at Jubany Station – Dallmann laboratory, sea water temperature and salinity, as well as chlorophyll-a and suspended particulate matter concentrations have been measured in Potter Cove, in the vicinity of the station. Several projects, using hydrographi-

cal data as central or complementary information were carried out. As a result, a 15-years series of data is available, although there are many gaps, especially in the winter months, when difficult weather conditions made sampling impossible. In the present paper, a preliminary analysis of these series is presented and discussed in the face of climatic change (warming) observed in the WAP coastal ecosystems.

Materials and methods

Air temperature data, obtained by the Servicio Meteorológico Nacional from the Argentinean Air Force at Jubany, will be presented in order to compare them with the hydrographic information. Monthly averages were calculated.

Sampling was conducted on a weekly basis during the summer season and two-weekly during the winter in the inner Potter Cove (King George Island, South Shetlands, Antarctica, 62°14'S, 58°38'W), close to Jubany Station. Average depth in the inner cove is around 30 m (maximum depth: 50 m). Water samples (4.7 l Niskin bottles) and CTD data were collected using Zodiac boats over the entire water column. However, in this paper, only surface water data (0, 5, and 10 m) will be considered. All the data were averaged over the upper 10 m of the water column. This depth was chosen based on the depth of the summer pycnocline, which is found at very shallow depths, usually around 10 m (Schloss et al., 2002).

Sea water temperature and salinity: Over the years, several instruments, calibrated to salinity standard and temperature were used to measure temperature and salinity in seawater. They are summarised in Table 1.

Table 1. Sensors and/or methods used for the sea water temperature and salinity data

Year	Temperature	Salinity
1991-1992	Inversion thermometer	Beckman RS9 induction salinometer
1993-1996	ME-ECO219 mini-CTD	ME-ECO219 mini-CTD
1997-2000	CTD - ECO PROBE ISITEC, (General Oceanics)	CTD - ECO PROBE ISITEC, (General Oceanics)
2001	FSI 3" micro CTD model MBP-S.	FSI 3" micro CTD model MBP-S.
2002-2004	Sea-Bird SBE 38 sensor	Sea-Bird SBE 37 sensor
2005	FSI 3" micro CTD model MBP-S.	FSI 3" micro CTD model MBP-S.

Chlorophyll-a. Seawater (0.25 - 2 l) was filtered onto 0.45-mm Millipore (1991-1992) or Whatman GF/F filters (all other seasons). Photosynthetic pigments were extracted in 90% acetone over 24 h at 4°C in the dark . Readings were made with a Hitachi Perkin Elmer UV-VIS 139 spectrophotometer (1991 – 1999)

or a Shimadzu RF-1501 (2000-2005) and chlorophyll-*a* (Chl-*a*) concentration was calculated after Strickland and Parsons (1972).

Total suspended particulate matter concentrations. Total suspended particulate matter (TPM) was measured gravimetrically after filtering 0.25 - 2 l seawater through combusted pre-weighed Whatman GF/F filters. After filtration, filters were rinsed twice with distilled water in order to remove salts, then dried for 24 h at 60°C, and weighed again.

Monthly averages for of all the data were calculated. They were then classified into winter (from April to September) and summer (from October to March). Linear regressions and the significance of the correlation with the data were calculated using Statistica (StatSoft) Software.

Results

Average annual air temperature at Jubany Station increased by 1.2^{o} C during the 1991 – 2005 period. The augmentation calculated for the winter months only amounted to 1.66^{o} C, whereas air temperature rose by 0.4^{o} C if only the summer months are considered (figure 1). The slopes for the linear regressions corresponding to the whole data set (not shown) as well as for the summer and winter months separately are presented in Table 2.

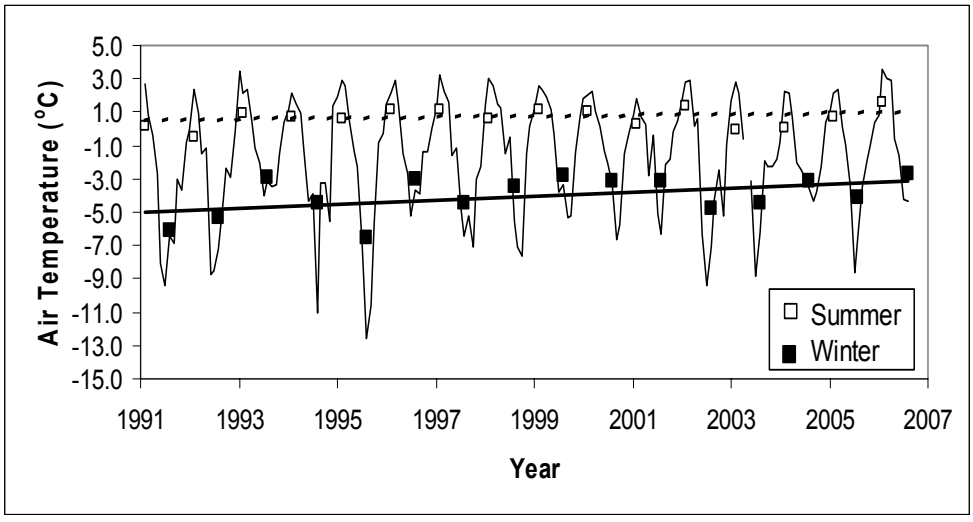

Figure 1: Monthly air temperature means, at Jubany Station (King-George Island), measured by the Servicio Meteorológico Nacional of the Argentinean Air Force. The lines show the linear regressions for summer (dashed) and winter data (solid), respectively. Slopes for the regression lines are presented in Table 2.

A significant increase was observed in sea water temperature within both seasons (figure 2). In spite of many gaps in the series, especially in the winter data from the years 2001 to 2005, water temperature increase was significant in winter data from April and June (Slope: 0.08, for April and 0.10 for June, R = 0.76 and R = 0.89, respectively, $p < 0.05$).

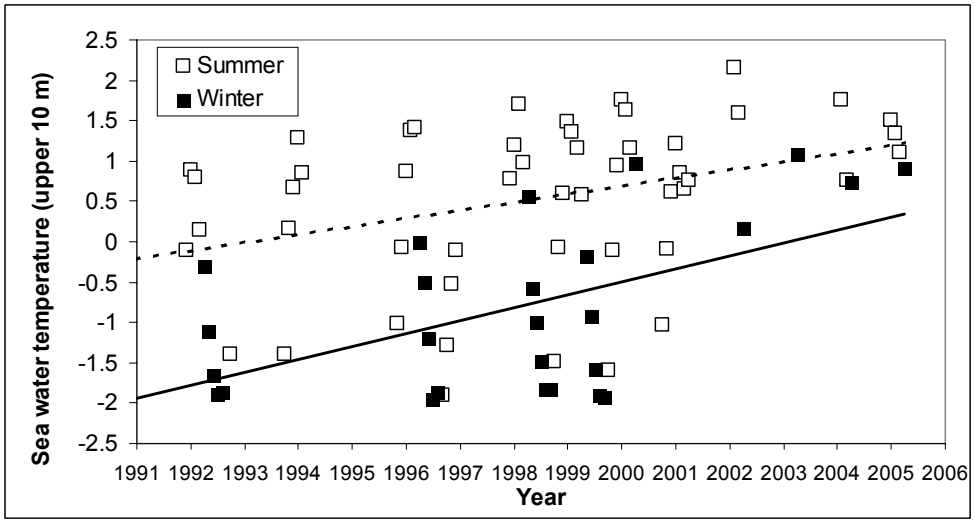

Figure 2: Monthly sea water temperature in the inner Potter Cove (averaged over the upper 10 m). The lines show the linear regressions for summer (dashed) data and winter (solid), respectively. Slopes for the regression lines are presented in Table 2.

Average annual surface water salinity in the upper 10 m water column (figure 3) decreased significantly ($R = 0.26$; $p < 0.05$) over the years, although this was not significant for the summer and winter data, if separately analyzed ($p = 0.20$ for winter and $p = 0.13$ for summer data). A significant negative decrease was found for the months of July ($R = 0.88$; $p < 0.05$) over the years, whereas not enough data were available for May, June and September.

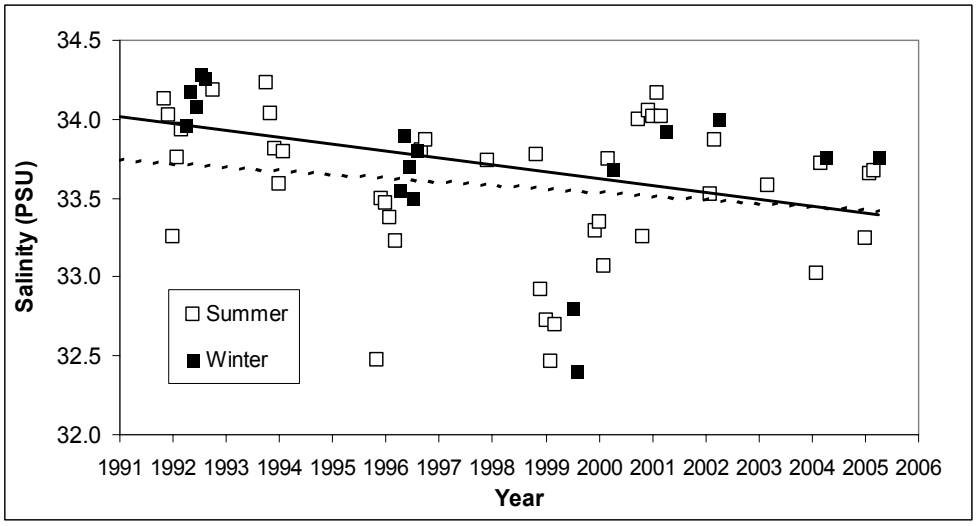

Figure 3: Monthly salinity data in the inner Potter Cove (averaged over the upper 10 m). The lines show the linear regressions for summer (dashed) data and winter (solid), respectively. Slopes for the regression lines are presented in Table 2.

No significant change of water column Chl-*a* concentrations was observed for any single month throughout the studied years (figure 4). However, a significant decrease became evident within the winter values (solid line, in figure 4, R =

0.46; $p < 0.05$; see slopes in Table 2). Monthly averages were consistently low (< 2 mg m^{-3}), with the maximum values corresponding to either November or late March.

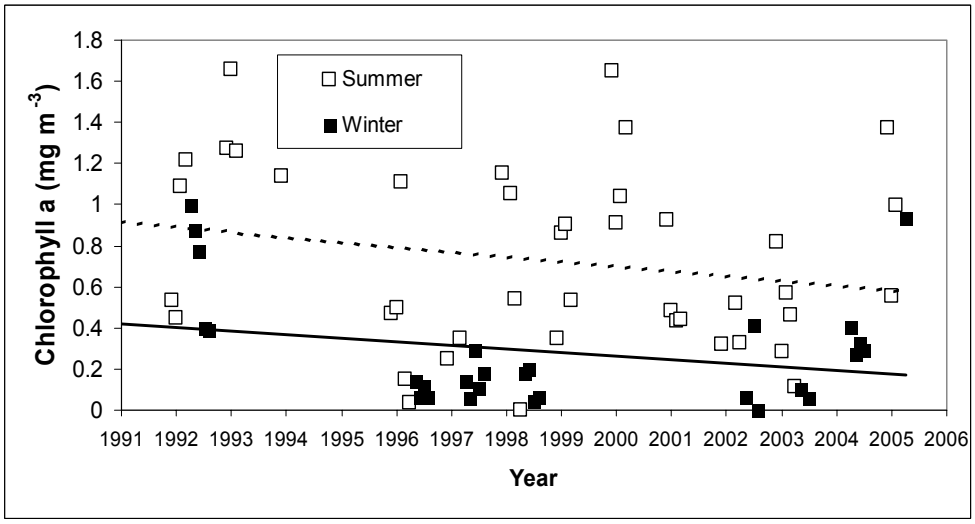

Figure 4: Monthly chlorophyll-*a* concentrations in the inner Potter Cove (averaged over the upper 10 m). The lines show the linear regressions for summer (dashed) data and winter (solid), respectively. Slopes for the regression lines are presented in Table 2.

The concentrations of TPM (figure 5) showed a significant increase over the years ($R = 0.29$; $p < 0.05$). Whereas there was no change during the summer months ($p = 0.12$), a significant increase was found in winter (dashed line in figure 5; $R = 0.46$; $p < 0.05$). Slopes are presented in Table 2.

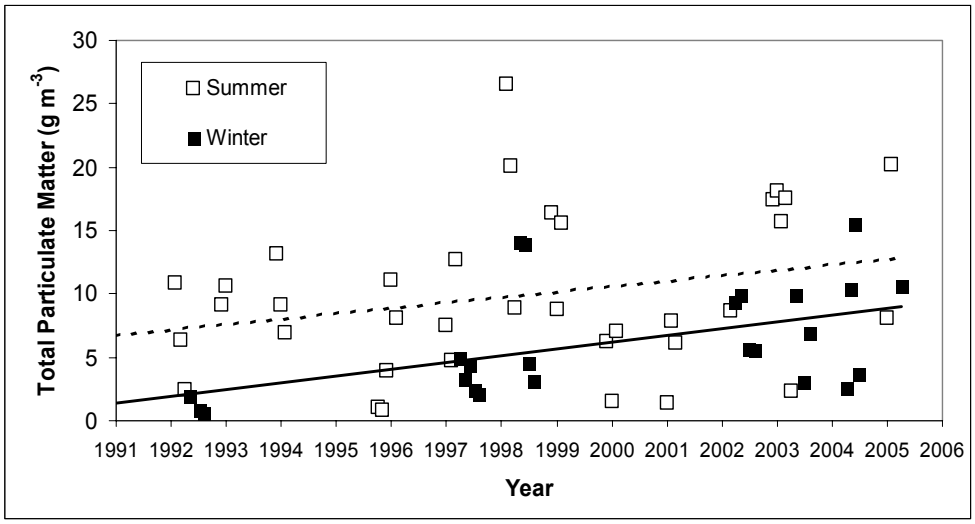

Figure 5: Monthly average total particulate matter concentrations in the inner Potter Cove (upper 10 m). The lines show the linear regressions for summer (dashed) data and winter (solid), respectively. Slopes for the regression lines are presented in Table 2.

For the complete data set, there is a significant inverse correlation between salinity and the amount of particles in the water column ($R = 0.5$; $p < 0.01$) and

a significant positive correlation between particles and sea water temperature ($R = 0.49$; $p < 0.01$). Also, we found a significant correlation between temperature and salinity for the complete data set ($R = 0.25$; $p < 0.05$), within the summer ($R = 0.35$; $p < 0.05$) but not in winter months ($p = 0.62$), indicating that warm, fresh water with high particle load is entering the cove. This is probably melt water from land ice masses which is flowing over the ice free areas into the cove, whereby warming up.

Chl-*a* was positively and significantly correlated with water temperature ($R = 0.42$; $p < 0.01$), but not with salinity ($p = 0.55$). Finally, no significant correlation was found between Chl-*a* content and TPM concentration ($p = 0.06$).

Discussion

Both, air and surface sea water temperature in Potter Cove (King George Island) increased significantly over the past 15 years. Although for the water column the time series is not complete, the records of monthly means from April and June suggest an effect of climate warming on sea water temperature in the WAP coastal environment.

Decreasing salinity was mainly observed in spring: October and November series showed lower salinity values at the end of the 1990's than in the beginning, indicating the melting process to start earlier in the spring season in the last years. This trend was not observed during either December or January, summer months in which melt waters occur on a regular basis. Although the series is not complete, very low surface salinity values (around 32 PSU) have been recorded in August, especially in 1999 (the end of the warmest decade of the last millennium, IPCC, 2001), a month typically characterised by the largest monthly sea ice cover and low air temperatures in the South Shetlands region (Ferron et al., 2004). Cold temperatures would prevent glaciers from melting. If the observed trend continues, the addition of melt water starting earlier in the season could have important effects on water column stabilization and, consequently, affect phytoplankton dynamics. This could have induced changes in species composition. Moline et al. (2004) found that salinity favoured the dominance of Cryptomonads in the phytoplankton community, a finding which is still under debate (Garibotti et al., 2005). The effects of salinity on phytoplankton and thereby on the whole Potter Cove food web are therefore subject to future studies.

TPM and Chl-*a* are negatively correlated in the area during the spring-summer season (Schloss et al., 2002). This was mainly due to the optical effect of particles in the water column, which limited light needed for photosynthesis. A high particle load in an environment as shallow as Potter Cove could be also indicative of re-suspension processes, which further contribute to light limitation of pelagic and benthic primary production. These processes respond to the observed heavy wind driven water column mixing (Schloss et al., 2002). In the long term, the lack of a consistent inverse correlation between TPM and Chl-*a* could be due to the low values of both variables that characterise the early

winter situation, when glacier melting has ceased and photosynthesis is constrained by the reduced winter light climate.

The observed positive correlation between Chl-*a* and temperature could be an artifact, related to the higher summer irradiances that are accompanied by higher sea water temperatures. No phytoplankton increase was observed along the studied years. However, in the long term an increase in water temperature could be beneficial for phytoplankton photosynthesis. Although photochemical reactions are not directly related to temperature (Jacques, 1983), biochemical reactions are (Cloern, 1979), and could benefit from temperatures somewhat higher than those of the polar environments, as shown among others by Smayda (1969) for Arctic diatom species. More studies are needed in order to understand the effect of water temperature increase on phytoplankton. For instance, for benthic diatom species it has been shown that tolerance varies among species (Longhi et al., 2003). Although correlations were not significant, our results show a negative trend along the studied years with regards to phytoplankton biomass (as Chl *a*; figure 4), indicating that environmental conditions were negatively affecting phytoplankton growth. Here we hypothesise that the earlier water column stabilization might be uncoupled with the adequate light environment, which would lead conditions for phytoplankton growth to become unfavourable.

Table 2: Slopes of the linear regressions for the different parameters analysed, considering the whole data set and Winter and Summer seasons separately. Significance is indicated: *: p <0.05; **: p < 0.01; N.S: not significant.

Variable	Slope	p
Air Temperature	0.02	N.S
Winter	0.13	**
Summer	0.06	N.S
Sea water Temperature	0.13	**
Winter	0.16	**
Summer	0.1	*
Salinity	-0.03	**
Winter	-0.04	N.S
Summer	-0.02	0.20
Chlorophyll-a	-0.02	N.S
Winter	-0.03	*
Summer	-0.02	N.S
TPM	0.45	*
Winter	0.51	*
Summer	0.43	N.S

The amount of particulate matter in the water column has augmented during the studied period (figure 5). If this is a consequence of increased glacial melting which is accompanied by the entrance of land-originated particles early in the spring summer season, light could be critically limiting photosynthesis. This will certainly affect not only shallow coastal environments like Potter Cove, but other coastal environments in the WAP, where warming is most evident (Turner et al.,

2005). The balance between the physiologically better temperature and worse irradiance conditions will finally determine the impact of global change processes on phytoplankton in shallow coastal Antarctic areas.

Acknowledgements
We would like to thank to all the persons who participated during the different years in the collection of data in the field, especially to Leonardo Cantoni, Alejandro Ulrich, Gustavo Latorre, Santiago de la Vega and Alejandro Olariaga.

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Long-term measurements of the atmospheric carbon dioxide concentration measured at Jubany Station indicate a relationship with "El Niño"

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Introduction

The role of the atmospheric CO_2 concentration for the radiative budget of the Earth and its atmosphere was discovered in the 19th century by J. Tyndall, S.A. Arrhenius and, later on, by G.S. Callendar. But only in the second half of the 20th century some systematic and coordinated programmes began to monitor this parameter. The basic criteria of such programmes carried out on a planetary scale are as follows: the measure in the sampling site must be representative for a geographic area as large as possible, scarcely influenced by anthropogenic or natural factors such as large sources and sinks of CO_2 in the surroundings, i.e. densely inhabited areas or areas with a very pronounced vegetative cycle, and finally must not be subject to substantial changes in the land use during the investigation period.

A second aspect of great importance is tied up to the procedures used in the routine measurements and the precision obtained in the field. The results obtained by sites conveniently placed over the whole planet need to be comparable in order to study sources and sinks, fluxes of greenhouse gases, which are absorbed and released by the biosphere, seas etc. Data collected around the whole planet form the bases for the inverse modelling technique for constraining global and regional budgets of atmospheric trace gases (Mikaloff et al. 2004, Wouter et al. 2004).

WMO launched an international program in the seventies last century named GAW (Global Atmospheric Watch), which is the atmospheric module of the Global Climate Observing System (GCOS). The precision requested to participants is at least 0.1 µmole/moles CO_2 (dry air) and this is checked by periodical round robin test coordinated by WMO. Previously the unit ppm by volume has been used. The Data Center is presently the WDCGG (World Data Center for Greenhouse Gases) operated in Tokyo by the Japan Meteorological Agency. Further initiatives by USA led to the establishing of CDIAC (Carbon Dioxide Information Analysis Center) by the Department of Energy of USA at Oak Ridge (Tn) and of the GLOBALVIEW-CO_2 by NOAA-GMD at Boulder (Co). The NOAA-GMD at Boulder also acts as a reference Laboratory (CCL, Central CO_2 Laboratory) for standards (CO_2 in air cylinders) used for calibrating the scale of the instruments, usually Non-Dispersive-Infra-Red (NDIR) analyzers. The CCL at Boulder uses a very accurate manometric calibration system (Zhao et al. 1997).

The Italian and Argentine contribution to Global Atmospheric Watch

At the beginning of nineties, the Italian National Antarctic Research Program (PNRA) and Argentinean Institute (IAA-DNA) started a monitoring program in the frame of the above described activity at the site of Belgrano

(77° 52' S , 34° 38' W) along the southern coast of Weddell sea. The station of Belgrano is located on a nunatak (Bertraub nunatak), at 10 km from the coast line, at an altitude of 255 m a.s.l. In January 1992, a Brewer spectrophotometer was installed, still operational, measuring total Ozone and UV radiation. In February 1994, a second activity began at Jubany station (62° 14' S, 58° 40' W), King George, in the South Shetland archipelago. The site description and details on instrumentation are described in Ciattaglia et al. (1997) and Ciattaglia et al. (1999). The monitoring programme running at Jubany station consists in continuous air sampling and measuring of the CO_2 concentration. The station complies with the criterion of remoteness being far enough from sources and sinks directly connected to human activity. Fig.1 shows the entire record from February 1994 to December 2006, reporting a trend similar to what is observed in other Antarctic stations.

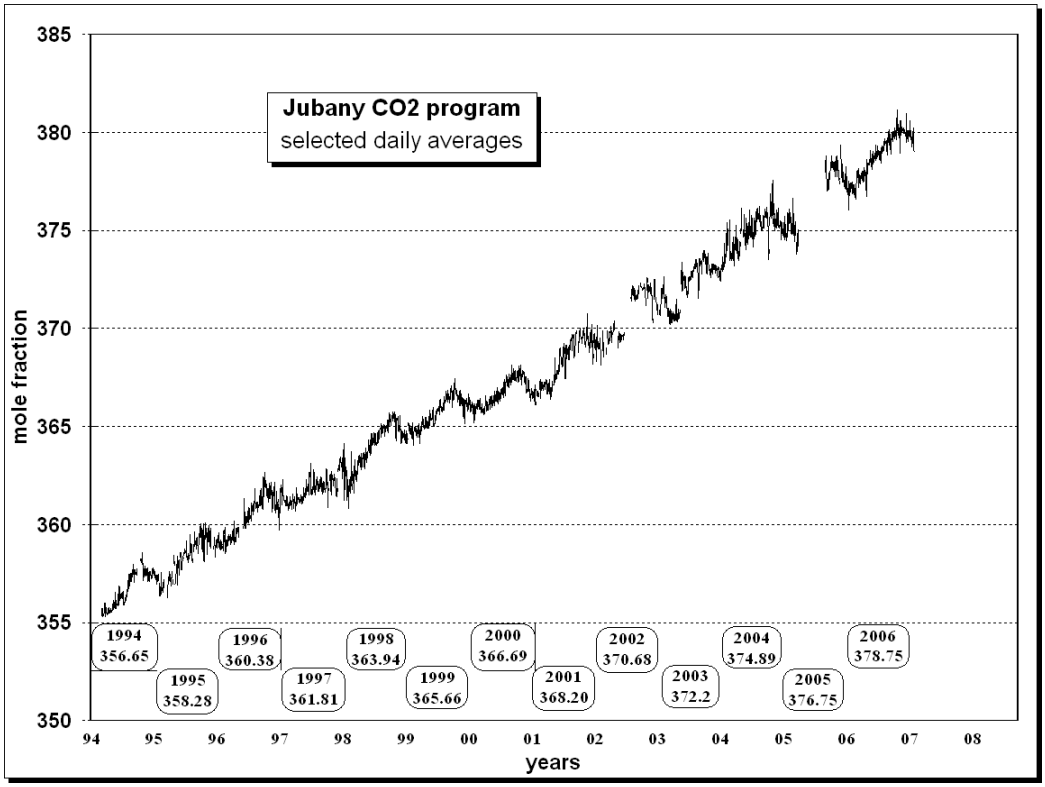

Fig.1: CO_2 daily mean values (mole fraction) selected according to wind and standard dev.

The mean values of each year are indicated at the bottom. The ascendant trend (from 356 µmole/mole of the year 1994 to around 379 µmole/mole of the current year 2006) shows a winter-summer inside oscillation in the order to 2-3 µmole/mole as expected at such latitudinal belt, see Colombo et al. (2000) for a comparison in a wider area. At present 3 stations in Antarctica sample CO_2 continuously: the oldest record are from to South Pole (base Amundsen Scott), the second from Syowa (Japan) and Jubany is the third site hosting a program of this type. Other sites follow monitoring programmes based on flask sampling method, with a weekly frequency usually.

Analysis of some characteristics of CO_2 concentration using a statistical approach

Since the beginning our activity was focussed, besides studies about the trend, on the identification of some relationships with large scale phenomena like El Niño Southern Oscillation (ENSO). A first result is reported in Anav et al. (1999). In the paper the authors interpret the anomalies of the CO_2 content observed during 1997-1998 and conclude that the increase of the air temperature, mostly in the Southern Hemisphere and equatorial regions, is caused by El Niño 1997-1998. Moreover the CO_2 decline observed during 2nd part of 1997 and 1st part of 1998, could explain as enhancement of CO_2 capture by the biosphere. A comparable anomaly of CO_2 trend during the El Niño 1991-1994, was interpreted by R. Feely et al.(1999) in the same way. In particular they ascribe the lessening of the atmospheric CO_2 to stopping or decreasing of the release of carbon dioxide by the upwelling currents rich of CO_2 along the South American west coast. In any case both mechanisms indicate El Niño as a possible cause, a large scale phenomenon which had in 1997-1998 an intensity that was claimed as a record. Mc Phaden M. (1999), attributes to the driving physical mechanism the modification of bottom current of Pacific Ocean to a sort of mix of possibilities related to weather, like Madden-Julian Oscillation (MJO), or to the Pacific Decadal Oscillation (PDO), a sort of course oscillation of the coupling ocean-atmosphere system in Pacific ocean area.

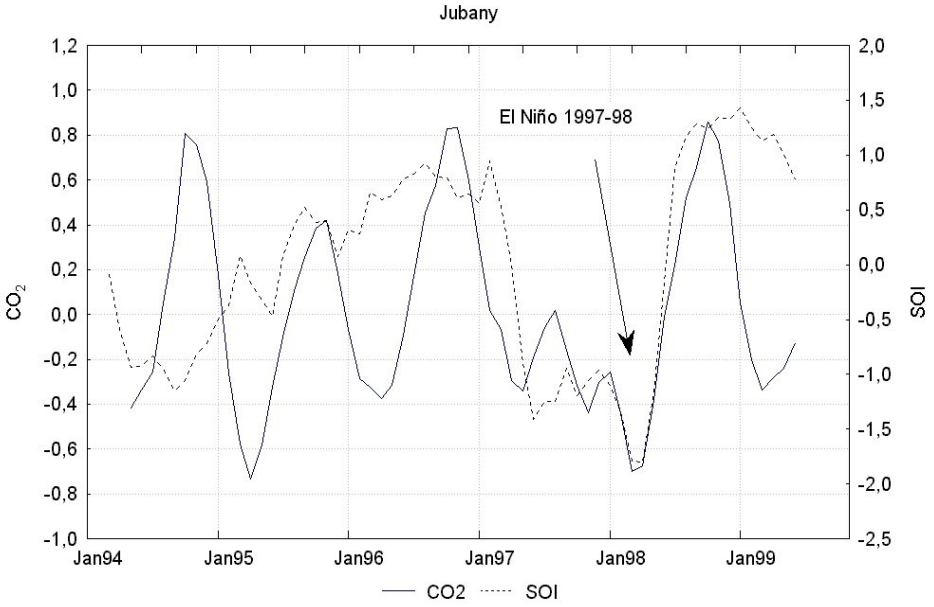

Fig.2: Jubany, SOI and CO_2 normalized and detrended.

The graph in Fig. 2 confirms at first sight that something must connect the two parameters SOI (Southern Oscillation Index), an index which is calculated from the monthly or seasonal fluctuations in the air pressure difference between Tahiti and Darwin areas (see also Trenberth K.E. 1984, Trenberth K.E. et al.1996, and Ropelewski C.F. et al.1987), and the atmospheric carbon dioxide. The two curves (both normalized and detrended by statistical tools) represent the SOI and CO_2 for a period including the 1997-1998 episode.

We want to show here the results of a different approach mainly based on a statistical treatment of the data base we gathered so far. Let's consider the series of 365 (or 366) days of a year and their daily CO_2 mean value, previously filtered according to the criteria described in the papers cited (standard deviation of measure, wind direction and speed, and technical reason are the factors), and calculate the day per day differences: i.e. January 1st 1998 minus January 1st 1997 and so on and repeat this calculation for all the years of the record. Then compute the averages of all these differences for the whole record: i.e. a sort of cumulative year where the day per day differences are the mean of all the years from 1994 to 2005. In the following graph, Fig.3, the two curves representing the 1998-1997 day per day differences (El Niño year), solid line, and the mean 1994-2006 of day per day differences, dashed line, are shown.

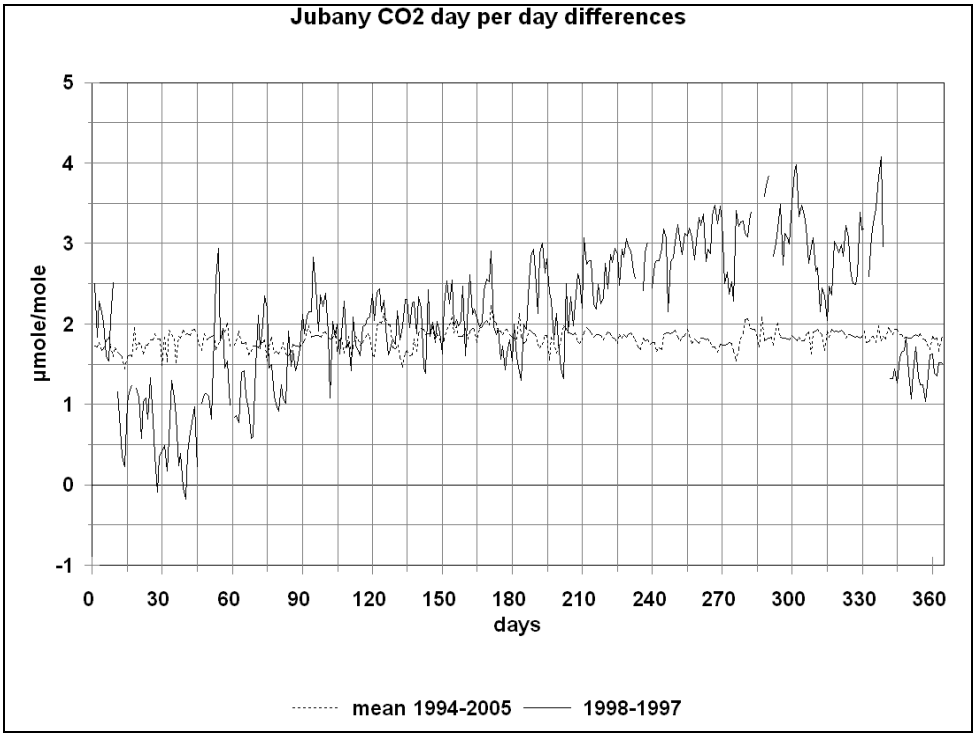

Fig.3. Day per day CO_2 differences: whole period (1994-2005) and El Niño 1997-1998

The values 1998-1997 in the first part of the year (days 0-180) appear smaller than the average of the cumulative year curve, on the other hand in the period corresponding to the days 180-365, values are higher respect to the long-term curve. This can be easily interpreted as a consequence of the decreasing of the CO_2 concentration due to El Niño (for the reasons cited above) during the first 6 month of 1998 (therefore $CO_{2(1998)} < CO_{2(1997)}$) whereas the subsequent recovery in the second part of the year is also explained by the stop of the influence of El Niño itself. This simple algorithm gives us the possibility to have at first glance an idea of what is going on during the year. Another simple and immediate index, which highlights the possibility that some physical factor is influencing the behaviour of the CO_2 measured during the year, is the correlation index calculated for the daily mean values for each pair of contiguous years.

years	Correlation index
1996 vs. 1995	0.85
1997 vs. 1996	**0.62**
1998 vs. 1997	**0.67**
1999 vs. 1998	0.87
2000 vs. 1999	0.86
2001 vs. 2000	0.87
2002 vs. 2001	0.88
2003 vs. 2002	0.76
2004 vs. 2003	0.72
2005 vs. 2004	0.79
2006 vs. 2005	0.91

Table.1 - CO_2 - correlation index for contiguous years

In the Tab. 1 quite high correlation indexes appear for the whole period but the years 1997 vs. 1996 and 1998 vs. 1997 have values notably smaller. Also this simple calculation could give us some information, in quasi real time, during the year.

Comparison of CO_2 data collected in very remote stations in the S.H.

Finally, using the possibility to access the data base described in the foreword, we compared the CO_2 data collected at Jubany Station with some other Antarctic records like South Pole, Palmer Station (both USA) and Amsterdam Island (France) and Indian Ocean. The Fig. 4 shows the results of a comparison between Jubany and Amsterdam Island, where the differences of values (daily mean) for the period 1995-2004 are drawn. The differences are in the order of few µmole/mole, as it should be expected when comparing CO_2 data belonging to very remote sites in the same hemisphere.

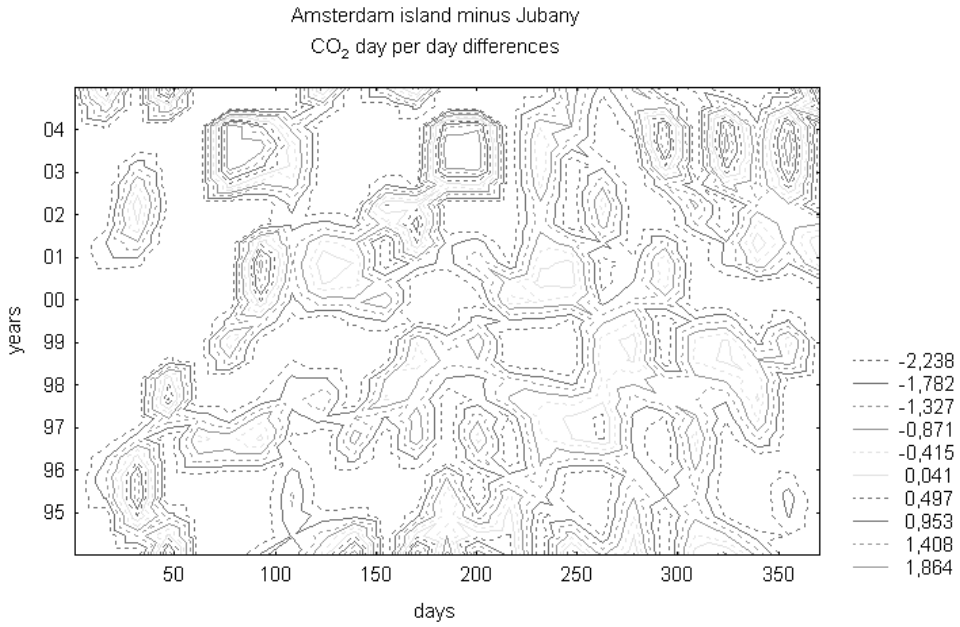

Fig.4 : CO_2 differences between daily mean values recorded at Amsterdam island and Jubany

In Fig. 5 the smoothed trends of three Antarctic sites: JBN(Jubany), SPO (base Amundsen Scott) and PSA (Palmer station) and the concentrations of so called MBL (marine boundary layer) - as a reference - are reported for the period 1994-2004.

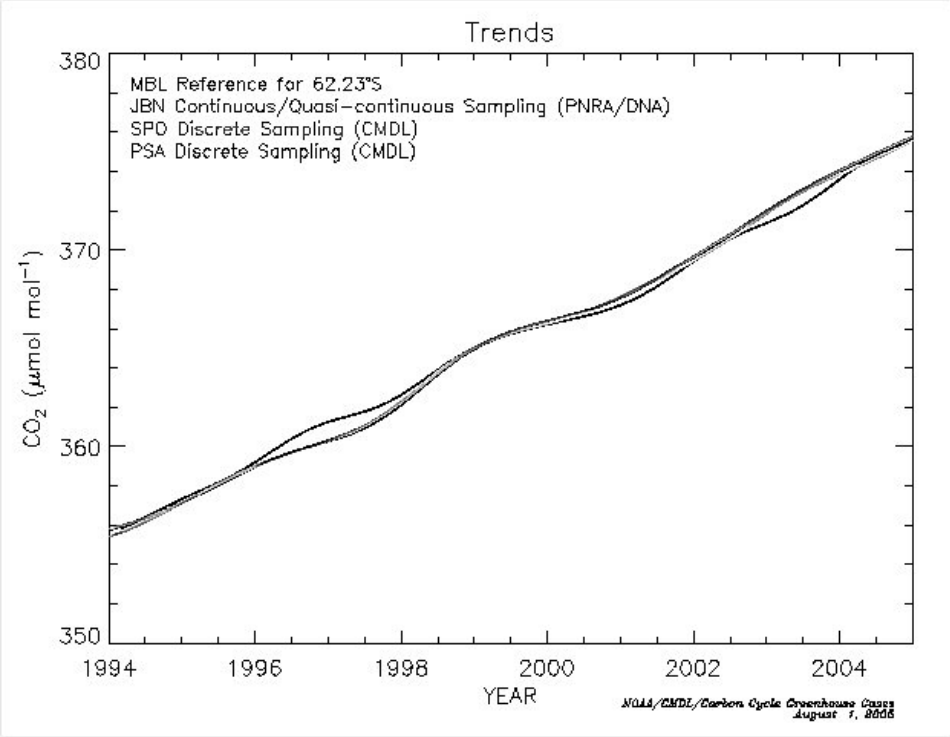

Fig.5: CO_2 smoothed trends of Jubany, South Pole and Palmer station and MBL (marine boundary layer) , courtesy of NOAA-GMD Boulder Co, USA.

Both Fig. 4 and Fig.5 make us quite confident on the quality of our data at Jubany station, despite the difficulties in logistics for a laboratory at those latitudes, especially when timely technical supply and spare parts are needed during the austral winter.

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APPENDIX

List of articles based on investigations performed in the Dallmann Laboratory published in peer-reviewed scientific journals in the years 1999-2007

2007

Abele D, Philipp E, Gonzalez P, Puntarulo S (2007): Marine invertebrate mitochondria and oxidative stress. Frontiers in Bioscience 12, 933-946

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Andrade S, Carlini A, Vodopivez C, Poljak S (2007): Heavy metals in molted fur of the southern elephant seal *Mirounga leonine*. Baseline/ Marine Pollution Bulletin 54, 602-605

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Dick D, Philipp E, Kriews M, Abele D (2007): Is the umbo matrix of bivalve shells (*Laternula elliptica*) a climate archive? Aquatic Toxicology 84. 450-456

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Ritz M (2007): Sex-specific mass loss in chick-rearing South Polar Skuas *Stercorarius maccormicki* stress induced or adaptive? Ibis 149, 156–165

Roleda M Y, Zacher K, Wulff A, Hanelt D, Wiencke C (2007): Susceptibility of spores isolated from different life history stages of Antarctic *Gigartina skottsbergii* (Gigartinales, Rhodophyta) to ultraviolet radiation. Phycologia, submitted

Roleda MY, Zacher K, Wulff A, Hanelt D, Wiencke C (2007): Photosynthetic performance, DNA damage and repair in gametes of the endemic Antarctic brown alga *Ascoseira mirabilis* exposed to ultraviolet radiation. Austral Ecology 32, 917-926

Ruberto L, Vazquez SC, Mac Cormack WP (2007): Bacteriology of extremely cold soils exposed to hydrocarbon pollution. In: Dion P, Nautiyal C S (eds): Microbiology of Extreme Soils. Series Soil Biology, Springer, New York, in press

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