A new experimental setup for UV-exposure experiments on phytoplankton

First results of UV-susceptibility tests on 6 Antarctic phytoplankton species

Steven Benjamins
Supervisor: A.G.J.Buma

Micro Botany group
Department of Marine Biology
Faculty of Mathematics & Natural Sciences
University of Groningen (RUG)
May 1998

ACKNOWLEDGEMENTS

Many people have contributed to the eventual succes of this research project. First and foremost, I would like to thank my supervisor, Dr. A.G.J.Buma, for her good advice and assistance as much as for her patience explaining techniques and protocols.
I would like to thank Drs. P.Boelen for keeping a close eye on my proceedings during Anita's absence, and for always making time to answer questions. From the (semi-) weekly meetings with other members of the UV-group, in particular with Dr. H.Pakker, often came welcome advice. Furthermore I would like to thank everybody at the Department of Marine Biology, for making my (short) stay such a pleasant one.
Last but not least, I would like to thank my fellow students, especially Nancy de Bakker for her vast stores of peptalk.

CONTENTS

ABSTRACT

We designed a culture chamber for phytoplankton in which we attempted to imitate natural temperature and lighting conditions as strictly as possible. In this chamber, PAR doses varied from 144 $\mu E/m^2/s$ to 266 $\mu E/m^2/s$. PAR was generated by 10 TL fluorescent lights, UVR by 4 UV-A and 1 UV-B TL fluorescent light.This combination produced a spectrum comparable to conditions in the field. In this culture chamber, six species of Antarctic phytoplankton were tested for their susceptibility to growth inhibition caused by UV-radiation. In addition, cells were tested for UV-B-induced DNA damage (specifically thymine dimers) by the immunoslot blotting technique. Results indicate that, for several species, UV-B can influence growth rates. In other species, no effect could be found; at least two species failed to grow under these circumstances. Thymine dimer detection proved more difficult than expected. Summarising, this experimental setup has proven a good addition to the equipment of this laboratory.

INTRODUCTION

Ultraviolet radiation (100-400 nm) is an integral, if often disregarded, part of the natural environment as we know it on Earth. It reaches everywhere on the surface, and can penetrate to several tens of meters in water (actual dose influenced by cloud cover, water turbidity, etc.). Only environments in deeper water, as well as subterranean ones, are shielded from its effects. The total UV-spectrum is usually divided into three parts (fig.1):

UV-C 100-280 nm. This is the most reactive type of UV-radiation emitted by the Sun. It is totally blocked by the Earth's stratosphere, in particular by the ozone layer.

UV-B 280-320 nm. This type is less reactive, but causes damage to living cells and tissues. Most of the incoming UV-B-radiation is succesfully blocked by the ozone layer, but a fraction reaches the Earth's surface and shallow waters.

UV-A 320-400 nm. This type of radiation is the least reactive. Effectively all of the incoming fraction reaches Earth's surface and penetrates shallow waters. The lower frequencies are utilised, among others, by many plants for photosynthesis.

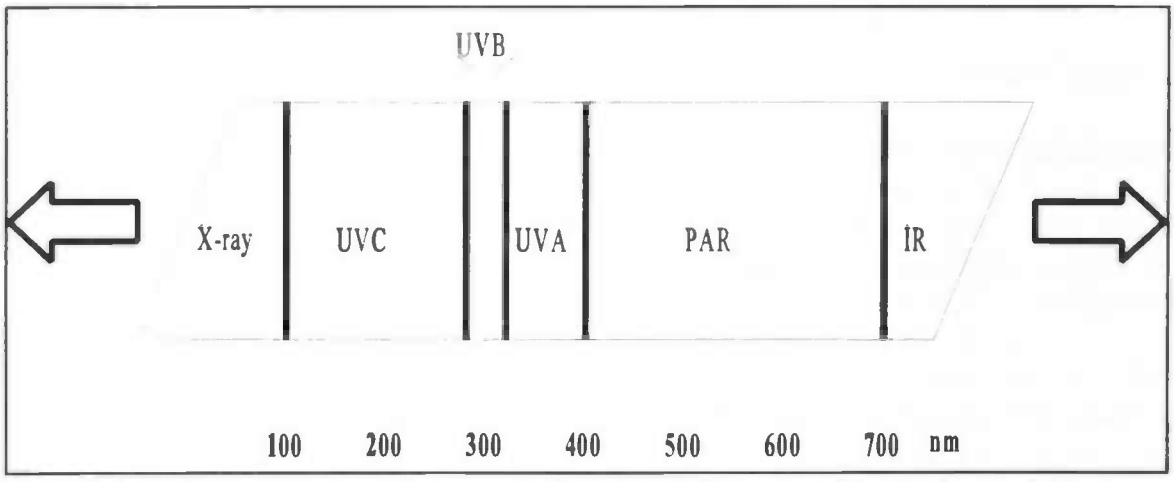
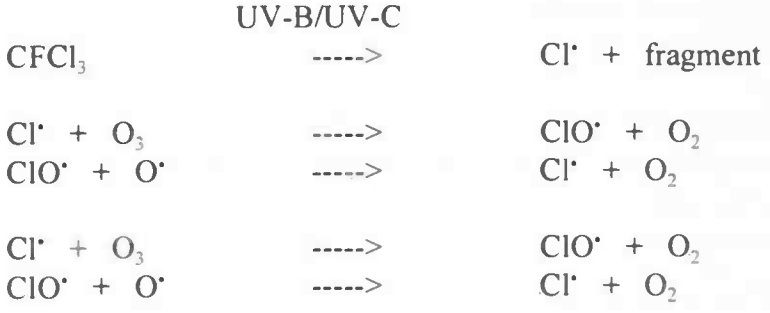


Figure 1: A simplified electromagnetic spectrum. Most abbreviations are explained in the text. PAR = Photosynthetically Active Radiation (visible light), IR = infrared.

In the stratosphere, ozone (O_3) is both formed and broken down by incoming UV radiation (Booth *et al.*, 1997). This ozone layer does not have the same density at all locations; rather, ozone concentrations (measured in Dobson Units) are highest near the poles (350 DU) and become progressively lower when moving towards the equator (200 DU). This is a direct result of lower irradiation levels at the poles: not only is radiation inhibited for several months of the year, but, due to the low angle of incidence, the same amount of incoming radiation energy must be divided over a larger area.
In general, incoming doses of UV-A and UV-B represent approximately 6.3 and 1.5 %, respectively, of total solar irradiance prior to atmospheric entry (Frederick *et al.*, 1989).

In recent years there has been growing concern over possible deleterious effects of increased UV-radiation, due to accelerated breakup of stratospheric ozone (O_3) by man-made molecules, in particular chlorofluorocarbons (CFCs). Over the years, these substances have had several different functions, the best known of which are perhaps as propellants in spraying cans and coolants in refrigerators. Until recently, the fact that these substances are so stable and non-reactive was largely viewed as a good thing. But the chemical inertness of these molecules practically guarantees their eventual accumulation in the upper stratosphere, where ultraviolet radiation breaks the molecules apart into, among others, chloride and fluoride radicals. These are, in contrast, highly reactive and immediately start acting as a catalyst, breaking apart O_3 into molecular oxygen, O_2, in the following reaction pathway:



Etc. (from Veen *et al.*, 1995)

It has been estimated that a single CFC molecule is able to degrade 100,000 molecules of O_3 before its radicals are removed from the upper atmosphere as the non-reactive hydrogen chloride, HCl and hydrogen fluoride, HF (Veen *et al.*, 1995).
The net result of this human-induced ozone depletion is a significant increase in the amount of ultraviolet radiation that reaches the surface (Madronich *et al*, 1995). Not only does the total amount of incoming radiation increase, but there is also a significant increase in UV-B/PAR ratio[1]. Also, the total incoming UV-B spectrum is increasingly composed of shorter wavelengths (Frederick & Lubin, 1994), which are damaging to living cells and tissues (a high Biological Effective Dosis).

The deleterious effects of CFCs - a significant ozone reduction in relatively short periods of time - are even more marked at the Earth's poles. This is partially caused by the high amounts of ozone in these regions (see above), but also by the low ambient temperatures, which form a good environment for ozone degradation (Veen *et al.*, 1995). For the last 15-20 years, the significant (50%; Gleason *et al.*, 1993) reduction in ozone levels at high austral latitudes during Oct-Jan has appeared every year, becoming widely known as the "Ozone Hole" (Gleason *et al*, 1993). This ozone-depleted section of the stratosphere forms in early austral Spring (Sept.-Oct.) and lasts until early Summer (Dec.-Jan.). It varies widely in size, is usually asymmetrical in shape and usually rotates above the Antarctic continent once every

[1] For all practical purposes, it is assumed that the *total* UV-output of the Sun has remained constant when compared to the total PAR-output

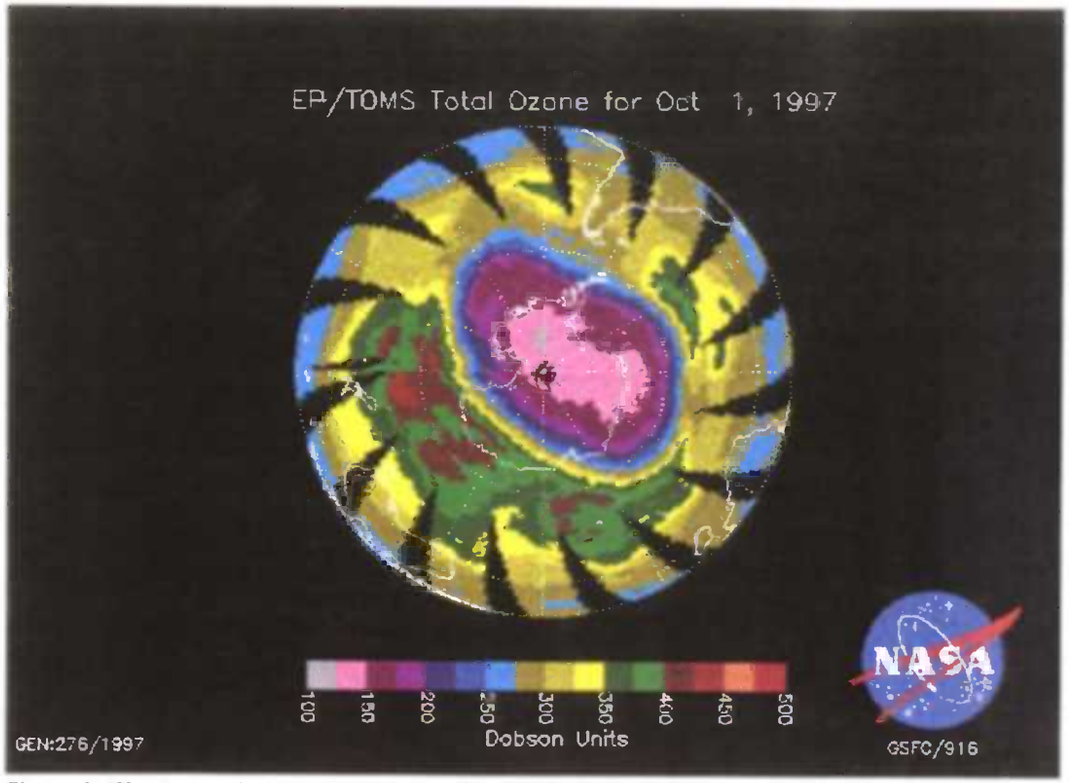


Figure 2: The Antarctic ozone hole, imaged by NASA's Earth Probe on October 1st, 1997. Courtesy NASA.

few days (Booth *et al*, 1997; data from NASA).
This means that the dosage of UV-radiation that reaches the surface at any one given location will often fluctuate in the course of less than a week.

UV-B radiation is a potential threat to most biota; although the intensity of incoming radiation is low, its effects are marked. It is capable of doing severe damage to living cells (Hoeijmakers *et al*, 1994), particularly to the DNA. Since DNA is an effective absorber of ultraviolet photons (230-310 nm, with peak absorbance at 260 nm; Friedberg *et al*., 1995), it is extremely sensitive to this type of radiation (Alberts *et al*., 1994), and damage in the molecular structure is often the result (Buma *et al*., 1997; Sage, 1993).
The most common types of damages are a result of dimerisation, when two adjacent (pyrimidine) bases are induced by the absorption of a UV-photon to form hydrogen bonds with each other, instead of with their "own" complementary base. Most of these are cyclobutane pyrimidine dimers, the most common type of which are thymine dimers (fig.3) and pyrimidine (6-4) pyrimidone dimers. These dimers prohibit the replication of the afflicted DNA molecule by DNA-replicase, which does not seem to be able to fit itself onto the altered structure. Other ways in which UV-B-radiation can influence the functioning and/or survival of marine phytoplankton include, among others, growth reduction, inhibition of photosynthesis, and inhibition of motility in the watercolumn (Buma *et al*., 1996; Häder, 1994; Kramer, 1990 for a more complete listing).

Once inflicted, DNA damage can be repaired in several different ways (Friedberg *et al*., 1995; Sage, 1993). In one method, known as *photoreactivation*, the hydrogen bonds within the dimer are broken apart by an enzyme known as DNA photolyase, under influence of

irradiation in the 300-500 nm-range (which is why this method is often called "light repair". Another way in which such damage can be restored is by *nucleotide excision repair*, in which the entire dimer is removed from its DNA strand by a group of co-working enzymes. Since this process can take place without input of radiation, it is also known as "dark repair". Finally, the information contained in the damaged DNA strand can also be restored by its complementary; this last process is known as *recombinational repair*. Some species are also capable of synthesizing intra- or extracellular substances which give a certain amount of protection against UV-B, such as MAA's (Mycosporine-like Amino Acids; Karentz *et al*, 1991; Xiong *et al.*, 1997).

The effects of UV-B radiation on living cells and tissues is usually quantified by use of an action spectrum. Such a spectrum expresses the relative effectiveness of UV-B radiation of different wavelengths in eliciting a certain response, usually normalised to 1 at the most effective wavelength. Over the years, several such action spectra have been developed (Rundel, 1983); in this case, the Setlow DNA damage spectrum, normalised at 300 nm, is used (Setlow, 1974).

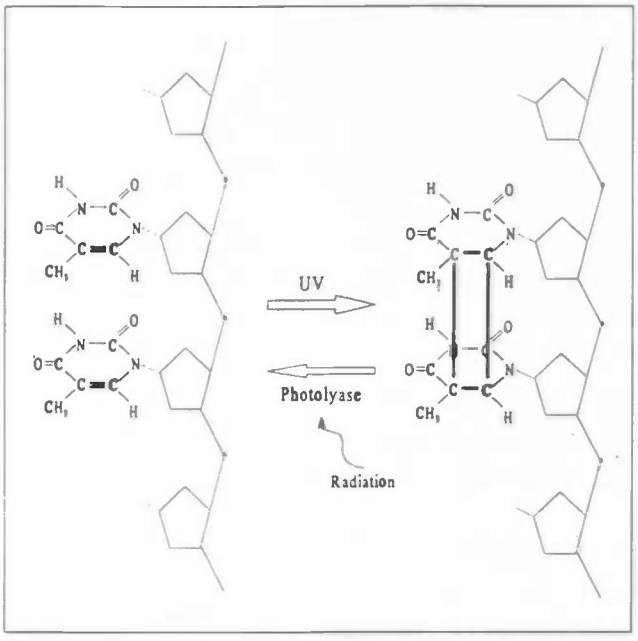


Figure 3. Schematic overview of cyclobutane thymine dimer formation/deactivation. Courtesy of Chantal Beekman.

In the Antarctic, primary production is usually quite low, with only coastal areas and the Marginal Ice Zone exhibiting high densities of biomass (Buma, 1992). In these locations, circumstances may favour the occurrence of phytoplankton blooms, which in turn attract the large schools of krill (*Euphausiidae*) so well-known in these regions. A high spatial and temporal variability in phytoplankton distribution and abundance has been recorded in the field (Helbling *et al.*, 1995; Clark & Leakey, 1996; Villafañe *et al.*, 1995). In general, the phytoplankton composition is characterised by central and pennate diatoms, dinoflagellates, (nano-)flagellates and prymnesiophytes.
Because the Ozone Hole is such a new phenomenon, it seems unlikely that any adaptation to increased UV-levels has already occured (Helbling *et al.*, 1996; Villafañe *et al.*, 1995). Since unicellular algae, such as the ones used in this particular experiment, stand at the very basis of the Antarctic food chain (and are locally capable of sustaining high concentrations of animal life as the famous "algal blooms"), it is of vital importance to reach a scientific understanding of the possible effects of increased radiation levels on these algae, in order to make safe predictions on the future of the Antarctic marine ecosystem as a whole (Davidson *et al.*, 1996; Karentz *et al.*, 1991; Smith *et al.*, 1992).

Phytoplankton research has been carried out for many years, but many species, including many from relatively extreme (and thus interesting) habitats, are still difficult to study. The main reason for this is the lack of success in cultivating these species in the laboratory. This is especially true for Antarctic species of phytoplankton. In many laboratory experiments, cultures are being kept under relatively low amounts of PAR, although doses *in situ* are much higher. Also, the amount of UV-A and/or UV-B that is applied in such experiments often deviates from natural conditions. We attempted to construct an experimental setup in which doses and ratios of PAR, UV-A and UV-B would match ratios measured in the field as closely as possible (Sage, 1993). This setup was designed with Antarctic phytoplankton in mind, but can be readily adapted to accomodate species from more temperate waters. In this setup, several species of Antarctic micro-algae of different taxonomic groups were exposed to different lighting conditions, to test their susceptibility to DNA-damage and growth inhibition due to UV-radiation.

Since pyrimidine dimers are formed only and specifically when DNA is irradiated with UV-B-radiation, the amount of dimers present in any given sample of DNA will probably give a good estimate of the total amount of UV-B-induced damage in that sample.
A good test for the detection and quantification of pyrimidine dimers has been developed over the years (Buma *et al.*, 1995; and Appendix 4). This test will be applied here, to attempt quantification of DNA damage by ultraviolet radiation. In addition, cultures will be sampled on a daily basis to acquire growth curves (by the old and trusted method of cell counting "by eye").

MATERIALS & METHODS

Used species:

The Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP; part of Bigelow Laboratory for Ocean Sciences) maintains stocks of many Antarctic phytoplankton species. Samples of these cultures are readily available to researchers at other institutes for a small fee. These samples are generally sent in small vials, which are cooled in ice and packed into thermos bottles.

Prior to starting this particular experiment, stock cultures of several species had been received in this fashion from the Bigelow Culture collection (for details, check Appendix 7). Since most of these stocks readily adapted to local laboratory growing conditions (a 4°C climate chamber), a relatively large choice of species was available. A selection was made on the basis of cell density, growth rate, taxonomic group and relative fragility (that is, resistance to stirring). The species that were finally chosen were the following:

No:		species:
A	-	*Chaetoceros brevis* (central diatom, labculture, previously used by Nancy de Bakker; originally CCMP163)
B	-	*Porosira glacialis* (central diatom, CCMP1099)
C	-	*Pyramimonas* sp. (*Prasinophyceae*, flagellate, isolated by A.G.J.Buma at Weddell-Scotia Confluence, 1988)
D	-	*Phaeocystis* sp. (*Prymnesiophyceae*, CCMP1374)
E	-	*Gymnodinium* sp. (dinoflagellate, CCMP1383)
F	-	*Fragilariopsis cylindrus* (pennate diatom, CCMP1102)

These six species combined a reasonable growth rate (surveyed by the naked eye in the respective serum bottles) with "reasonably" high cell densities prior to dilution; in addition, all species were relatively robust in shape. Other species, such as the large diatom *Corethron criophylum* were ultimately left out of this experiment partially because it was feared that it would not be able to withstand the stress of mixing.

The only two species that had previously been cultured in this laboratory were *Ch.brevis* (which had also been used in UV-irradiation experiments some months before; De Bakker, 1997) and *Pyramimonas* sp., which had been used in photoadaptation experiments (Buma, 1992, doctoral thesis). The other four species were newcomers to this laboratory: part of this experiment was, in fact, centered around learning more about the culturing of these species under laboratory conditions. In this way, these species might become valuable research subjects for later experiments.

One other important factor in choosing these six species was that they represented a diverse sample of the total Antarctic phytoplankton community. Although diatoms are often by far the most abundant species (Buma, 1992), flagellates (prasinophytes), cryptophytes and dinophytes also occur. In this regard, it looked promising to compare these representatives of such different taxonomic groups in the way they were affected by UV-radiation, both in growth rate and in DNA damage.

Culturing conditions:

The cultures arrived in our laboratory in small sealed-off containers, packed together in a thermos bottle. Each sample was brought into its own separate 250-ml erlenmeyer, to which 200 ml of F/2-growth medium had previously been added. These cultures were then incubated in a 4°C-chamber under an 8/16 hr light regime (light emitted by a fluorescent light). In due course, a secondary culture collection was established in a Fridina - Refrigerator, to prepare for contingencies. These cultures were exposed to a 10/14 hr light/dark regime.
Pre-experiment culturing usually started a week before the start of any experiment. Cells were sampled (depending on estimated growth rate) in amounts ranging from 25 ml (*Chaetoceros brevis*) to 30 ml (*Fragilariopsis cylindrus*) and transferred to 500 ml F/2-medium.

Experimental setup

At the start of this experiment, the initial culturing plan was to conduct the entire experiment in a Fridina refrigerator. The TL-armature was specifically designed with this in mind.
To test the capability of the refrigerator to sustain these species, regular measurements were taken using TinyTalks, which were placed at various locations in the refrigerator and recorded temperatures every few minutes for 2-3 days. These measurements indicated that, because such a large number of TL lamps (10) was used, inside temperatures could not be kept constant. In particular, the temperature sometimes rose 2 - 3°C in the course of less than 2 hours (fig.4 for an example of TinyTalk data). Such large fluctuations ultimately posed insurmountable problems for the culturing of Antarctic species under these light conditions.

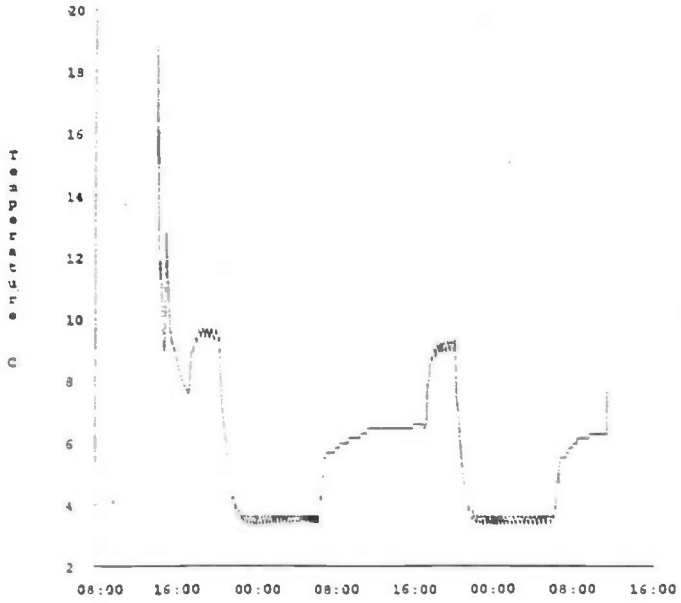


Figure 4: TinyTalk data printout after ± 2 days of incubation in the Fridina refrigerator. Note the strong rise in temperature around 05:00 hrs. The temperature "spike" between 17.00 hrs and 21.00 hrs is due to a technical error.

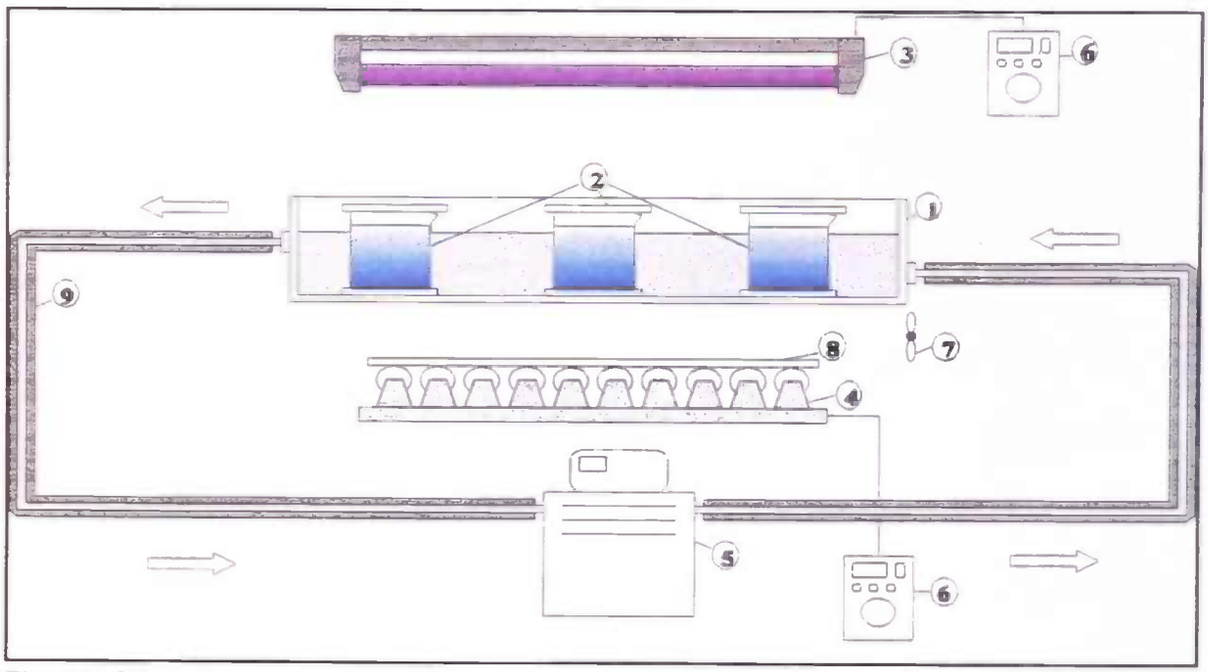


Figure 5. Schematic overview of experimental setup, sideway view. 1 = perspex incubator, 2 = culture containers, covered by cutoff filters and positioned on petri dishes, 3 = UV light source (5 lamps), 4 = PAR source (10 lamps), 5 = cryostat, 6 = digital time switch, 7 = ventilator, 8 = protection plate (perspex), 9 = insulated tubing. Arrows denote direction of current.

After these results, it was decided that further experiments would be done in a more seasoned fashion. A large perspex incubator (81.5 x 61.5 x 9.5 cm, 0.3 cm thick; outside measurements) was filled with tapwater to which several liters of 0.25M ethyleneglycol were added; this lowered the freezing point sufficiently to simulate Antarctic temperatures. The incubator was divided into four compartments, each one of which had its own in- and outflow piece. Through these, the compartments were all connected by flexible, insulated tubing to a Neolab RTE 220 cryostate, which maintained a constant flow of water and cooled it to an average of 4.5°C ±0.5°C (fig.6 for details).

The incubator was placed inside a large metal frame, which also supported PAR lights below the incubator, and UV lights suspended above. The positions of the lights were adjustable (with some difficulty) by means of either changing the length of the chain supporting the UV-armature, or changing the position of the supporting beams on which the PAR armature rested. In addition, a small ventilator, placed on a separate beam, provided the necessary air current to prevent excess condensation.

Photosynthetically Active Radiation (400-700 nm) was provided by 10 Osram L 18W TL (fluorescent lights) lamps, shining from ca. 20 cms below, through the perspex bottom. These lamps shone constantly every day, under a 14hr light/10 hr dark regime; they were switched on at 03.00 hr a.m. and switched off at 17.00 hr p.m., by an Elro digital time switch (type No. 739). It was assumed that no extinction of PAR took place as it passed through the perspex, but that all traces of emitted UV-A-radiation would be blocked.

The lamps were placed at a right angle to the long axis of the incubator, to ensure that no one category would receive a significantly higher dose of PAR (This position was also demanded by the construction specifics of the entire setup). The assumption here was that levels of PAR would be high enough to permit both growth and photosynthesis, but would not be high enough to induce photoinhibition.
Ca. 31 cms above the perspex incubator, a combination of UV-lamps provided ultraviolet light; a combination of 1 Philips UV-B 20W-lamp surrounded by 4 Philips R-UV-A 40W-lamps was applied in this case, since previous experience had shown such a combination to provide a spectrum approximately comparable to the solar spectrum. This combination was controlled by an Elro digital time switch and shone for 3 hours/day during the 3 days of each irradiation experiment.

In each compartment, three different culture containers were placed at fixed positions: 1 in the middle and two on the side (center of cultivation container ± 18 cm from the far sides, fig. 6). These positions were used throughout the entire set of experiments. Compartments were assigned letters according to distance from the front (A,B,C,D) and positions were numbered from left to right (1,2,3).

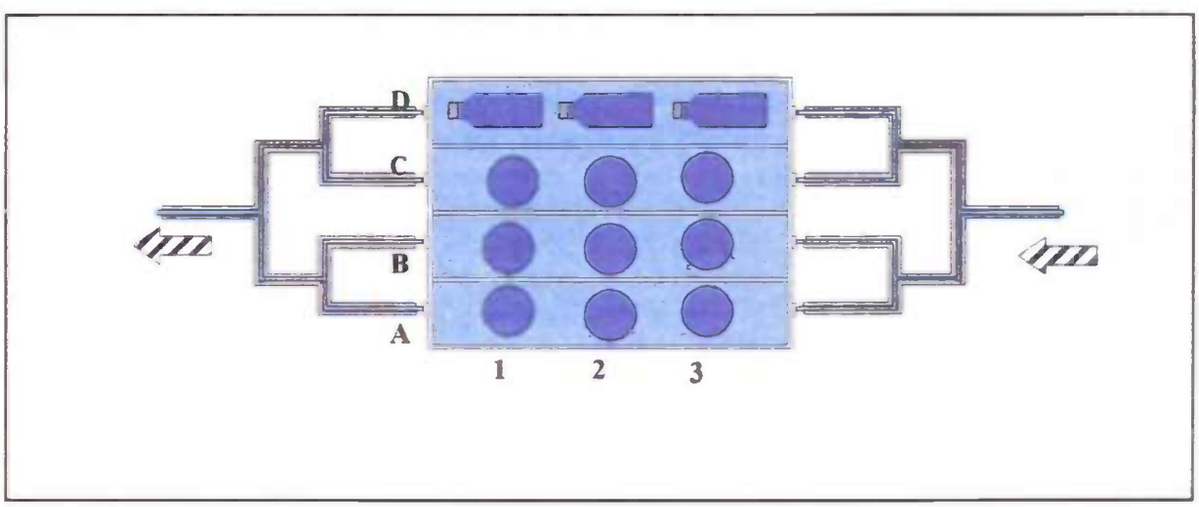


Figure 6. Schematic overview of experimental setup, top view. Letters (A,B,C,D) denote compartments, numbers (1,2,3) denote positions. Circles stand for culture containers, shapes in compartment D stand for preculturing bottles. Hatched arrows denote direction of current.

A Macam SR9910 spectroradiometer was used to measure the light spectra; measurements were conducted using a 4π collector. The collector was attached to the Macam instrument by means of a 1-m quartz cable. The entire spectrum, ranging from 280 nm to 700 nm, was measured at 1 nm-intervals. The Setlow action spectrum was employed to calculate the effective daily UV-doses (based on exposure periods of 3 hrs/d). These measurements were taken by positioning the 4π collector in the center of a collection container and moving this container from one position to another. Each measurement was conducted at least twice; in some cases, a third measurement was taken to decrease spreading between values.

The cultures in the front compartment were exposed only to PAR. This was achieved by using small sheets of UV-opaque perspex to block out any incoming UV-radiation.
In the second compartment, cultures were exposed to both PAR and UV-A, by using (simple, windowpane-type) glass plates to cut off all UV-B.
Finally, cultures in the third compartment received PAR, UV-A and UV-B radiation, but were protected from traces of UV-C radiation by 305 Schott cut-off filters. Every day, culture containers were switched around in each compartment, so that no one culture would receive a significantly higher dosage of either PAR, UV-A or UV-B.
The fourth compartment was generally left empty, and was only used to acclimatise new cultures before starting new experiments (fig. 5 for schematic overview).

Only one exeption was made to the scheme outlined above: during the first experiment (using *C.brevis*, *P.glacialis* and *Pyramimonas* sp.), cultures in Compartment A (which were supposed to be irradiated solely with PAR) were accidentally covered with UV-*transparent* perspex sheets, rather than with UV-opaque ones.
Macam measurements revealed no significant difference in irradiation regime (Appendix 5); the UV-lamps had apparantly been too far away to cause much damage. Nevertheless, these cultures will from now on be referred to as "PAR"-cultures instead of PAR-cultures.

The species mentioned above were grown in purified and autoclaved seawater with a salinity of 35 ‰; nutrients were added accordingly to create F/2 growth medium (Guillard, 1975). All cultures were sampled (25 - 30 ml) from small collection vials in the 4°C-chamber, which were kept in storage for eventualities. Each species was initially grown in 500 ml serum bottles, which were put in the experimental setup, in water of 4.5 °C ±0.5°C. Just before the start of each experiment, the 500 ml culture stocks were mixed with 1600 ml F/2 medium, in sterile 3 l-serum bottles. The resulting ±2100 ml mixture was then divided over three 1L-glass containers, each of which then contained approximately 700 ml of culture. Water levels in each culture container were all approx. 7 cms. To prevent the glass containers from floating away from their fixed positions, each one was placed on an upside-down petri dish lying on the bottom of the incubator.

In the experimental setup, three species were incubated at the same time (fig.6 for details). UV-radiation was applied during 3 days, after which all cultures were subsampled.
These subsamples (approx. 100 ml, in sterile 100ml serum bottles) were then given the opportunity to start DNA repair (at the same positions), under a constant dose of PAR, for another 1.5 weeks. Every day, 2 ml-samples were taken to establish growth curves.
All experiments were repeated once, so that for each measurement, two data sets were available. From this point onward, all experiments will either be referred to as "Batch 1" or "Batch 2", signifying either the 1st or the 2nd experiment in which the species in question was used.

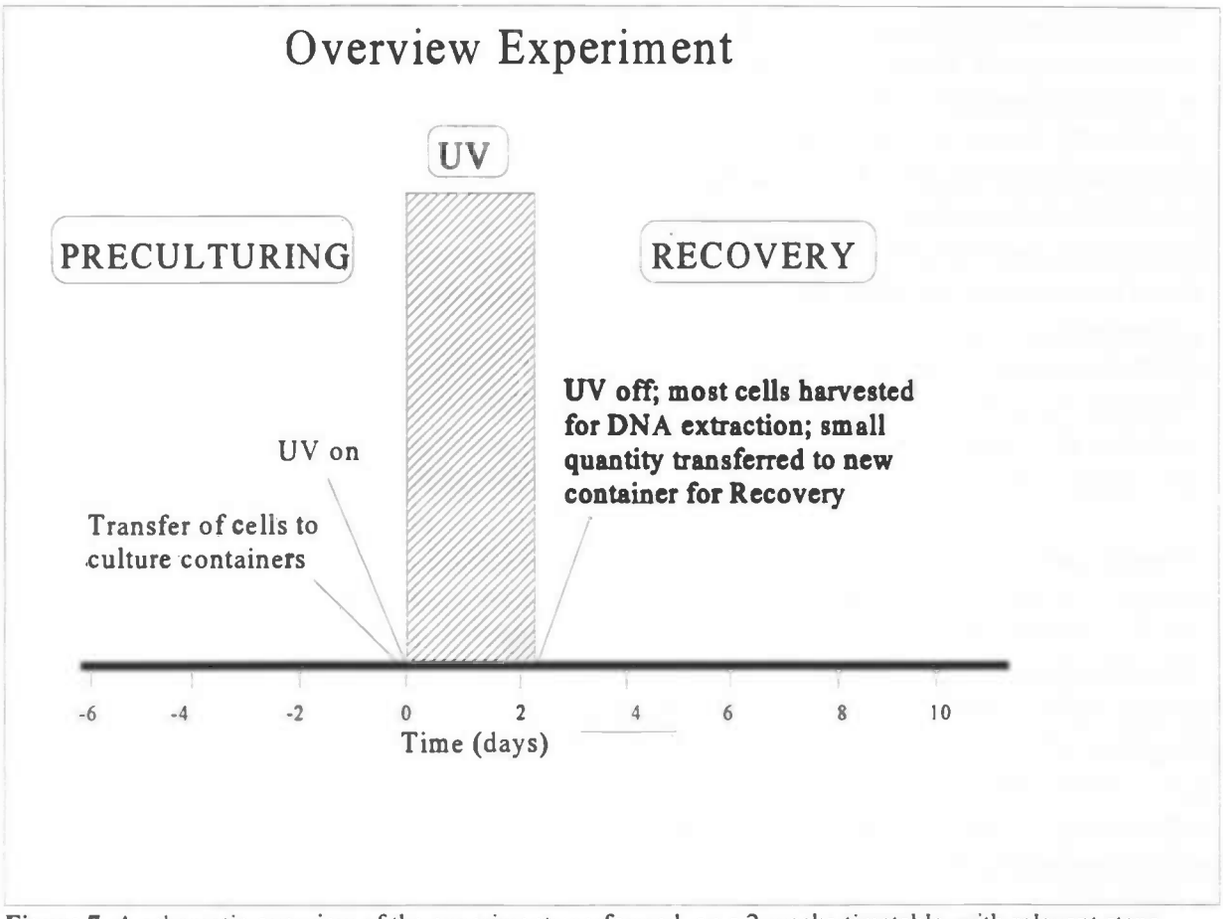


Figure 7: A schematic overview of the experiments performed, on a 2-weeks-timetable, with relevant steps included. The hatched area denotes the period in which UVR is supplied, in three 3-hr-periods (in other words, no continuous irradiation).

Used techniques:

- Cell counts: Each day, directly after UV irradiation had ended, a 2-ml sample was taken from each culture container. These samples were then fixed by use of 10 µl formaline (37%) and stored at approximately 2°C. Directly prior to sampling, containers were gently stirred for approx. one minute. These samples were then used in cell counting experiments.

- DNA extraction: Cells were filtrated over a GF/F glassfiber filter and immediately frozen in liquid nitrogen. DNA was extracted according to the CTAB protocol (of H.Klerks, modified from Maniatis *et al*, 1982). A short protocol is added as Appendix 2; Spoelstra, 1996; Riegel, 1996 for details.

- DNA quantification: DNA quantification was performed by using a nucleic acid stain for double-stranded DNA, PicoGreen® dsDNA (P-7581) from Molecular Probes. This substance emits a fluorescent light when in contact with dsDNA, which can be measured and quantified. In this case, measurements were done on a Victor 1420 multilabel from Wallac (courtesy of the National Institute for Coastal and Marine Management, or RIKZ). A protocol is added (Appendix 3).

- Immunoslot blotting: In this technique, DNA (isolated by the CTAB-procedure) was brought onto a membrane. The adding of a blocking agent (milk powder) ensured that all sites where no DNA was present were successfully blocked. An antibody was added, which bound specifically to thymine dimers (Roza *et al.*, 1988). Another antibody, which possessed a HorseRadish Peroxidase (HRP) group, was bound to the first. This group then served as a reactive site for the ECL light reaction (used to be Lumiphos in the old protocol), in which a P group is removed from the ECL molecule while emitting a photon. The results of this reaction could be made visible by adding ECL to the blot under low-light conditions and exposing a photosensitive sheet to it.This sheet was scanned and processed, during which the amount of DNA damage could be quantified.
The blotting protocol used in this experiment, according to Roza *et al.*, (1988) (Appendix 4), has been modified in several ways. The new protocol differs from the old protocol in the following:

- membrane: In the new protocol, samples are filtrated over a nitrocellulose membrane, instead of a nylon one. At the same time, pore size has decreased from 0.45 μm to 0.1 μm.

- the Lumiphos® solution has been exchanged for a new kit provided by ECL Systems. This system works with HRP (or Horseradish Peroxidase) as the 2nd antibody, using ECL as the reagens.

- the Kodak X-AR 5 photosensitive sheet has been replaced with a new, ECL-approved type of photosensitive sheet (Hyperfilm™ECL™).

- the usage of HRP instead of Lumiphos eliminates the need for repeated immersion of the membrane in buffer C, so this step has been removed from the new protocol.

These changes in protocol had some evident effects. First of all, the smaller pore size increased the amount of DNA left on the membrane. Also, the use of HRP turned out to be an improvement, because the AP used in the old protocol is also commonly found in bacteria. This means that if a sample is not completely sterile, bacteria may also contribute to the staining. Since HRP is largely confined to horseradish (genus *Taraxacum*), less aspecific staining can be expected. All in all, the new protocol increased blotting sensitivity.

- Dimer quantification: The photosensitive sheets containing spots of varying darkness were analysed using an Image Quant™ version 4.2 computer system, to which an Umax scanner was linked. The sheets were scanned using Photo Adobe; Image Quant was used to measure spot size, average blackness, volume etc. These quantifications were used to calculate the amounts of thymine dimers which were present in each sample (calculations kindly provided by drs. P.Boelen, 1998).

EXPERIMENTS & ANALYSES

Growth rate experiment

The objective of this experiment was to examine the effects of the different UV-treatments on growth rate of algae cultures, as determined by daily cell counts. Cultures had been adapted to grow at 14 hours of PAR a day, at an average temperature of 2°C. This regime was continued during the experiment, as this was deemed a fair approximation of natural circumstances during the austral summer.
Every culture was regularly sampled (2 ml) before the start of the experiment. This served to produce standard growth curves. During the experiment, cultures were sampled every day at approximately the same time (13.00 hrs), to establish whether a change in growth rate had occured. Just prior to sampling culture containers were stirred (using a stirring bar) for approximately 1 min.
The 2 ml-samples were fixed with 10 µl 37%-formaline solution, so that they could safely be stored (refrigerated) for extended periods of time.
At the end of each UV-irradiation period, the cultures were harvested through filtration. Prior to this, approximately 100 ml of culture was transferred to 100 ml serum bottles, to examine whether recovery would take place. Day-to-day sampling was continued from these bottles (mixing was now done manually instead of by a stirring bar). The rest of each culture was filtrated over GF/F Whitman glassfiber filters. The filters were then transferred to 2 ml-Eppendorf cups and frozen in liquid nitrogen. Finally, the cups were stored at -80°C.

The 2 ml-samples were primarily used for cell counts. Approximately 1 ml subsample was examined in a Sedgewick Rafter counting chamber on an Olympus Inverted Research Microscope, model IMT-2. Each chamber was left alone for at least 20 minutes, to give cells the opportunity to settle on the bottom. Every sample was counted at least twice, to obtain several cell counts of at least 250 cells. From these cell counts, growth rates were calculated by using the following equation:

growth rate:

$$\mu(d^{-1}) = 2.303/t \cdot \log_{10}(N_t/N_0)$$

The objective of this particular experiment was to compare growth rates prior to, during and after UV-radiation. While not exactly quantifiable, a significant difference between these growth rates should be indicative of UV stress.

Immunoslot blotting experiment

After irradiation, cells were collected on a GF/F filter. The harvested cells were submitted to DNA-extraction procedures according to the CTAB protocol (Appendix 2). The extracted DNA samples were then blotted according to the revised Immunoslot blotting protocol (Appendix 4). Each sample was transferred in amounts of both 100 and 200 µl, to examine what the ideal amount for transfer was. The resulting sheets were scanned by a Umax scanner and subsequently analysed using SigmaPlot 3.0, MS-Excel 5.0 and ImageQuant. Results are added in Appendices 11 - 12.

RESULTS

Lighting conditions

From initial measurements with the Macam Photospectrometer it became clear that the dose of incoming UV-B radiation varied widely within compartments: radiation was highest in compartment C, directly under the UV-B-TL lamp. Although cultures in compartments A and B were shielded from UV-B-radiation by means of protective filters (perspex and glass, respectively), a low dose of UV-B was still measured. These values probably represent inaccuracies in the Macam collector, a kind of background noise (Appendix 5).

Position	A(all)-av	B(all)-av	C(all)-av	
PAR (LiCor)	94.5	144.9667	136.6	$\mu E/m^2/s$
PAR (Macam)	153.6559	196.5114	227.6252	$\mu E/m^2/s$
PAR (Macam)	34.78069	44.47963	51.53661	W/m^2
UV-A	1.443696	5.868598	7.965268	W/m^2
UV-B	0.010008	0.042435	0.541559	W/m^2
UV-B (Setlow)	14.82799	7.592502	1342.479	$J/m^2/d$
UV-B/UV-A	0.593496	0.723949	6.795746	(%)
UV-B/PAR	0.024966	0.095625	1.061978	(%)
UV-B/UV-A(N)			2.7	(%)
UV-B/PAR(N)			0.19	(%)

Table 1: Overview of spectrometer data and UV-B/UV-A & UV-B/PAR; both Licor and Macam measurements are included. All values averaged over 3 locations within compartments; A(all)-av = averaged over 6 measurements due to differences in perspex cover. Measurements marked with "(N)" represent Natural ratios as measured on the roof of the Biological Center (A.G.J.Buma, pers.comm., 1998).

The C compartment (directly under the UV-B lamp, under a Schott 305nm cutoff filter) received the highest dose of UV-B (UVB/PAR ratio). PAR doses were measured with both Macam and LiCor photospectrometer. On average, large differences between these two measurements were obvious (Table 1). A possible explanation for these differences might be the different types of collector being used (4π in the Macam, cosine in the LiCor).In this table, several differences between compartments are visible. The first compartment (Compartment A), in which cultures were (supposed to be) exposed only to PAR, received significantly less incoming PAR than Compartments B and C. Still, the standard errors in compartment A were smaller than in either Compartment B or C. Because all culture chambers were switched around daily (from position 3 to 2 to 1 to 3 again), they were exposed to varying amounts of PAR. These PAR levels were significantly lower than outside measurements (± 1500 - 2000 $\mu E/m^2/s$; Dr.A.G.J.Buma, pers.comm., 1998); this was partly a result of practical considerations (originally, the PAR armature was designed for use inside a Fridina refrigerator, and surface area was thus limited) and partly because such high levels of PAR frequently lead to photoinhibition in phytoplankton - a condition we wished to avoid. Moreover, it was assumed that such levels of PAR would be sufficient for the species used in this experiment to exhibit both photosynthesis and growth.

Cell counting

Average growth rates of each species were plotted in figs 9.1. - 9.6 (Appendix 9). In general, some species were better able to adapt to culturing conditions than others. It turned out that good pre-culturing growth in the 4°C-chamber (or the Fridina refrigerator) was no guarantee for good growth in the actual experimental setup. A case in point was formed by the *Phaeocystis* sp.- culture: no growth occured in the 4°C-chamber, good growth occured in the Fridina refrigerator, and hardly any growth at all during the actual experiment. Apparently, more research into the particular growing conditions of these species is required.
The practical consequences of this lack of knowledge were cultures of several species that were not growing at all. In some cases, cell counts indicated quick deterioration and subsequent starvation of the cultures; in other cases, however, cell counts stayed more or less the same during the 1.5 weeks of DNA damage repair time.

Chaetoceros brevis

This particular species of central diatom had previously been cultured in this laboratory, e.g. by N.V.J.de Bakker in 1997. It was known to exhibit good growth in culture, which was one reason for including it in this experiment. Single cells were most abundant, but sometimes short chains of cells were also observed.
In preculturing, no great differences were found in growth rates: on average, batches exhibited growth rates of resp. 0.38 and 0.47 per day. After dilution (before application of UV), all cultures contained ± the same cell densities (38.8 - 92.9 cells/μl) so results would be comparable. During the actual radiation experiment, differences between batches began to establish themselves. At this stage, all cultures still exhibited moderate growth, regardless of their specific circumstances (the type of irradiation they were exposed to). Only later, during the "repair" experiments, did real differences emerge: in both batches, the (UV-B+UV-A+PAR)- irradiated cultures lagged behind in growth (-0.04 and 0.07 per day, respectively; Appendix 10) when compared to both PAR- and (UV-A+PAR)- irradiated cultures. The differences between these two were not significant in either batch.

Porosira glacialis

This central diatom was one of the "new arrivals" from Bigelow. As such, no culturing experience was available, but the species was selected because it had adapted well to culturing conditions in the 4°C- chamber, where it exhibited high cell densities (as seen by the naked eye). It, too, was usually found as single cells, or as two cells that had not yet completed division.
Unfortunately, cell densities remained low during the entire experiment; in Batch I, cell densities rose from 3.83 cells/µl to 13.4 cells/µl in preculturing. After dilution, cell densities in all Batch I-cultures remained at an extremely low level, hardly growing at all. At the end of the "repair" experiment, cell densities in UV-A and UV-B cultures had finally risen near the original preculturing range (growth rates of 0.16 and 0.13 per day, respectively; Appendix 10) whereas growth in the "PAR"-culture had effectively stopped (growth rate of -0.07 per day).

In Batch II, the results were even worse: the culture which could have been expected to grow best (the PAR-culture) failed to exhibit any growth at all. Preculturing cell counts remained at the same basic level of approx. 2.5 cells/µl, and after dilution, no increase in cell densities was observed (fig. 9.2; growth rate of -0.007 per day). Comparable cell counts were found when testing irradiated samples from the very end of the Recovery experiment, indicating that neither culture had exhibited growth. All in all, culturing of *P.glacialis* in Batch II can be considered a failure.

Pyramimonas sp.

This flagellate was originally isolated by Dr. A.G.J. Buma in 1988/89 during Leg II of the European Polarstern Study in the Weddell-Scotia Confluence Area (Buma, 1992). This species had originally been used in growth and photoadaptation kinetics experiments (Buma, 1992). It exhibited good growth under laboratory circumstances.
In preculturing, both batches hardly grew at all (growth rates -0.09 and -0.1, resp.); this might indicate that maximum cell densities had already been reached and growth was halted by nutrient limitation. During UV-irradiation, a difference in growth rate between the UV-B-irradiated cultures of Batches 1 and 2 was found. Although the UV-B-irradiated culture from Batch 1 did show surpressed growth when compared to PAR (GR of 0.27 versus 0.66 per day), the UV-B-irradiated culture from Batch 2 effectively stopped growth when compared to PAR (GR of 0.14 versus 0.42 per day) until several days after UV-treatment had ended. From that point onward, this culture started growing again, reaching pre-culturing densities at the end of the Repair-experiment. In this phase of the experiment, growth rates no longer differed as much: UVB vs. PAR (Batch 1) = 0.37 vs. 0.33,
and UVB vs. PAR (Batch 2) = 0.38 vs. 0.36
From these results, it can be gathered that growth inhibition by UV-B, in whatever form, was not irreversible in this species, because all cultures eventually reached densities higher than, or comparable to, pre-culturing densities.

Phaeocystis sp.

Another new arrival, this prymnesiophyte species grew rapidly in the 4°C-chamber. Although several small spherical colonies were observed under the microscope, single cells were most common. When brought in the incubator for preculturing, however, cell densities remained at more or less the same level. After dilution, cell counts rapidly dropped and no recovery took place in any culture. The only difference between different treatments was that some cultures deteriorated more rapidly than others (. Apparently, some other factor than the irradiation regime kept these cultures from adapting to incubator circumstances.

Gymnodinium sp.

This dinoflagellate had never before been cultured in this laboratory. It frequently occurred in small "clusters" or "lumps" of 2-6 cells lying close to each other.
Although growth in the 4°C-chamber seemed to be reasonable, in preculturing conditions no growth occured. Instead, cell densities in all cultures remained remarkably stable during the entire experiment, neither growing rapidly nor dying off. A clear difference can be seen between Batches 1 and 2: from the beginning, Batch 2 contains more cells than Batch 1, and these differences remain throughout the entire experiment. Cell numbers in both Batches remained stable, between 1-10 cells/µl.

Fragilariopsis cylindrus

This pennate diatom was another recent acquisition from Bigelow. It most often was found as a single-cell species, but this might have had something to do with the intensity of stirring which was applied. In both Batches, initial growth (during preculturing) lagged as if maximum densities had already been attained. During UV-irradiation, both UV-B-irradiated cultures showed strong decrease in cell densities after prolonged exposure (GR of -0.46 and -0.81, resp.); a similar pattern was found in the UV-A (Batch 2)-culture, where cell densities dropped during the irradiation experiment (GR = -0.42). Another apparently anomalous result (the sudden drop in cell densities in the PAR-(Batch 2) culture) might be explained by the transfer of cultures to new containers for the Recovery experiment. All cultures were quick to respond to shutdown of UV-radiation; at the end of the repair experiment, all had reached cell densities comparable to preculturing values.

Immunoslot blotting

From the results of the PicoGreen analysis it became clear that DNA was, in fact, present in all samples. Blotting of these samples was carried out in 4 sessions (in which each sample was blotted at least once), and films were illuminated for different amouts of time. On each blot, at least one reference series was included. These reference series consisted of 0-100 ng/ml UV-irradiated calf thymus DNA, of which the concentration of thymine dimers was known (146.6 T<>T per 10^6 nucleotides of reference DNA, drs. P.Boelen, pers.comm., 1998). In most cases, blotting accuracy increased with extended illumination time, although longer exposure tended to increase the average blackness of some samples to a point where they could no longer be analysed accurately. Blotting results for thymine dimers are shown in Appendix 12. Blotting results were quantified using reference series values to calculate the actual amounts of thymine dimers present: these reference series results are shown in Appendix 11.
After exposure and subsequent development, most blots showed hardly any signal at all; longer exposure times (30 min. - 45 min.) were needed to show any reaction. As could have been expected, only UV-B-irradiated samples showed any sign of increased blackness (indicating the presence of thymine dimers).
Some samples were actually blotted twice, to increase accuracy. This became necessary when, in several cases, strange results were found in the initial blots. For instance, when samples of

the first 2 batches (containing *Ch.brevis*, *P.glacialis* and *Pyramimonas*sp.) were blotted for the first time, high levels of thymine dimers were indicated - but this seemed to be species-dependent, not irradiation-dependent. As can be seen in Appendix 12 (Blot 1), *all* samples of *Ch.brevis* exhibit low thymine dimer concentrations, all *Porosira* samples exhibit high concentrations, and all *Pyramimonas* samples are roughly in the middle, regardless of the type of irradiation actually received.

DISCUSSION

Technical background

At the very beginning of this project, the plan was to attempt cultivation and experimental incubation in the Fridina refrigerator. As explained previously, temperatures would rise far above desired levels in a very short time. The amount of heat produced by 10 TL-lights was the main cause of this, and evidently stands in the way of broad implementation of these light armatures in refrigerators. If another light source (with approximatly the same intensity) can be found, these refrigerators will become a useful addition to incubators currently in use.

From the beginning, it was a quandary whether the six used species would actually grow under incubator conditions. When algae are transferred from one set of culturing conditions to another, the resulting "adaptation shock" often kills of a sizeable fraction of cells. In this case, mere bad luck seems to have been "aided" by some other factor: whereas all species exhibited modest to good growth rates in either the 4°C-chamber or the Fridina refrigerator, fully three out of six species (*Phaeocystis* sp., *Gymnodinium* sp., and *Porosira glacialis*) failed to grow in the incubator (in the case of *P.glacialis*, the case is ambiguous). The question, of course, is a simple: why? The low growth rate in these species could have any of a number of reasons, including the following:

- Light: In the incubator, cultures were illuminated by 10 TL-lamps, receiving (150 - 250 $\mu E/m^2/s$). We did not attempt to supply the cultures with an approximation of natural PAR levels (± 1500 $\mu E/m^2/s$); when exposed to such high levels of PAR, the algae would have experienced photoinhibition. We assumed that all species would be able to adapt to these conditions.
Although a fair approximation of natural light conditions, these algae were preadapted to growth in the 4°C-chamber (2 TL lights) and/or even the Fridina refrigerator (4 TL lights). Cultures exposed to these (relatively) low lighting conditions for a long period of time might experience trouble readapting to high intensities as experienced in the incubator. This might even lead to the extreme of failing to grow at all. In this case, this possibility is a non-issue, since all species did show (some) growth during preculturing.
The fact remains, however, that the ratios of UV radiation given in this experiment do not correspond with natural values, but are significantly higher. The daily dose of UV-B (1200 - 1500 $J/m^2/d$) corresponds with a significant O_3-depletion (Dr.A.G.J.Buma, pers.comm., 1998) of 30 % or so. Unfortunately, the UVA component is relatively underestimated.

- Medium: All cultures were kept in filtered natural seawater, to which nutrients, vitamins and trace elements were added according to the F/2-medium-protocol by Guillard (Guillard, 1975). It is possible that some error occurred during the adding of these substances (personal error). Furthermore, the substances used might not have been completely pure; vitamin solutions in particular were in doubt. If a contamination of some sort would have been added to the growth medium in this fashion, it probably would have inhibited cell growth.
Finally, there is the (distant) possibility that some contaminant might have arrived in the seawater itself. Since this water is regularly sampled in the North Atlantic (near Iceland, by the R.V. *Pelagia*) and regularly tested for impurities, this possibility seems somewhat remote.

- Temperature: All in all, temperatures were probably most stable in the 4°C-chamber. Temperature control had been set with a upper limit of 6°C, which it never surpassed. Likewise, the Fridina refrigerator operated within limits of 3°C and 6°C, rarely exceeding either value. In contrast, the room in which the incubator was located was airconditioned at 16°C, even though the cryostate cooled the incubator's contents to an average of 4.5°C. This was regularly checked by two thermometers. The large temperature gradient was obvious as large drops of condensation formed under the bottom pane of the incubator: to protect the PAR-lamps shining below, a perspex sheet was put over them, while a small fan provided air circulation to combat condensation. Even though surrounding water temperatures remained constant (Appendix 6), these might have been too high for some species.

- Stirring: As has been stressed before, the mechanical (electromagnetic) stirring might have had a profound effect on the growth of some species (e.g. *Phaeocystis*). Even at the minimum stirring intensity, a "maelstrom"-like eddy appeared in the water column, sometimes reaching nearly to the bottom of the culture container. For large cells, this might have been a distinct disadvantage; they might have experineced high levels of physical stress by this treatment. Unfortunately, there is, for now, no easy implementable alternative to the use of these stirring bars. It is only regrettable that no increase in growth rate took place after mechanical stirring was replaced by manual stirring (at the start of the Recovery experiment) in *Phaeocystis*.

- Initial concentrations: It has been a common observation for years that, in alga cultivation, a minimum amount of preculture must be transferred to the growth medium to ensure good growth (Dr.A.G.J.Buma, pers.comm., 1998). This is necessary because such a transfer invariably causes adaptation problems and subsequent starvation in a certain percentage of the cells. In this case, 25 to 30 ml of stock culture was used to start preculturing (that is, added to 500 ml F/2 growth medium). Because so little is actually known of these species' requirements, it might very well be that some need nigher initial densities to start good growth.

Summarising, it is not yet clear whether the bad culturing results for these 3 species were caused by any of the circumstances cited above. Perhaps it was a combination of two (or possibly more) factors which inhibited growth. The only way in which this issue can be clarified further is through future, more focused research.

Irradiation

Growth rates in (UV-A + PAR)-irradiated cultures were comparable to cultures irradiated solely with PAR. This is probably caused by the low dose of PAR emitted by the UV-A- and UV-B-lamps (which, obviously, makes it possible to see whether they are switched on); cultures closer to these lamps would receive more PAR. Also, Compartment A (where all the PAR-irradiated cultures were kept) received a lower dose of PAR from the PAR-lamps than either Compartments B or C, which probably inhibited growth.

Survival

Of the six species used in this experiment, only three can be said to respond to irradiation with UV-B as was initially expected. Two species of diatoms (*C.brevis* and *F.cylindrus*), as well as *Pyramimonas* sp., showed good growth during preculturing, and retarded growth when exposed to UV-B.

The *C.brevis* cultures that were exposed to UV-B were growth-inhibited. No repair took place during this experiment. All other cultures showed good growth. The results seem to indicate that the deleterious effects of UV-B irradiation do not occurr instantaneously, but that prolonged irradiation exposure must take place before it has any effect. Also, the effects appear to be both inhibiting growth and permanent, because cell counts in both UV-B-irradiated cultures remained stable during the subsequent Recovery experiment.

In the case of *F.cylindrus*, growth in UVR-cultures did not begin until the end of the irradiation experiments, when UV was switched off. From that day onward, all cultures exhibited rapid growth; after 1 week, all cultures had attained the same (high) densities (comparable to preculturing). Cell densities in UV-B-irradiated cultures dropped to a minimum after 3 irradiation sessions, but recovered quickly (Appendix 9, fig. 9.6). In this species, UV-A does also seem to have an effect on growth; although not as dangerous as UV-B, some inhibition does seem to occur. Again, the effect appears to be completely reversible.

When looking at *Pyramimonas* sp., UV-B did have an evident effect in one of the two Batches; Batch 1 did grow during irradiation, albeit slowly when compared to others, but growth in Batch 2 was inhibited and did not restart until well after UVR had been switched off. Both UV-A-irradiated cultures seemed to experience some difficulties in adapting to experimental conditions, but all cultures managed to regain original pre-culturing cell densities. As in the previous species, no permanent (irrepairable) damage seems to have occured.

In *P.glacialis*, growth was observed, although only in 1 batch. This species did not seem to be influenced by UV-B, but the "PAR"-irradiated cultures did not grow well. Whether this can be attributed to the presence of UV-B, or to some other cause, is not completely clear, although there is room for speculation (see below). Growth rate was slow, and this species cannot be said to be such a good candidate for future experiments as previously thought.

In the case of *Phaeocystis*, no growth at all occured in preculturing, and cell numbers dropped rapidly in all cultures after starting irradiation. Possible agents might include increased light intensity when compared to culture chambers, stress caused by stirring, nutrient limitation of some kind, or ambient temperatures. Since this genus has a reputation for quick growth and subsequent starvation, the transfer to culturing vessels might not have been quick enough. Instead of the large, sheet-like colonies found in the Fridina refrigerator, only small, whitish colonies were found in the incubator.

No growth at all was observed in the *Gymnodinium* culture; Instead, all cultures remained at a basic density level. It might be that high doses of PAR-radiation inhibited growth, not by DNA damage, but perhaps by influencing metabolism, so that replication would be inhibited. As in all species, further research is required.

Thymine dimers

From the blotting results (Appendix 12), some tentative conclusions regarding sensitivity to UV-B radiation can be drawn. First of all, high amounts (> 2 - 3) of dimers were, in fact, detected in (at least) three species using this technique, although dimers were the norm in this experiment. Secondly, relatively large differences can be noted between blotting of duplo samples between two blots (or even on the same blot). It is not entirely clear what is the cause of these differences, but an important factor may be the differences in references series (Appendix 11 for reference series data).
Finally, when the blotting results are compared to the results from the cell counting experiments, results are ambiguous. For instance, growth rates in *Pyramimonas* sp.-cultures which received UV-B-irradiation were not very divergent from other cultures' growth rates, even though in at least one sample a high concentration of thymine dimers was measured. On the other hand, although nearly all UV-B-irradiated *Phaeocystis* cultures contained thymine dimers, cell counts showed no great differences in growth rates between such cultures, and others with a more benevolent irradiation regime. Crudely speaking, *Phaeocystis* cells were not hampered in their growth because of the presence of thymine dimers, because they were not growing anyway. Clearly, cell growth (or rather the lack of it) is influenced by many factors and variations cannot solely be attributed to radiation; similarly, UVR (and thymine dimers) can have many effects on cell metabolism which are not visible in cell counts.
In general, blotting results are still somewhat ambiguous and await further study. The high margin of error in these samples was linked to several uncertainties in the blotting method; in particular, the high levels of thymine dimers indicated in the 1st blot (Appendix 11, 12) might be explained because an important step in the overall procedure (the adding of RNase to degrade any RNA present) was omitted. The anomalous results could very well be explained by differences in RNA content between species. Another problem was that results such as these from the first blots led us to believe that DNA amounts brought onto the blotting apparatus gave sufficient blackness, while later data showed that this was not the case.

CONCLUSIONS

Possible suggestions & improvements

In many ways, this experiment was a "pilot": the used species were largely selected from cultures new to this laboratory, the experimental setup had to be built (sometimes literally) from scratch, and the blotting protocol had also just been improved. As such, there are several adaptations possible in the (near) future:

The experimental setup might be improved in that more culture chambers would be subjected to the same treatment, to average out great differences in growth rate. This might mean that a new incubator is necessary, to accomodate more culturing vessels.

Another aspect of the experimental setup that might be subject to improvement is the arrangement of the TL lights. From both LiCor and Macam data (Appendix 5) it is evident that not all compartments (or, for that matter, all locations within compartments) receive the same dose of PAR. Variation within compartments has been countered by switching culture containers around in a standard fashion each day, so that each culture would, in the course of the entire experiment, be subjected to an "average" dose of PAR. Still, variation *between* compartments remained high enough to possibly influence measurements. This was probably caused by the fact that the two middle compartments (B and C) were placed directly above the middle of all 10 TL-lights, whereas the two peripheral compartments (A and D) were placed above the ends of the TL lights. Obviously, these lights give off more intense radiation near their centers; this might have influenced growth rates of preculturing bottles (which were kept in Compartment D) and/or PAR cultures (which were kept in Compartment A).
A possible solution might be installing longer (80 cm or so) TL lights, so that the amounts of received PAR in each compartment would be more comparable. A clue that this might be less than optimal can be found in cell counts of *P.glacialis*, in which PAR-irradiated cultures exhibited less growth than either UV-A- or (UV-B+UV-A)-irradiated cultures. Very little is known about light requirements in this (and other) species, but an unspoken assumption during this experiment was that the difference in effects between 140 $\mu E/m^2/s$ and 260 $\mu E/m^2/s$ was negligible; that is, that all containers received enough PAR for continued growth. It can be conjectured that the level of illumination in the PAR-irradiated subcompartment failed to reach a certain "threshold value", below which growth could not be sustained. This means, of course, that data from the PAR-irradiated subcompartment are only partly comparable to data from other compartments: there is no telling what growth rates could have been achieved by these cultures, had they also been exposed to such high levels of PAR.
As has already been stressed, the ratios of both UVB/UVA and UVB/PAR were significantly higher than encountered in a natural setting. Clearly, the arrangement of 10 PAR lights -- 4 UV-A lights -- 1 UV-B light might be less than optimal. Perhaps an increased number of PAR and UV-A lights might improve ratios to a more natural level.

The problem outlined above is primarily a case of lack of acquaintance with these species. More knowledge of general culturing requirements is therefor necessary; for example, the establishment of P/I-curves for each species would make the incubator design relatively simple.

An important improvement in this setup would be the installment of some new method of stirring, to replace the mechanical stirring using magnetic stirring bars. Perhaps some method of continuous (slow) stirring can be installed using either aeration in each separate culturing vessel or some sort of stirring arm slowly rotating in each vessel. However, UV irradiance should not be impaired.

Irradiation results show that light regimes were not comparable in the different subcompartments, and not even within subcompartments. The latter variance was corrected for by switching the culture chambers around each day, thereby assuming that each culture had, by the end of the experiment, received the same irradiation regime. Further analysis suggests that this might not be prudent; instead, containers might be left in fixed positions, correllated with received irradiation regime by means of a covariant analysis, or ANCOVA (Th.Reusch, pers.comm., 1998), because at least growth rates seem to have been dependent not only of the UV-regime, but also of the variance in PAR-dose received.

When examining growth rates, cell numbers often show a precipitous drop the first day after transfer to the culturing containers. This increased cell mortality is induced by initial adaptation problems rather than by UVR, because this phenomenon is also found in PAR-irradiated cultures. In following experiments, these effects might be separated if cells were transferred to their culture containers ± 1 day prior to UV-irradiation, rather than less than an hour in some cases. In this fashion, cells will have the opportunity to adapt to their new surroundings and most adaptation problems will have passed before the start of the actual experiment.

One possible improvement, when considering blotting procedures in these species, concerns the amount of DNA brought onto the blot. In general, results were difficult to quantify at best, because of the vagueness of the spots on the photosensitive sheet. To increase these signals, illumination time had to be extended, thereby risking the loss of some points of the reference series' samples because of overillumination. It might be best, when working with these species in the future, to consider increasing the amounts of DNA when using this technique.

Finally, a substantial amount of available species has not yet been tested in this type of experiment. If comparable experiments are done with other species, it might give an indication whether UV-vulnerability is somehow different in distinct taxonomic groups.

Survival experiments:

As can be seen in Appendices 9 - 10, there were several possible reactions to the different irradiation treatments:

- growth inhibition by UV-B, without subsequent recovery. In the case of *C.brevis*, all cultures exhibited growth - except the cultures irradiated with UV-B. These cultures were unable to sustain growth after UV-B irradiation had ended and this damage was not reversed.

- growth inhibition by UV-B, followed by recovery. This pattern was observed in the *F.cylindrus* culture. As was expected, the PAR cultures were hardly affected. Results for the UV-A irradiated cultures are ambiguous. Both UV-B cultures showed drops in cell densities. After 1.5 week of repair time, all cultures had reached roughly the same densities, indicating that some other factor (e.g. nutrient limitation) was limiting further growth.
More or less the same was observed in the *Pyramimonas* cultures; one of the 2 UV-B-irradiated cultures was growth-inhibited, and recovery started even later than in the preceding species. The other UV-B irradiated culture showed no real effects. Still, damage was repaired during the course of the Recovery experiment.

- growth inhibition in nearly all cultures. In the case of *P.glacialis*, the entire Batch 2 failed to adapt. In Batch 1, the only culture which did not grow well was the PAR-culture, while both UV-A and UV-B-irradiated cultures showed (comparable) growth rates. These unexpected results might be caused by the photosynthetic requirements of this species, in that the PAR-culture (being at the outer end of the incubator) simply did not receive sufficient PAR to sustain growth. Thus, negative effects of UV-B and/or UV-A on growth could not be discerned, although the presence of thymine dimers in these cultures (Appendix 12) indicates that such effects should have been present.

- general growth inhibition in all cultures without any recovery. In the *Gymnodinium* culture, no growth of any magnitude occurred either in preculturing, during UV-irradiation or during repair. These cultures probably had insurmountable difficulties in adapting to incubator conditions, caused by one of the reasons cited above. Still, cell densities remained at pretty much the same level, neither rising nor falling. This might indicate that some other system, preventing replication, was damaged by the transfer.

- growth during preculturing, but growth inhibition in all cultures without recovery during UV. This scheme was found in the *Phaeocystis* cultures. No culture apparently withstood the transition from preculturing vessel to culture chambers. After incubation, cell densities quickly dropped to a fraction of initial numbers. Several possible reasons for this phenomenon have already been cited above; another cause could be that the pre-culture *Phaeocystis*, which has a reputation for quick growth and subsequent collapse (Dr. A.G.J.Buma, pers.comm., 1998), was already dying before transfer to the culturing vessels.

This limited performance in the actual experiment is quite different from the experiences gained in preculturing, where all used species showed modest to good growth rates. This was not indepedently tested by sampling and counting, to keep the cultures as sterile as possible.

There was a great difference between cell densities (between species), depending on whether the species adapted to incubator conditions. All in all, it will probably take more research, focused on one or two particular species, to decide whether these species will eventually become useful research subjects.

Blotting experiments:

The results of the diverse blotting experiments are somewhat hard to interpret. On the one hand, staining evidently occurred, showing that thymine dimers were, in fact, present. When studying the trendline through the reference series, however, it becomes clear that, in some cases, results are not to be trusted blindly outside the trendline range (through reference series data, Appendix 11).
Another problem encountered when studying these samples was the issue of reproductability. In most cases, 4 different samples (from every one irradiation treatment) were blotted at the same time, producing an n of 4. These values often displayed a high margin of error (see also Appendix 12 for original data). This was particularly obvious in those cultures that actually contained thymine dimers, because rare was the case in which all duplos actually contained more or less the same amounts of dimers. Even so, many samples did display such damage (Appendix 12).
Summarising, this experiment has served solely as a short pilot study, and more extensive research is required to answer the question whether these six species are actually vulnerable to ultraviolet radiation. It cannot conclusively be said that UV-B radiation leads directly to either growth inhibition or higher levels of thymine dimers in every species.

Ecological context

In their natural setting, it is probably quite rare for Antarctic phytoplankton to be exposed to elevated levels of UV-radiation for extended periods of time, due to the occurrence of vertical mixing (Buma, 1992; Helbling *et al*, 1994). This is by no means restricted to the Antarctic: in fact, few marine phytoplanktonts are constantly irradiated by UVR, wherever they are. Furthermore, the ozone hole itself rotates above/across the continent, thereby exposing the same area to different amounts of UV-radiation within a relatively short time period. From this point of view, a three-day UV-illumination period, as in this experiment, seems comparable to a natural situation.

It has been the assumption that a "sudden" increase in biologically harmful UV-radiation would lead to a change in phytoplankton species composition. From the results found in this experiment, few (if any) true predictions can be made for a natural setting, where cell densities are governed by many other factors not accounted for in our laboratory. Furthermore, phytoplankton species are not oblivious to each other's presence: they compete with each other for nutrients. Any prediction of the phytoplankton composition in ozone-depleted conditions will also have to take into account the possible competitive (or perhaps symbiotic) effects the algae in question have on each other. Such a study has already been initiated for *Phaeocystis* sp. (Davidson & Marchant, 1994). This species has a significant

effect on the microbial community in its immediate surroundings, due to the production of prolific amounts of mucilage during its colonial stage. This substance apparently attracts or stimulates microheterotrophs, while deterring autotrophs and most bacteria. Because *Phaeocystis* is such an abundant species, it is possible that an increase in UV-irradiation will indirectly induce widespread changes in the phytoplankton community, due to the effects it has on this species.

Planctonic algae have several possible mechanisms to protect themselves against harmful UV-radiation (Karentz, 1994). The best known of these is the increased production of compounds which strongly absorb in the UV-B-part of the spectrum (280-320 nm). A large group of such substances is known as Mycosporine-like Amino Acids, or MAA's. Such substances may be produced specifically against UV, or they may have a different primary function. *Phaeocystis* sp. is an example of a species which contains high concentrations of MAA's (at least in its colonial stage; Davidson & Marchant, 1994). As such, it would be protected rather well against increased UV-irradiation, particularly because there would now be selective pressure to step up MAA formation. Other species, such as diatoms, possess only modest amounts of MAAs, but recent research suggests that they may contain other substances which can act as solar screens (Davidson & Marchant, 1994). Such species would, therefore, probably experience only limited, if any, UV-related stress at present fluctuating ozone levels.

From the results obtained in this experiment, it is difficult to say anything about the possible changes in species composition by increased UVB-radiation. Perhaps it is possible to discern a trend in taxonomical groups: 2 of the 3 species which exhibited good growth in both batches were diatoms (1 central, 1 pennate), and the third species of diatom present did grow in one batch. A tentative prediction for the natural situation, partly based on literature (Davidson & Marchant, 1994) might be that diatoms and prymnesiophytes would be only mildly affected, possibly at the expense of dinoflagellates. Contradictory results are also reported from field studies (Vernet *et al.*, 1994). It thus remains to be seen whether the ozone hole will, in fact, wreak havoc in the Antarctic marine food chain, or that the system is resilient enough to be able to respond with only minor shifts in species composition.

CITATION INDEX

Alberts, B., D.Bray, J.Lewis, M.Raff, K.Roberts, J.D.Watson (1994) Molecular Biology of the Cell. 3rd Edition, Garland Publising, Inc., NY, USA.

de Bakker, N.V.J., (1997) Effects of UV-radiation on DNA damage and growth in the Antarctic diatom *Chaetoceros brevis*. Department of Marine Biology, RUG, internal report.

Booth, C.R., J.H.Morrow, T.P.Coohill, J.J.Cullen, J.E.Frederick, D.-P.Häder, O.Holm-Hansen, W.H.Jeffrey, D.L.Mitchell, P.J.Neale, I.Sobolev, J.van der Leun, R.C.Worrest (1997) Impacts of Solar UV on Aquatic Microorganisms. Invited review in *Photochemistry & Photobiology*, 1997, 65(2): 252-269

Buma, A.G.J. (1992) Factors controlling phytoplankton growth and species composition in the Antarctic Ocean. Doctoral thesis.

Buma, A.G.J., E.J.van Hannen, L.Roza, M.J.W.Veldhuis, W.W.C.Gieskes (1995) Monitoring ultraviolet-B-induced DNA damage in individual diatom cells by immunofluorescent thymine dimer detection. *Journal of Phycology*, 1995 (31): 314-321

Buma, A.G.J., H.J. Zemmelink, K.Sjollema, W.W.C.Gieskes (1996) UVB radiation modifies protein and photosynthetic pigment content, volume and ultrastructure of marine diatoms. Marine Ecology Progress Series, 1996, Vol.142: 47-54

Buma, A.G.J., A. H. Engelen, W. W. C. Gieskes (1991) Wavelength-dependent induction of thymine dimers and growth rate reduction in the marine diatom Cyclotella sp. Exposed to ultraviolet radiation. Marine Ecology Progress Series, 1997, Vol. 153: 91-97

Clarke, A., & R. J. G. Leakey (1996) The seasonal cycle of phytoplankton, macronutrients, and the microbial community in a nearshore Antarctic marine ecosystem. Limnol. Oceanogr., 1996, Vol.41(6): 1281-1294

Davidson,A.T,, H.J.Marchant, W.K. de la Mare (1996) Natural UVB exposure changes the species composition of Antarctic phytoplankton in mixed culturing. *Aquatic Microbial Ecology*, 1996, vol.10: 299-305

Frederick, J.E. & D. Lubin (1994) Solar Ultraviolet Irradiance at Palmer Station, Antarctica. In: Weiler, C.S. and Penhale, P.A. [Eds.] *Ultraviolet Radiation in Antarctica: Measurements and Biological Effects.* Antarctic Research series, American Geophysical Union, Washington D.C., 62: 43-52

Frederick, J.H., H.E.Snell and E.K.Haywood (1989) Solar ultraviolet radiation at the Earth's surface. *Photochemistry & Photobiology*, 1989, Vol.50 (8): 443-450

Friedberg, E.C., G.C.Walker and W.Siede (1995) DNA Repair and mutagenesis. 1st Edition, ASM Press, Washington DC, USA.

Gleason, J.F., P.K.Bhartia, J.R.Herman, R.McPeters, P.Newman, R.S.Stolarski, L.Flynn, G.Labow, D.Larko, C.Seftor, C.Wellemeyer, W.D.Komhyr, A.J.Miller, W.Planet (1993) Record Low Global Ozone in 1992. *Science*, 1993, Vol. 260: 523-526

Guillard, R. R. L. (1975) Culture of phytoplankton for feeding marine invertebrates. *Woods Hole Oceanographic Institution Collected Reprints* 1975-1, 45-60

Häder, D.P. (1994) UV-B effects on aquatic systems. In: Hilton Biggs, R. And Joyner, M.E.B. [Eds.] *Stratospheric Ozone Depletion/UV-B radiation in the Biosphere.* Springer-Verlag 155-161

Helbling, E.W., V.E.Fillafañe, O. Holm-Hansen (1994) Effects of ultraviolet radiation on Antarctic marine phytoplankton photosynthesis with particular attention to the influence of mixing. In: Weiler, C.S. and Penhale, P.A. [Eds.] *Ultraviolet Radiation in Antarctica: Measurements and Biological Effects*. Antarctic Research series, American Geophysical Union, Washington D.C., 62: 207-227

Helbling, E.W., B.E.Chalker, W.C. Dunlap, O.Holm-Hansen, V.E.Fillafañe (1996) Photoacclimation of antarctic marine diatoms to solar ultraviolet radiation. *Journal of Experimental Marine Biology and Ecology*, 1996, 204: 85-101

Hoeijmakers, J.H.J, & A.R.Lehmann (1994) Nucleotide Excision-Repair Among Species. In: R.G.Tardiff, P.H.M.Lohmann, G.N.Wogan (eds.): *Methods to assess DNA damage and repair: Interspecies comparisons*, pp. 57-82. Prepared by Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC). SCOPE 52, IPCS Joint Activity 19, SGOMSEC 8.

Karentz, D. (1994) Ultraviolet tolerance mechanisms in Antarctic marine organisms. In: Weiler, C.S. and Penhale, P.A. [Eds.] *Ultraviolet Radiation in Antarctica: Measurements and Biological Effects*. Antarctic Research series, American Geophysical Union, Washington D.C., 62: 93-110

Karentz, D., F.S. McEuen, M.C. Land, W.C. Dunlap (1991) Survey of mycosporine-like amino acid compounds in antarctic marine organisms: potential protection from ultraviolet exposure. *Marine Biology*, 1991, 108: 157-166

Karentz, D., J. E. Cleaver, D. L. Mitchell (1991) Cell survival charcteristics and molecular responses of Antarctic phytoplankton to ultraviolet-B radiation. *Journal of Phycology*, 1991, Vol.27: 326-341

Karentz, D., & L.H. Lutze (1990) Evaluation of biologically harmful ultraviolet radiation in Antarctica with a biological dosimeter designed for aquatic environments. *Limnology and Oceanography*, 1990, 35 (3): 549-561

Kramer, K.J.M. (1990) Effects of increased solar UV-B radiation on coastal marine ecosystems: an overview. In: Beukema, J.J. [Eds.] *Expected effects of climate change on marine coastal ecosystems*. Kluwer academic publishers 195-210

Madronich, S., R.L. McKenzie, M.M. Caldwell, L.O. Björn (1995) Changes in Ultraviolet Radiation Reaching the Earth's Surface. *Ambio* Vol. 24 No. 3 (May 1995): 143-152

Maniatis, T., E.F.Fritsch, J.Sambrook (1982) Molecular cloning: A laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 545 pp.

Roza, L., K.J.M. van der Wulp, S.J. MacFarlane, P.H.M. Lohamn, R.A.Baan (1988) Detection of cyclobutane thymine dimers in DNA of human cells with monoclonal antibodies raised against a thymine dimer-containing tetranucleotide. *Photochemistry & Photobiology* (1988), 48: 627-633

Rundel, R.D. (1983) Action spectra and estimation of biologically effective UV radiation. *Physiologia Plantarum*, 1983, 58: 360-366

Sage, E. (1993) *Yearly review*: Distribution and repair of photolesions in DNA: genetic consequences and the role of sequence context. *Photochemistry & Photobiology*, 1993, Vol.57 (1): 163-174

Setlow, R.B. (1974) The Wavelengths in Sunlight Effective in Producing Skin Cancer: a Theoretical Analysis. *Proc. Nat. Acad. Sci. USA*, 1974, Vol.71: 3363-3366

Smith, R.C., B.B.Prézelin, K.S.Baker, R.R.Bidigare, N.P.Boucher, T.Coley, D.Karentz, S.MacIntyre, H.A.Matclick, D.Menzies, M.Ondrusek, Z.Wan, K.J.Waters (1992) Ozone Depletion:Ultraviolet Radiation and Phytoplankton Biology in Antarctic Waters. *Science*, 1992, Vol. 255: 952-959

Veen, A., A.G.J.Buma, W.W.C.Gieskes, L.van Liere (1995) Effects of ozone related UV-B enhancement on aquatic ecosystems. *RIVM* report No.731054002.

Vernet, M., E.A. Brody, O. Holm-Hansen, B.G. Mitchell (1994) The response of Antarctic phytoplankton to Ultraviolet radiation: absorption, photosynthesis, and taxonomic composition. In: Weiler, C.S. and Penhale, P.A. [Eds.] *Ultraviolet Radiation in Antarctica: Measurements and Biological Effects*. Antarctic Research series, American Geophysical Union, Washington D.C., 62: 143-158

Villafañe, V.E., E.W. Helbling, O.Holm-Hansen (1995) Spatial and temporal variability of phytoplankton biomass and taxonomic composition around Elephant Island, Antarctica, during the summers of 1990-1993. *Marine Biology*, 1995, Vol.123: 677-686

Villafañe, V.E., E.W. Helbling, O.Holm-Hansen, B.E.Chalker (1995) Acclimatization of Antarctic natural phytoplankton assemblages when exposed to solar ultraviolet radiation. *Journal of Plankton Research*, 1995, Vol.17(12): 2295-2306

Xiong, F., J.Komenda, J.Kopecký, L.Nedbal (1997) Strategies of ultraviolet-B protection in microscopic algae. *Physiologia Plantarum*, 1997, Vol.100: 378-388

APPENDIX 1: Buffers and stock solutions

10 M ammonium acetate
Dissolve 385.4 g ammonium acetate in 150 ml H2O.
Add H2O to 500 ml

CIA (chloroform:isoamylalcohol 24:1)

CTAB-extractionbuffer (for 100 ml):
2% CTAB (2g)
1.4 M NaCl (35 ml 4M stock)
20 mM EDTA (4 ml 0.5 M stock)
100 mM Tris-HCl pH 8.0 (10 ml 1M stock)
Add sterile H2O to 100 ml

0.5 M EDTA (ethylenediamine tetraacetic acid)
Dissolve 186.1 g Na2EDTA·H2O in 700 ml H2O
Adjust pH to 8.0 with 10 M NaOH (~50 ml)
Add H2O to 1 liter

1 M KCl
74.6 g Kcl
Add sterile H2O to 1 liter

1 M MgCl2
20.3 g MgCl2·6H2O
Add sterile H2O to 100 ml

5 M NaCl
292 g NaCl
Add sterile H2O to 1 liter

10 M NaOH
Dissolve 400g NaOH in 450 ml H2O
Add sterile H2O to 1 liter

PBS:

Dissolve in 500 ml MilliQ:

4 g NaCl
0.1 g KCl
0.72 g $Na_2HPO_4 \cdot 2H_2O$
0.165 g $NaH_2PO_4 \cdot H_2O$

10x PBS (10x stock solution, 1 liter):

80 NaCl
2 g Kcl
11.5 g $Na_2HPO_4 \cdot 7H_2O$
2 g KH_2PO_4

Working solution:

137 mM NaCl
2.7 mM KCl
4.3 mM $Na_2HPO_4 \cdot 7H_2O$
1.4 mM KH_2PO_4

TE buffer, pH = 7.4, 7.5, 8.0

10 mM Tris-HCl, pH 7.4, 7.5, 8.0
1 mM EDTA, pH 8.0

1 M Tris-Cl [Tris(hydroxymethyl)aminomethane]

Dissolve 121 g Tris base in 800 ml H_2O
Adjust to desired pH with concentrated Hcl
Mix and add H_2O to 1 liter

Desired pH values can also be obtained by mixing the indicated amounts of 0.1 M Hcl with 100 ml of 0.1 M Tris base using the following table:

pH, 25°	0.1 M HCl (ml)	pH, 25°	0.1 M HCl (ml)	pH, 25°	0.1 M HCl (ml)
7.2	89.4	7.8	69.0	8.4	34.4
7.3	86.8	7.9	64.0	8.5	29.4
7.4	84.0	8.0	58.4	8.6	24.8
7.5	80.6	8.1	52.4	8.7	20.6
7.6	77.0	8.2	45.8	8.8	17.0
7.7	73.2	8.3	39.8	8.9	14.0

Note: The pH of Tris buffers changes significantly with temperature, decreasing approximately 0.028 pH units per 1°C. Tris-buffered solutions should be adjusted to the desired pH at the temperature at which they will be used. Since the pKa of Tris is 8.08, Tris should not be used as a buffer below pH ~ 7.2 or above ~ 9.0.

APPENDIX 2: DNA-isolation procedure (CTAB)

- Filtrate 30 - 50 ml of the culture over a GFF-filter; put the filter in a 2 ml Eppendorf cup. Freeze it briefly in liquid nitrogen and store at -80°C.
- OR centrifugate 20 - 50 ml of culture in a sterile 50 ml tube for 15 minutes using a table centrifuge (preferrably in a 4°C-environment); put ± 2 ml in a 2 ml-Eppendorf cup and centrifugate it at maximum speed for 5 minutes (at a temperature of 4°C); freeze pellet in liquid nitrogen and store cup at -80°C.

- Warm 750 µl CTAB-lysis buffer (for each sample), with 2 µl β-mercapto-ethanol/ml CTAB lysis buffer, to 60°C.
- Put the Eppendorf cups from -80°C in a 60°C-"dry-bath"; add 750 µl of the lysis buffer to the cups and incubate them for 30 minutes at 60°C under occasional vortexing.
- After incubation add 750 µl CIA-mixture (to separate the DNA from the cell debris and proteins), mix shortly and centrifugate for 10 minutes at maximum speed at 4°Cin an Eppendorf centrifuge (N.B. the polycarbonate filter will dissolve during this step).
- The Eppendorf cup will by now contain two phases. Remove the upper (liquid) phase (which contains relatively pure DNA) by using a clean and sterile pipet tip and place it in a clean and sterile Eppendorf cup.
- Add approximately 2/3 of the volume of the upper phase (±1 ml) of cold (4°C) isopropanol. Mix and incubate for 1 hour at 4°C (refrigerator).
- Centrifugate for 30 minutes at maximum speed at 4°C in an Eppendorf centrifuge.
- Wash the pellet with 80% EtOH (-20°C), leave it at -20°C for 15 minutes.
- Centrifugate for 30 minutes at maximum speed at 4°C in an Eppendorf centrifuge.
- Dry pellet.
- Dissolve pellet in 500 µl 0.1xTE.
- Add 25 µl RNase (boiled for at least 1 min.) to remove the RNA, and incubate for 1 hour at room temperature.
- Add 1/10 volume units of NaAc (3 M, pH = 5.2).
- Add 2 units of 100% EtOH (-20°C). Mix thoroughly and incubate for 1 hour at -20°C.
- Centrifugate sample for 30 minutes at maximum speed in an Eppendorf centrifuge.
- Remove liquid phase and wash pellet with 80% EtOH (centrifugate at maximum speed for 5 min.). If necessary, dry pellet as described above.
- Dissolve pellet in e.g. 500 µl 0.1xTE.
- Store at -80°C.

Requirements:

- Eppendorf cups, pipettips, buffers, etc. all DNase-free (autoclaved)

- 0.1xTE
- 80% EtOH (-20°C)
- β-mercapto-ethanol
- CIA (Chloroform:isoamylalcohol = 24:1)
- Isopropanol (4°C)
- RNase (DNA-free (cook briefly), 0.047 μg/ml)
- CTAB-lysis buffer (contains per 100 ml: 2%CTAB (2g), 1.4M NaCl (35 ml 4M stock), 20 mM EDTA (4 ml 0.5M stock), 100 mM Tris-HCl pH=8 (10 ml 1M stock)
- 100% EtOH (-20°C)
- 3 M NaAc pH 5.2

APPENDIX 3: DNA quantification with PicoGreen

- General method: pipette 100 µl sample (in either TE or 0.1TE) in a black microtray; add 100 µl PicoGreen-solution (diluted 200 times). Mix and measure sample on the VICTOR 1420 multilabel platereader. Expected DNA concentrations should lie between 10 - 900 ng/ml. If DNA concentration is unknown, test several different dilutions.

- Exact method:

- Prepare a DNA reference series in TE, using a standard DNA-solution such as 10 µg/ml calf thymus DNA solution. Dilute this solution 10 times by mixing 500 µl DNA-solution in 4.5 ml TE in a sterile plastic vial. This will suffice for a reference series. Prepare 10 sterile Eppendorf cups and pipette according to the scheme below:

DNA-solution (µl)	TE (µl)	Concentration (ng/ml)
0	1000	0
10	990	10
20	980	20
30	970	30
60	940	60
100	900	100
200	800	200
300	700	300
600	400	600
900	100	900

- Pipette 100 µl of this reference series in microtraywells. Freeze the rest of the reference series for later use.
- Thaw samples and dilute according to expected concentrations (for example 20 µl sample with 80 µl TE in microtraywell, diluted 5 times; or 10 µl sample with 90 µl TE in well, diluted 10 times). Always end with 100 µl in each well.
N.B. Vortex the extracts very well before taking a subsample!
- Make the PicoGreen solution by diluting it 1:200 with TE (for example: 50 µl PicoGreen + 10 ml TE will suffice for 90 samples and a reference series, in other words a completely filled tray).
- Take the microtray, the PicoGreen solution, a good 100 µl-pipette and the protocol to the VICTOR platereader. The PicoGreen solution should under no account be exposed to light!
- Adjust the Wallac VICTOR 1420 multilabel to the 'DNA'-protocol, which has an excitation of 485 nm and an emission of 535 nm. Check the protocol and the filter.
- Add 100 µl of the PicoGreen solution (in dim light) in every well, mix and place the tray in the VICTOR sample compartment. Start the scanning procedure on VICTOR.

APPENDIX 4: Immunoslot blotting

Transfer the amount of DNA you want to bring on the blot to 1.5 ml Eppendorf cups. Make a reference series of UV-irradiated calf thymus DNA of 0-100 ng/ml. Put 100 - 200 µl of this solution on the blot.

- Make the DNA single-stranded by boiling it for 10 minutes. Immediately after this put it on ice.
- Wet the blotting paper in PBS.
- Before usage, wash the membrane in Sterile Milli Q (SMQ) for as long as possible (at least 30 minutes); afterwards drench it in PBS. Mark the membrane by cutting off one or more of the corners.
- Apply the vacuum on the blotting apparatus. Place the blotting paper on the apparatus. Place the membrane on the apparatus, and remove bubbles of air onder the membrane by pressing it firmly against the blotting paper.
- Close blotting apparatus crosswise.

- Place DNA-samples on membrane in slots of apparatus. Now wait for samples to be sucked through the membrane, leaving DNA behind.
- Once the samples have been sucked through the membrane, wash every sample down with 200µl PBS. Make sure every sample is sucked completely dry before proceeding.
- Open the blotting apparatus (crosswise).
- Take out membrane (while pump is operating at low vacuum) and put it loosely between a folded sheet of filtration paper. Put this in a sterile container and transfer it to an oven. Let it dry for 1-2 hours at 80°C. This will immobilize the DNA.

From now on the membrane is kept in constant (gentle) motion.
- Take membrane out of oven and discard filtration paper. Pre-incubate membrane in a solution of 5%-milkpowder-PBS-0.1%Tween (that is, 5 g/100 ml), for ± 30 minutes at room temperature. The container is in a constant, rocking motion.The milkpowder serves as a *blocker*; it binds to those places on the membrane which would later become aspecific attachment sites for antibodies.
- Wash membrane in PBS-0.1%(Tween-20) (=PBS-T) for 10 minutes at room temperature. Repeat this step twice, to wash away all traces of excess blocker.
- Primary antibody incubation (H3) in either an 1/2000 or 1/4000 dilution, in 0.5%-milkpowder-PBS-T overnight at 4°C or 2 hours at room temperature. Keep membrane in constant motion. Seal off container with Parafilm. Alternatively, the membrane can be transferred to plastic tubes and placed on a rolling table.

The last step usually marks the end of the first day of procedures.

Day2:

- Wash the membrane three times with PBS-T, for 10 minutes each.
- Second antibody incubation (Rabbit Antimouse-HRP) in either an 1/2000 or 1/5000 dilution, in 0.5%-milkpowder-PBS-T for 2 hours at room temperature in container sealed off with Parafilm.
- Wash membrane in PBS-T for 15 minutes; repeat this step 3 times. Alternatively, after the first wash has taken place in a plastic tube, transfer the membrane to a sterile container to complete this step.

- Prepare solution of ECL reagens in clean container. Keep this solution in dim light (e.g. a dark room).
- In the dark room, incubate membrane in reagens solution for 1 minute at room temperature.
- Afterwards, place membrane between Photogene™ development sheets. Make sure membrane is as humid as possible. Seal in membrane.
- Switch off normal lights and switch on special light for the darkroom.
- Place Amersham Lifescience Hyperfilm™ ECL™ on sealed membrane and expose 2-60 minutes. The film should be put in a black container for this (to prevent light pollution). Also, a heavy weight should be placed on top of the film, to prevent light pollution and light reflection.
- Develop the film in the dark room with development and fixation fluids suitable for this film.
- Scan and quantify film with e.g. ImageQuant.

Requirements:

- Eppendorf cups, pipette-tips, buffer solutions etc.were autoclaved DNase- and AP-free.

- Schleicher & Schüll blot apparatus (type minifold 1, SRC 96D)
- Blotting paper (GB-300)
- Membrane (Amersham, Hybond-N Nylon 0.1µm)
- Sterile Milli Q
- PBS
- Tween-20 (= 0.1% polyoxyethylenesorbitan monolaurate from Sigma Chemical co.)
- PBS-T (PBS + 0.1% (v/v) Tween)
- Milkpowder (Elk instant powder, Campina Melkunie, Eindhoven)
- H3-antibody
- Rabbit Antimouse Alkaline Phosphatase antibody
- ECL
- Hyperfilm™ ECL™ film
- Gibco Brl Photogene development folders

APPENDIX 5: Macam & LiCor spectroradiometer data.

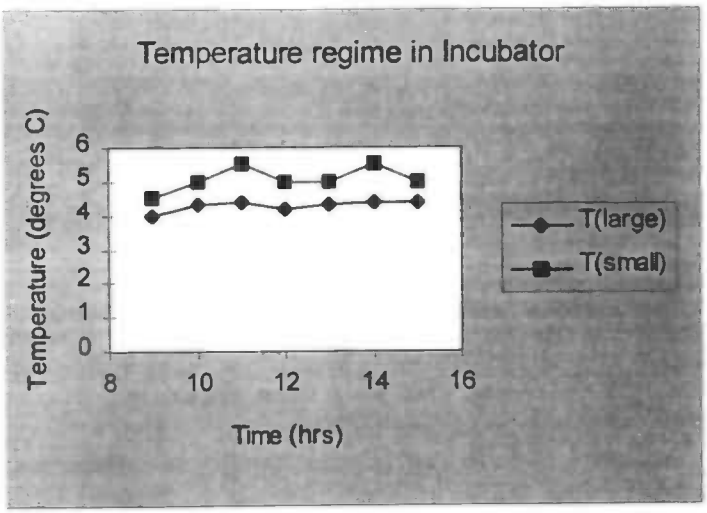
Position	A1(opq)1	A1(trnsp)1	A2(opq)av	A2(trnsp)av	A3(opq)av	A3(trnsp)av	
PAR (LiCor)	75.4	75.4	120.7	120.7	87.4	87.4	$\mu E/m^2/s$
PAR (Macam)	142.107	143.9899	165.9842	165.24799	150.5223	154.0835	$\mu E/m^2/s$
PAR (Macam)	32.1461	32.57942	37.57783	37.415769	34.09231	34.872764	W/m^2
UV-A	1.175	1.342136	1.486891	1.6529135	1.494266	1.5109691	W/m^2
UV-B	0.00548	0.003566	0.013361	0.0077148	0.020526	0.0094012	W/m^2
UV-B (Setlow)	0.34599	0.200344	5.959011	1.2567494	75.31984	5.8860202	(J/m2/d)
UV-B/UV-A	0.4664	0.265689	0.366044	0.4666809	1.374851	0.6213093	(%)
UV-B/PAR	0.01705	0.010945	0.013997	0.0206234	0.060219	0.0269625	(%)

Position	B1-av	B2-av	B3-av	C1-av	C2-av	C3-av	
PAR (LiCor)	111.7	178.5	144.7	119	171.1	119.7	uE/m2/s
PAR (Macam)	169.233	214.2917	206.009	200.9253	215.7551	266.19534	uE/m2/s
PAR (Macam)	38.2842	48.5419	46.61281	45.473612	48.85521	60.281014	W/m^2
UV-A	5.70778	6.288722	5.609295	7.6685922	8.701328	7.5258831	W/m^2
UV-B	0.03772	0.04486	0.044726	0.475166	0.603369	0.5461417	W/m^2
UV-B (Setlow)	3.63667	4.760696	14.38014	1329.101	1507.905	1190.4324	(J/m2/d)
UV-B/UV-A	0.66122	0.713196	0.79743	6.1964592	6.934216	7.2565625	(%)
UV-B/PAR	0.09856	0.092365	0.095952	1.0449276	1.235024	0.9059814	(%)

Natural levels	(on roof BC)
PAR (Macam)	1500 uE/m2/s
UVB/UVA	2.70 (%)
UVB/PAR	0.19 (%)

APPENDIX 6.Temperature regime (example) in the perspex incubator (15-12-'97). Measurements were taken with 2 thermometers ($T_{(large)}$ and $T_{(small)}$).

time	T(large)	T(small)
09:00	4	4.5
10:00	4.3	5
11:00	4.4	5.5
12:00	4.2	5
13:00	4.3	5
14:00	4.4	5.5
15:00	4.4	5



APPENDIX 7: An overview of species received from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts. (Growth rate, as observed in either 4°C-chamber or Fridina refrigerator, denoted in symbols: ++ = good growth (thick sheet on bottom), + = reasonable growth (thin sheet on bottom), - = compromised growth (a few patches across the bottom), -- = arrested growth (no growth at all)).

Registration Number	Species	Growth	Used (Y/N)
CCMP 106	*Actinocyclus actinochilus*	-	N
CCMP 163	*Chaetoceros brevis*	+/++	Y
CCMP 981	*Thalassiosira antarctica*	+	N
CCMP 1099	*Porosira glacialis*	++	Y
CCMP 1031	*Thalassiosira tumida*	-/+	N
CCMP 1102	*Fragilariopsis cylindrus*	+	Y
CCMP 1374	*Phaeocystis* sp.	-/+	Y
CCMP 1383	*Gymnodinium* sp.	-/+	Y
CCMP 1430	*Nitzschia curta*	+	N
CCMP 1437	*Nitzschia subcurvata*	--/-	N
CCMP 1452	*Eucampia antarctica*	--	N
CCMP 1458	*Thalassiosira gravida*	--/-	N
CCMP 1751	*Chaetoceros dichaeta*	-	N
CCMP 1754	*Corethron criophyllum*	-/+	N
PYR	*Pyramimonas* sp.	++	Y

APPENDIX 8: Description of used Antarctic phytoplankton species, as received from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP), Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine 04575, U.S.A. (Thus, no such information is available for the 6th species, *Pyramimonas* sp.).

Strain Number:	CCMP163
Species:	**Chaetoceros* *brevis*
Name Authority:	Schutt
Class:	Coscinodiscophyceae
Identified by:	Fryxell,G
Collected by:	
Collection Date:	
Collection Year:	1979
Collection Site:	Islas Orcadas Sta.6
Ocean:	Southern
Sea:	
Nearest Continent:	Antarctica
Other Information:	
Isolated by:	
Isolation Date:	
Deposited by:	Fryxell,G
Deposit Date:	November 20, 1990
Axenic by:	
Axenic Date:	
Culture medium:	f/2:
Low Temperature:	-2 degrees C
High Temperature:	2 degrees C
Cell length:	6 - 12 um
Cell width:	3 - 4 um
Strain Synonyms:	AA-49
Name Synonyms:	
Axenic (Yes/No):	No
Toxic (Yes only):	

Strain Number:	CCMP1099
Species:	**Porosira *glacialis*
Name Authority:	
Class:	Coscinodiscophyceae
Identified by:	Medlin,L
Collected by:	
Collection Date:	
Collection Year:	
Collection Site:	Islas Orcadas Stat. 21, 1979
Ocean:	Indian
Sea:	
Nearest Continent:	Antarctica
Other Information:	
Isolated by:	Fryxell,G
Isolation Date:	
Deposited by:	Fryxell,G
Deposit Date:	September 21, 1982
Axenic by:	
Axenic Date:	
Culture medium:	f/2agar:
Low Temperature:	-2 degrees C
High Temperature:	2 degrees C
Cell length:	36 - 45 um
Cell width:	9 - 21 um
Strain Synonyms:	AA-20
Name Synonyms:	
Axenic (Yes/No):	No
Toxic (Yes only):	

Strain Number:	CCMP1102
Species:	**Fragilariopsis *cylindrus*
Name Authority:	
Class:	Bacillariophyceae
Identified by:	Medlin,L
Collected by:	
Collection Date:	
Collection Year:	
Collection Site:	Islas Orcadas Stat. 12, 19/79
Ocean:	Indian
Sea:	
Nearest Continent:	Antarctica
Other Information:	
Isolated by:	Fryxell,G
Isolation Date:	
Deposited by:	Fryxell,G
Deposit Date:	August 5, 1982
Axenic by:	
Axenic Date:	
Culture medium:	f/2:
Low Temperature:	-2 degrees C
High Temperature:	2 degrees C
Cell length:	3 - 7 um
Cell width:	2 - 4 um
Strain Synonyms:	AA-88H
Name Synonyms:	
Axenic (Yes/No):	No
Toxic (Yes only):	

Strain Number:	CCMP1374
Species:	**Phaeocystis* sp.
Name Authority:	
Class:	Prymnesiophyceae
Identified by:	Jacobson,D
Collected by:	Putt,M
Collection Date:	1/91
Collection Year:	1991
Collection Site:	McMurdo Station, Antarctica
Ocean:	Southern
Sea:	Ross Sea
Nearest Continent:	Antarctica
Other Information:	near sea ice
Isolated by:	Brett,S/Jacobson,D
Isolation Date:	
Deposited by:	Jacobson,D
Deposit Date:	May 1, 1991
Axenic by:	
Axenic Date:	
Culture medium:	f/2-si:K,L/20
Low Temperature:	0 degrees C
High Temperature:	6 degrees C
Cell length:	0 - 0 um (no data)
Cell width:	0 - 0 um (no data)
Strain Synonyms:	B991K
Name Synonyms:	
Axenic (Yes/No):	Yes
Toxic (Yes only):	

Strain Number:	CCMP1383
Species:	**Gymnodinium* sp.
Name Authority:	
Class:	Dinophyceae
Identified by:	
Collected by:	Moisan,T
Collection Date:	1/91
Collection Year:	1991
Collection Site:	McMurdo Sound
Ocean:	Southern
Sea:	Ross Sea
Nearest Continent:	Antarctica
Other Information:	ice edge
Isolated by:	Jacobson,D
Isolation Date:	June 1, 1991
Deposited by:	Jacobson,D
Deposit Date:	June 1, 1991
Axenic by:	
Axenic Date:	
Culture medium:	Prov:
Low Temperature:	0 degrees C
High Temperature:	6 degrees C
Cell length:	10 - 15 um
Cell width:	4 - 8 um
Strain Synonyms:	
Name Synonyms:	
Axenic (Yes/No):	No
Toxic (Yes only):	

APPENDIX 9: Growth of specified cultures during the entire experiment. UVR was applied within the hatched area in each graph, in 3 periods of three hours each.

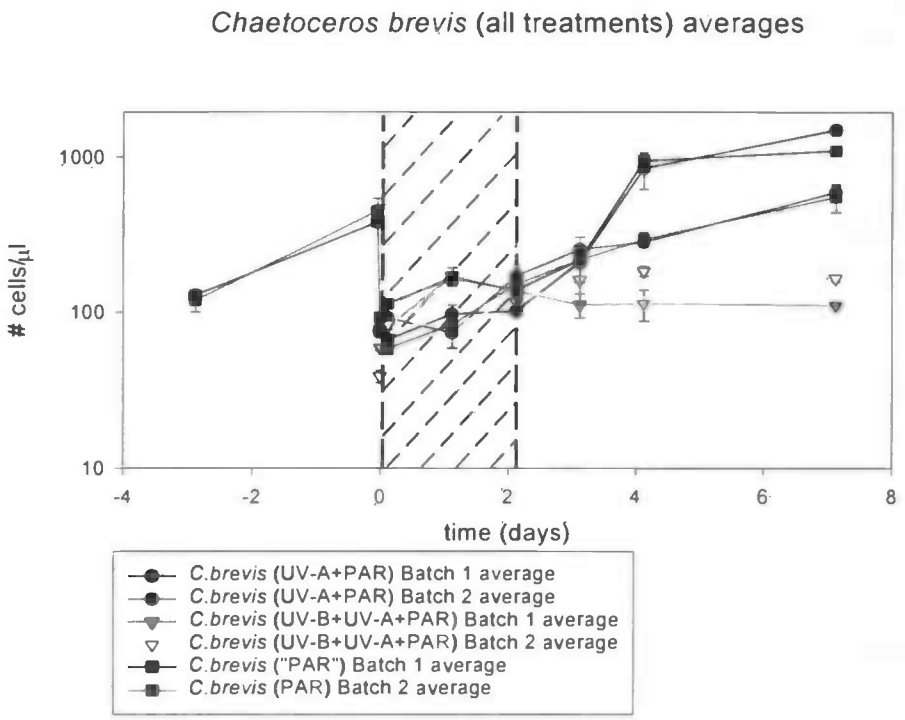


Fig. 9.1

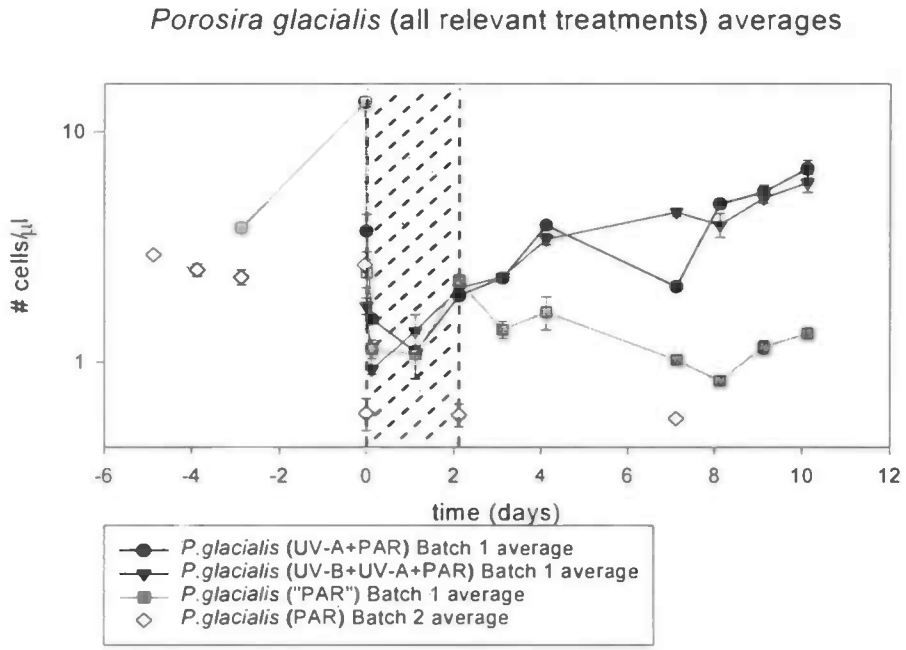


Fig. 9.2

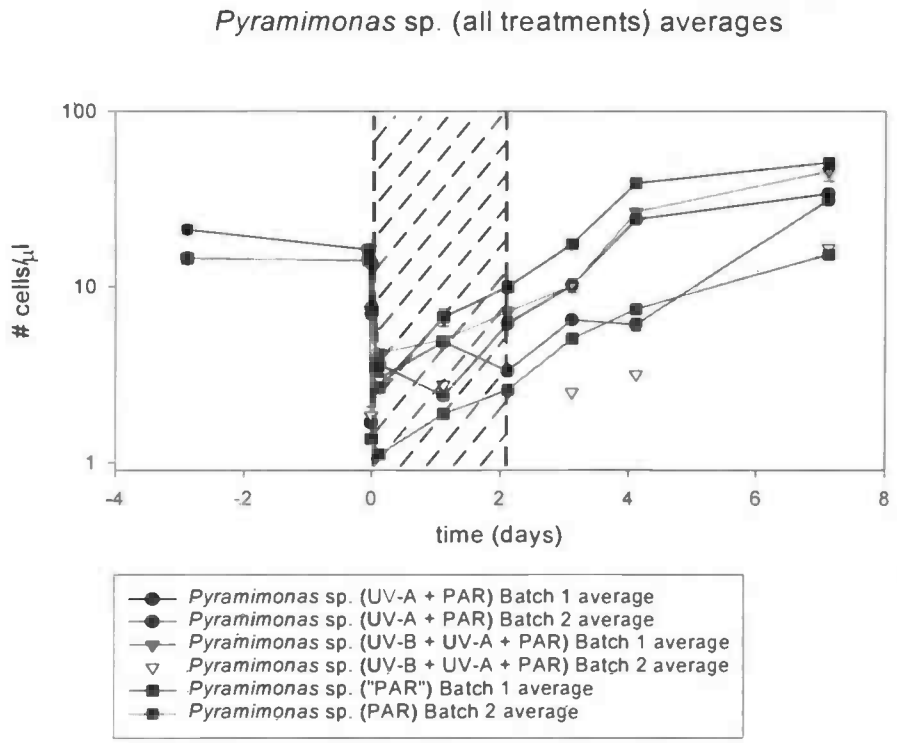

Pyramimonas sp. (all treatments) averages
100
10
1
cells/µl
-4
-2
0
2
4
6
8
time (days)
Pyramimonas sp. (UV-A + PAR) Batch 1 average
Pyramimonas sp. (UV-A + PAR) Batch 2 average
Pyramimonas sp. (UV-B + UV-A + PAR) Batch 1 average
Pyramimonas sp. (UV-B + UV-A + PAR) Batch 2 average
Pyramimonas sp. ("PAR") Batch 1 average
Pyramimonas sp. (PAR) Batch 2 average

Fig. 9.3

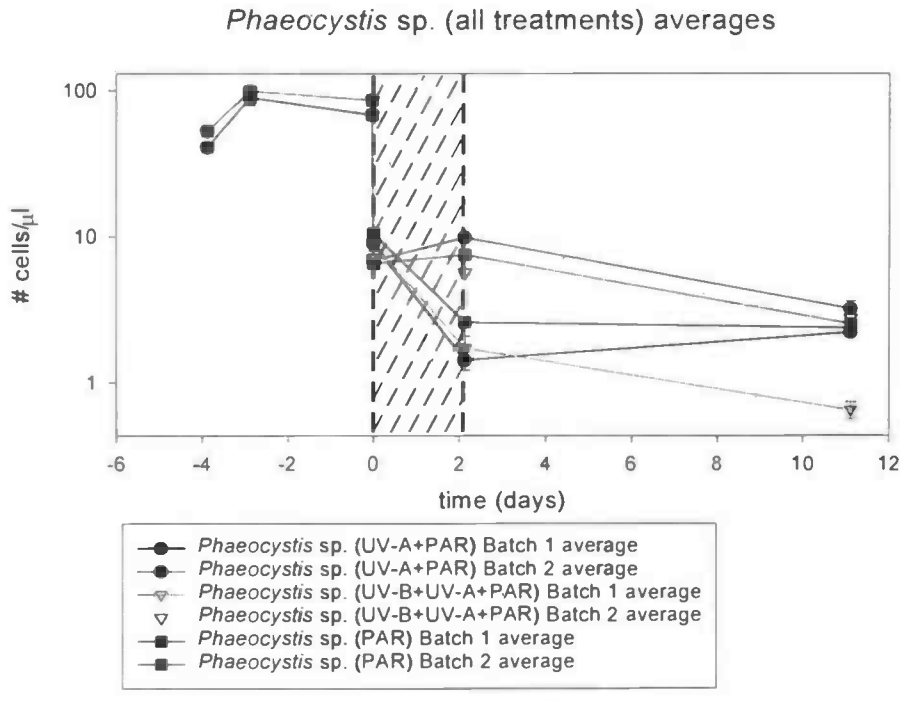

Phaeocystis sp. (all treatments) averages
100
10
1
cells/µl
-6
-4
-2
0
2
4
6
8
10
12
time (days)
Phaeocystis sp. (UV-A+PAR) Batch 1 average
Phaeocystis sp. (UV-A+PAR) Batch 2 average
Phaeocystis sp. (UV-B+UV-A+PAR) Batch 1 average
Phaeocystis sp. (UV-B+UV-A+PAR) Batch 2 average
Phaeocystis sp. (PAR) Batch 1 average
Phaeocystis sp. (PAR) Batch 2 average

Fig. 9.4

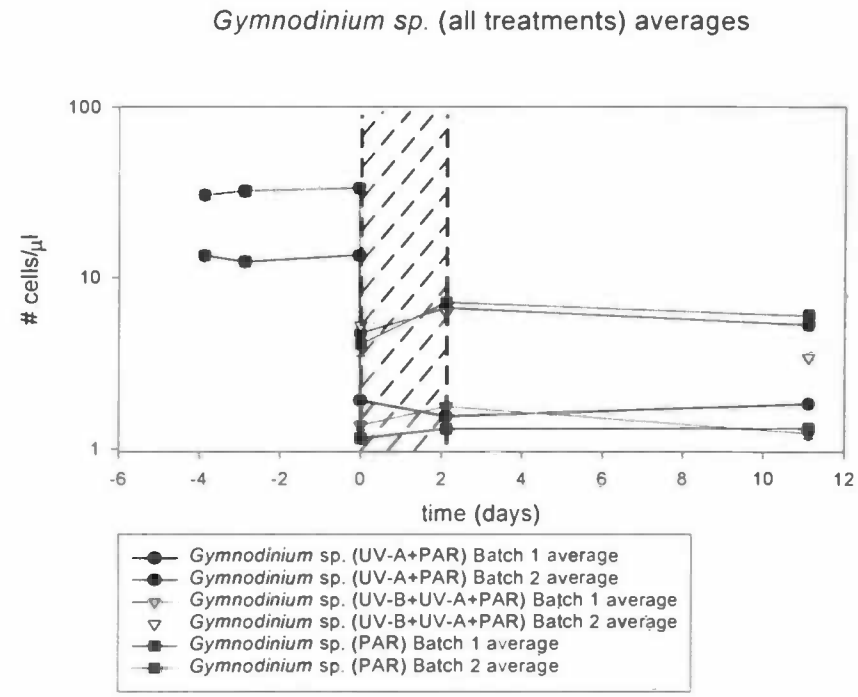

Gymnodinium sp. (all treatments) averages
100
10
1
cells/µl
-6
-4
-2
0
2
4
6
8
10
12
time (days)
Gymnodinium sp. (UV-A+PAR) Batch 1 average
Gymnodinium sp. (UV-A+PAR) Batch 2 average
Gymnodinium sp. (UV-B+UV-A+PAR) Batch 1 average
Gymnodinium sp. (UV-B+UV-A+PAR) Batch 2 average
Gymnodinium sp. (PAR) Batch 1 average
Gymnodinium sp. (PAR) Batch 2 average

Fig. 9.5

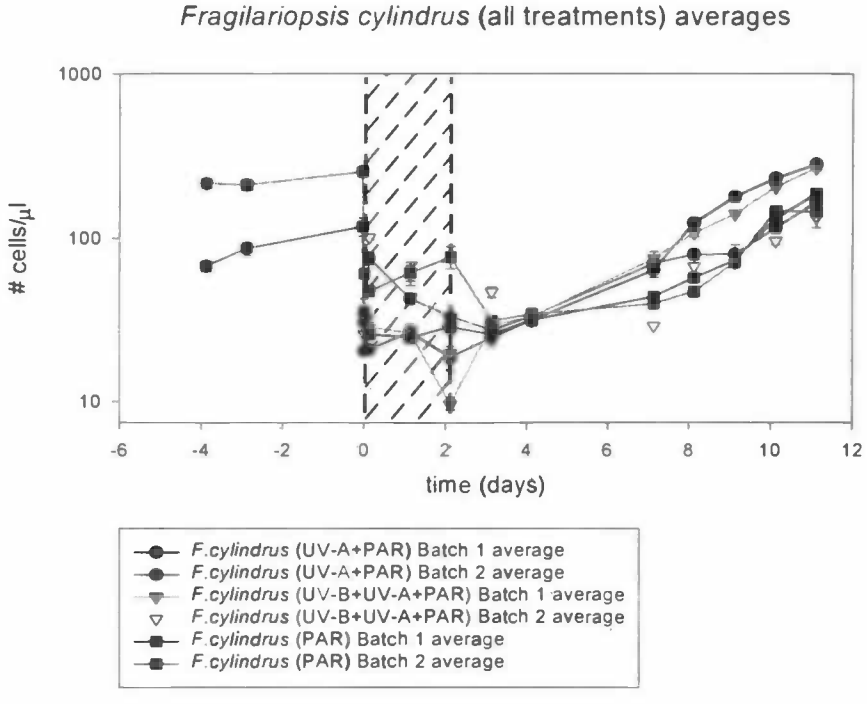
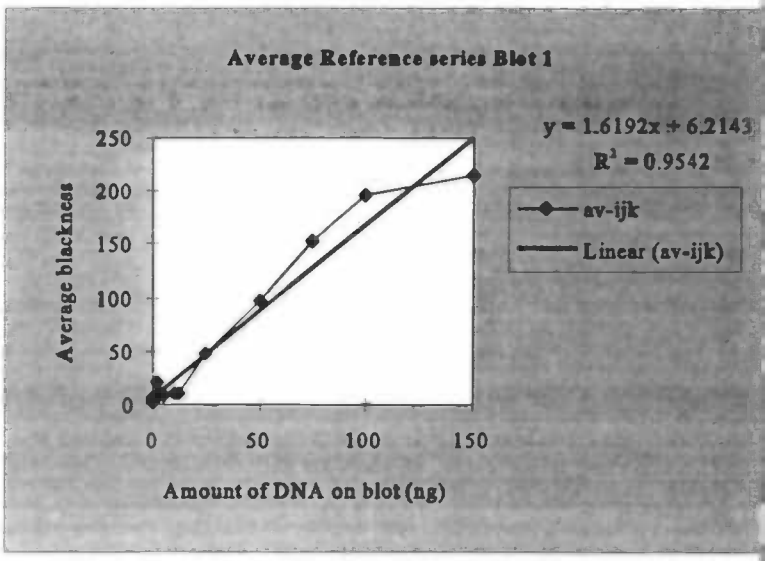

Fragilariopsis cylindrus (all treatments) averages
1000
100
10
cells/µl
-6
-4
-2
0
2
4
6
8
10
12
time (days)
F.cylindrus (UV-A+PAR) Batch 1 average
F.cylindrus (UV-A+PAR) Batch 2 average
F.cylindrus (UV-B+UV-A+PAR) Batch 1 average
F.cylindrus (UV-B+UV-A+PAR) Batch 2 average
F.cylindrus (PAR) Batch 1 average
F.cylindrus (PAR) Batch 2 average

Fig. 9.6

APPENDIX 10: Growth rates (GR, day^{-1}) of used species & treatments. Results are calculated from data in Appendix 9.

Species + Treatment	GR (precult.)	GR (UVR)	GR (Recov.)
C.brevis (UVA+PAR) Batch 1	0.3831398	0.2110535	0.5372356
C.brevis (UVA+PAR) Batch 2	0.4666842	0.3978916	0.2478455
C.brevis (UVB+UVA+PAR) Batch 1	0.3831398	0.3998231	-0.0436621
C.brevis (UVB+UVA+PAR) Batch 2	0.4666842	0.5222136	0.0689327
C.brevis ("PAR") Batch 1	0.3831398	0.2856571	0.4128954
C.brevis (PAR) Batch 2	0.4666842	0.4765666	0.2580796
P.glacialis (UVA+PAR) Batch 1	0.4419028	0.1166036	0.1582163
P.glacialis (UVB+UVA+PAR) Batch 1	0.4419028	0.4059677	0.1318379
P.glacialis ("PAR") Batch 1	0.4419028	0.3378237	-0.0647165
P.glacialis (PAR) Batch 2	0.0428338	-0.007278	-0.0069854
Pyramimonas sp. (UVA+PAR) Batch 1	-0.094955	0.2682933	0.3411953
Pyramimonas sp. (UVA+PAR) Batch 2	-0.010197	0.3162043	0.448303
Pyramimonas sp. (UVB+UVA+PAR) Batch 1	-0.094955	0.2668066	0.3692108
Pyramimonas sp. (UVB+UVA+PAR) Batch 2	-0.010197	0.1406312	0.3781715
Pyramimonas sp. ("PAR") Batch 1	-0.094955	0.6578538	0.3278609
Pyramimonas sp. (PAR) Batch 2	-0.010197	0.4205041	0.3545005
Phaeocystis sp. (UVA+PAR) Batch 1	0.779561	-0.866383	0.0479572
Phaeocystis sp. (UVA+PAR) Batch 2	0.6363394	0.1693246	-0.1252825
Phaeocystis sp. (UVB+UVA+PAR) Batch 1	0.779561	-0.782871	-0.1083552
Phaeocystis sp. (UVB+UVA+PAR) Batch 2	0.6363394	-0.10434	-0.081162
Phaeocystis sp. (PAR) Batch 1	0.779561	-0.656995	-0.0108415
Phaeocystis sp. (PAR) Batch 2	0.6363394	0.0635203	-0.1217951
Gymnodinium sp. (UVA+PAR) Batch 1	0.0002428	-0.100548	0.0186232
Gymnodinium sp. (UVA+PAR) Batch 2	0.0240069	0.160262	-0.0254274
Gymnodinium sp. (UVB+UVA+PAR) Batch 1	0.0002428	0.1203856	-0.0406536
Gymnodinium sp. (UVB+UVA+PAR) Batch 2	0.0240069	0.0828111	-0.0672495
Gymnodinium sp. (PAR) Batch 1	0.0002428	0.0603906	0.0002114
Gymnodinium sp. (PAR) Batch 2	0.0240069	0.2633274	-0.0199895
F.cylindrus (UVA+PAR) Batch 1	0.1447385	-0.047643	0.299908
F.cylindrus (UVA+PAR) Batch 2	0.0412857	-0.405793	0.1771581
F.cylindrus (UVB+UVA+PAR) Batch 1	0.1447385	-0.468627	0.3689633
F.cylindrus (UVB+UVA+PAR) Batch 2	0.0412857	-0.811884	0.2114271
F.cylindrus (PAR) Batch 1	0.1447385	0.0516297	0.2066016
F.cylindrus (PAR) Batch 2	0.0412857	0.2397093	0.0701492

APPENDIX 11: Reference series used in blotting sessions. Bold values indicate values used.

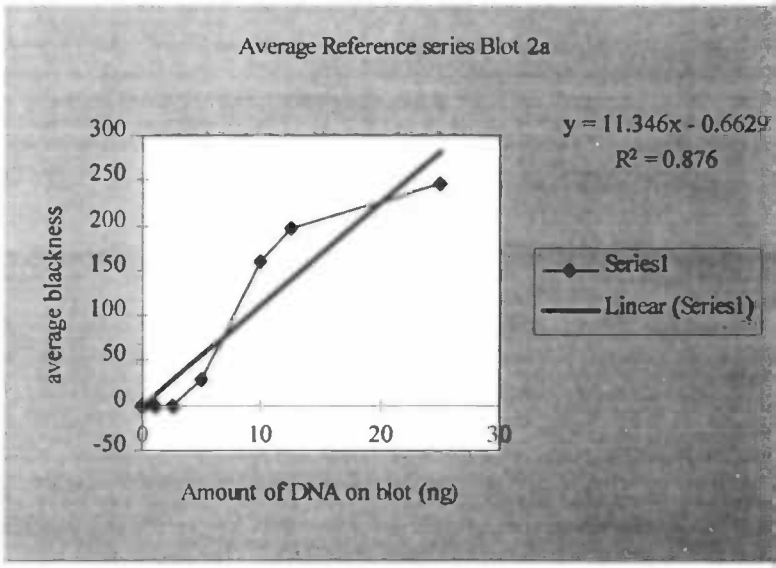


Blot 1:

amount DNA	average reference series
0	**2.377917**
0.5	**5.619417**
1	**5.407417**
2.5	**19.76042**
5	**8.503417**
10	**10.04292**
12.5	**11.01492**
25	**47.50642**
50	**96.47492**
75	**153.7449**
100	**197.1909**
150	**215.6034**

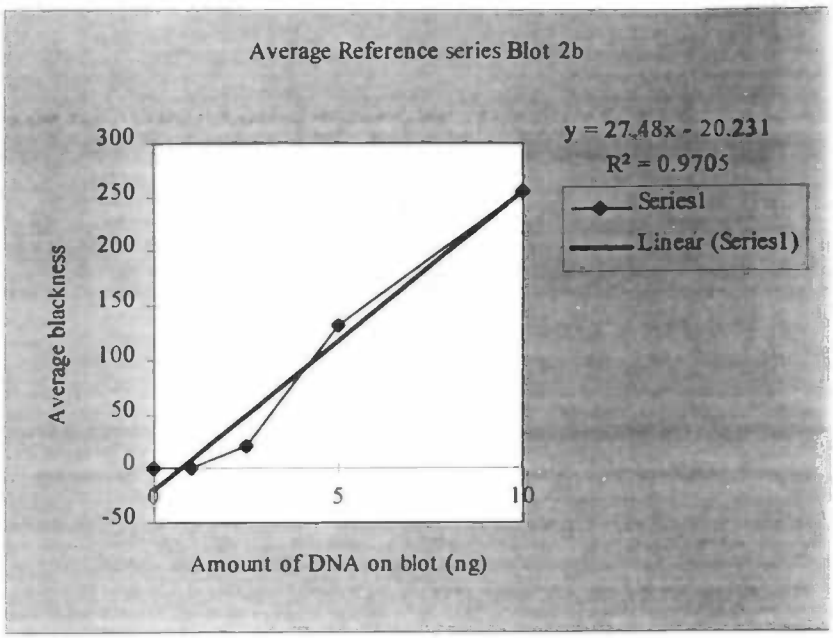
Blot 2: First Blot

amount DNA	average reference series
0	**0**
1	**0**
2.5	**0.014**
5	**28.588**
10	**160.5975**
12.5	**196.7975**
25	**244.746**
50	250.4545
75	250.8515
100	251.162
150	251.199
200	251.8115



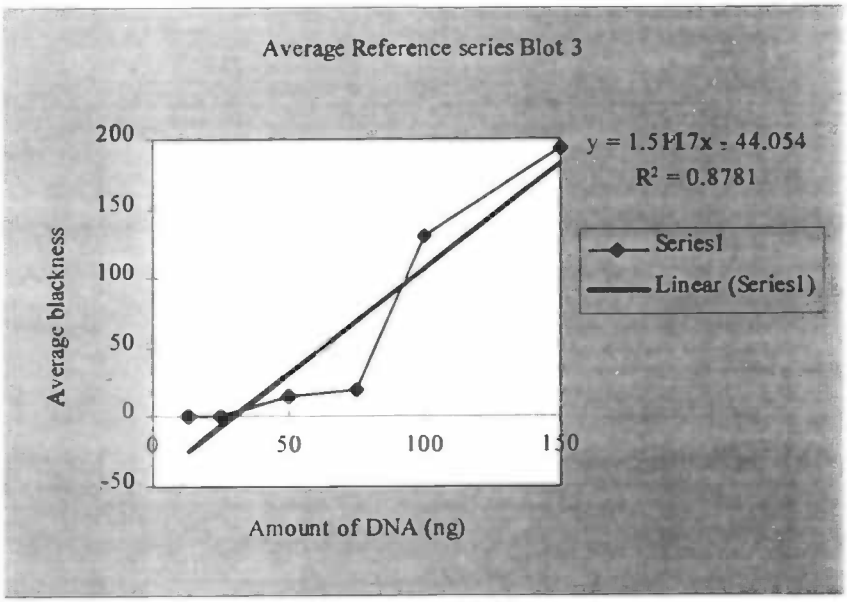
Blot 2: Second Blot

amount DNA	average reference series
0	**0**
1	**0**
2.5	**20.4135**
5	**131.8415**
10	**254.9765**
12.5	254.97
25	255
50	255
75	255
100	255
150	255
200	255



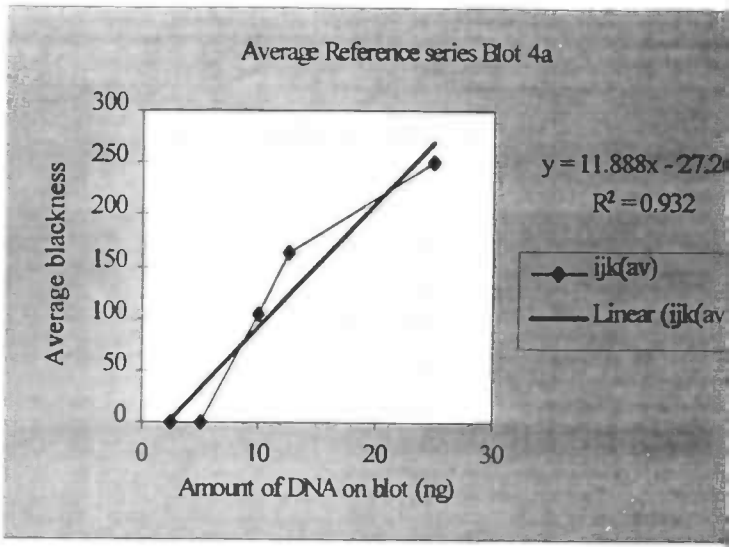
Blot 3:

Amount DNA	Average reference series
0	0.2895
0.5	0.023
1	0.24
5	0.0685
10	0
12.5	**0**
25	**0.0085**
50	**14.605**
75	**19.493**
100	**130.8765**
150	**194.2895**



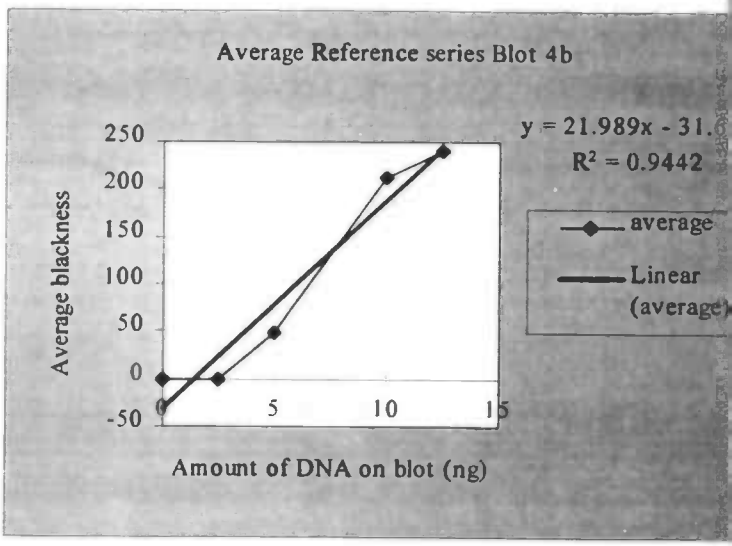
Blot 4: 1e Blot

amount	Average reference series
0	0.0005
1	0.0095
2.5	**0.0175**
5	**0.387**
10	**103.6555**
12.5	**162.138**
25	**251.3135**
50	245.9795
75	252.513
100	254.8745



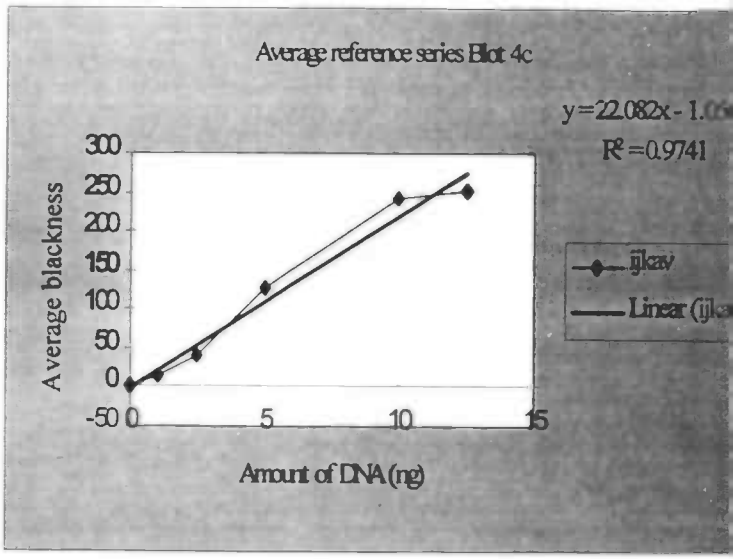
Blot 4: 2e Blot

amount	average reference series
0	0.0755
1	14.8655
2.5	0.16
5	48.074
10	212.871
12.5	240.2225
25	254.9825
50	254.823
75	254.9415
100	254.99



Blot 4: 3e Blot

amount	Average reference series
0	1.029
1	15.2105
2.5	38.39
5	128.152
10	242.707
12.5	252.7005
25	254.995
50	254.9425
75	255
100	255



APPENDIX 12: Blotting results. Tables contain all relevant steps used to calculate thymine dimer amounts (last column).

1e blot Batch 1,2 *C.brevis*, *P.glacialis*, *Pyramimonas* sp.

Sample	Volume	Average	Area	av-bl	ngijkdna/sample	opgebracht	ngijk/ngsample	T<>T/10^6n.
Ref.series-0	35329	30.247	1168	-3.11558	-1.707377909	0	#DIV/0!	#DIV/0!
Ref.series-0.5	37215	31.862	1168	-1.50058	-0.888080019	0.5	-1.776160038	-260.39
Ref.series-1	30925	26.477	1168	-6.88558	-3.619918493	1	-3.619918493	-530.68
Ref.series-2.5	48169	41.241	1168	7.878417	3.869935403	2.5	1.547974161	226.93
Ref.series- 5	48238	41.3	1168	7.937417	3.89986641	5	0.779973282	114.34
Ref.series-10	48831	41.807	1168	8.444417	4.157070143	10	0.415707014	60.94
Ref.series-12.5	59217	50.699	1168	17.33642	8.668027936	12.5	0.693442235	101.66
Ref.series-25	125087	107.095	1168	73.73242	37.2780117	25	1.491120468	218.60
Ref.series-50	207447	177.609	1168	144.2464	73.05013021	50	1.461002604	214.18
Ref.series-75	251241	215.104	1168	181.7414	92.07153849	75	1.227620513	179.97
Ref.series-100	289776	248.096	1168	214.7334	108.8085515	100	1.088085515	159.51
Ref.series-150	291879	249.896	1168	216.5334	109.7217008	150	0.731478006	107.23
Ref.series-0a	48161	41.234	1168	7.871417	3.866384267	0	#DIV/0!	#DIV/0!
Ref.series-0.5a	53847	46.102	1168	12.73942	6.335945955	0.5	12.67189191	1857.70
Ref.series-1a	59641	51.063	1168	17.70042	8.852687027	1	8.852687027	1297.80
Ref.series-2.5a	75926	65.005	1168	31.64242	15.92553605	2.5	6.370214421	933.87
Ref.series- 5a	49560	42.432	1168	9.069417	4.47413589	5	0.894827178	131.18
Ref.series-10a	52565	45.004	1168	11.64142	5.778924851	10	0.577892485	84.72
Ref.series-12.5a	44449	38.056	1168	4.693417	2.254168358	12.5	0.180333469	26.44
Ref.series-25a	63823	54.643	1168	21.28042	10.66883962	25	0.426753585	62.56
Ref.series-50a	95853	82.066	1168	48.70342	24.58066998	50	0.4916134	72.07
Ref.series-75a	185842	159.111	1168	125.7484	63.66599871	75	0.848879983	124.45
Ref.series-100a	248797	213.011	1168	179.6484	91.00974871	100	0.910097487	133.42
Ref.series-150a	289706	248.036	1168	214.6734	108.7781132	150	0.725187421	106.31
C.brevis (UVA+PAR) Batch 1	34139	29.229	1168	-4.13358	-2.223814597	100	-0.022238146	-3.26
C.brevis (UVA+PAR) Batch 1	35710	30.574	1168	-2.78858	-1.54148911	200	-0.007707446	-1.13
C.brevis (UVA+PAR) Batch 2	36006	30.827	1168	-2.53558	-1.413140896	100	-0.014131409	-2.07
C.brevis (UVA+PAR) Batch 2	41483	35.516	1168	2.153417	0.965613163	200	0.004828066	0.71
C.brevis (UVB+UVA+PAR) Batch 1	39984	34.233	1168	0.870417	0.314740598	100	0.003147406	0.46
C.brevis (UVB+UVA+PAR) Batch 1	41060	35.154	1168	1.791417	0.781968682	200	0.003909843	0.57
C.brevis (UVB+UVA+PAR) Batch 2	35638	30.512	1168	-2.85058	-1.572942032	100	-0.01572942	-2.31
C.brevis (UVB+UVA+PAR) Batch 2	41504	35.534	1168	2.171417	0.974744656	200	0.004873723	0.71
C.brevis ("PAR") Batch 1	34850	29.837	1168	-3.52558	-1.915373038	100	-0.01915373	-2.81
C.brevis ("PAR") Batch 1	31784	27.212	1168	-6.15058	-3.247049175	200	-0.016235246	-2.38
C.brevis (PAR) Batch 2	35056	30.014	1168	-3.34858	-1.825580019	100	-0.0182558	-2.68
C.brevis (PAR) Batch 2	32919	28.184	1168	-5.17858	-2.753948525	200	-0.013769743	-2.02
P.glacialis (UVA+PAR) Batch 1	235507	201.633	1168	168.2704	85.23763021	100	0.852376302	124.96
P.glacialis (UVA+PAR) Batch 1	289088	247.507	1168	214.1444	108.5097487	200	0.542548744	79.54
P.glacialis (UVA+PAR) Batch 2	232963	199.455	1168	166.0924	84.13271949	100	0.841327195	123.34
P.glacialis (UVA+PAR) Batch 2	284137	243.268	1168	209.9054	106.359282	200	0.53179641	77.96
P.glacialis (UVB+UVA+PAR) Batch 1	248789	213.004	1168	179.6414	91.00619758	100	0.910061976	133.42
P.glacialis (UVB+UVA+PAR) Batch 1	295584	253.068	1168	219.7054	111.3308729	200	0.556654365	81.61
P.glacialis (UVB+UVA+PAR) Batch 2	220713	188.967	1168	155.6044	78.81210261	100	0.788121026	115.54
P.glacialis (UVB+UVA+PAR) Batch 2	285014	244.019	1168	210.6564	106.7402682	200	0.533701341	78.24
P.glacialis ("PAR") Batch 1	100181	85.771	1168	52.40842	26.46023573	100	0.264602357	38.79
P.glacialis ("PAR") Batch 1	271999	232.876	1168	199.5134	101.0873664	200	0.505436832	74.10
P.glacialis (PAR) Batch 2	268830	230.163	1168	196.8004	99.71104742	100	0.997110474	146.18
P.glacialis (PAR) Batch 2	297401	254.624	1168	221.2614	112.1202398	200	0.560601199	82.18
Pyramimonas sp. (UVA+PAR) Batch 1	81359	69.657	1168	36.29442	18.28551982	100	0.182855198	26.81
Pyramimonas sp. (UVA+PAR) Batch 1	216324	185.209	1168	151.8464	76.90564969	200	0.384528248	56.37
Pyramimonas sp. (UVA+PAR) Batch 2	82949	71.018	1168	37.65542	18.97596219	100	0.189759622	27.82
Pyramimonas sp. (UVA+PAR) Batch 2	179924	154.045	1168	120.6824	61.0959906	200	0.305479953	44.78
Pyramimonas sp. (UVB+UVA+PAR) Batch 1	84628	72.455	1168	39.09242	19.70495975	100	0.197049598	28.89
Pyramimonas sp. (UVB+UVA+PAR) Batch 1	148467	127.112	1168	93.74942	47.43273979	200	0.237163699	34.77
Pyramimonas sp. (UVB+UVA+PAR) Batch 2	80162	68.632	1168	35.26942	17.76553199	100	0.17765532	26.04
Pyramimonas sp. (UVB+UVA+PAR) Batch 2	140541	120.326	1168	86.96342	43.99016673	200	0.219950834	32.24
Pyramimonas sp. ("PAR") Batch 1	79155	67.77	1168	34.40742	17.32823492	100	0.173282349	25.40
Pyramimonas sp. ("PAR") Batch 1	232349	198.929	1168	165.5664	83.86587696	200	0.419329385	61.47
Pyramimonas sp. (PAR) Batch 2	73045	62.539	1168	29.17642	14.67452144	100	0.146745214	21.51
Pyramimonas sp. (PAR) Batch 2	158395	135.612	1168	102.2494	51.74483394	200	0.25872417	37.93
blanco-1	29366	25.142	1168	-8.22058	-4.297170928	0	#DIV/0!	#DIV/0!
blanco-2	25705	22.008	1168	-11.3546	-5.887065409	0	#DIV/0!	#DIV/0!
blanco-3	27158	23.252	1168	-10.1106	-5.255977746	0	#DIV/0!	#DIV/0!
blanco-4	27330	23.399	1168	-9.96358	-5.181403883	0	#DIV/0!	#DIV/0!
blanco-5	27317	23.388	1168	-9.97458	-5.18698424	0	#DIV/0!	#DIV/0!
blanco-6	31984	27.384	1168	-5.97858	-3.159792681	0	#DIV/0!	#DIV/0!
blanco-7	36216	31.007	1168	-2.35558	-1.32182596	0	#DIV/0!	#DIV/0!
blanco-8	41080	35.171	1168	1.808417	0.790592871	0	#DIV/0!	#DIV/0!
blanco-9	51120	43.767	1168	10.40442	5.151388325	0	#DIV/0!	#DIV/0!
blanco-10	49125	42.059	1168	8.696417	4.284911052	0	#DIV/0!	#DIV/0!
blanco-11	59675	51.092	1168	17.72942	8.867398877	0	#DIV/0!	#DIV/0!
blanco-12	61532	52.682	1168	19.31942	9.674014137	0	#DIV/0!	#DIV/0!
	blanco-av	33.36258						

2nd Blot (Batch 1,2 *Phaeocystis*, *Gymnodinium*, *Fragilariopsis;* 1st illumination)

Sample	Volume	Average	Area		ngijkdna/sample	opgebracht	ngijk/ngsample	T<>T/10^6n.
Ref.series-0	0	0	833		0.736208151	0	#DIV/0!	#DIV/0!
Ref.series-1	0	0	833		0.736208151	1	0.736208151	107.93
Ref.series-2.5	34009	40.827	833		2.221906841	2.5	0.888762737	130.29
Ref.series- 5	102632	123.208	833		5.219759825	5	1.043951965	153.04
Ref.series-10	212376	254.953	833		10.0139738	10	1.00139738	146.80
Ref.series-12.5	212365	254.94	833		10.01350073	12.5	0.801080058	117.44
Ref.series-25	212415	255	833		10.01568413	25	0.400627365	58.73
Ref.series-50	212415	255	833		10.01568413	50	0.200313683	29.37
Ref.series-75	212415	255	833		10.01568413	75	0.133542455	19.58
Ref.series-100	212415	255	833		10.01568413	100	0.100156841	14.68
Ref.series-150	212415	255	833		10.01568413	150	0.066771228	9.79
Ref.series-200	212415	255	833		10.01568413	200	0.050078421	7.34
Ref.series-0a	0	0	833		0.736208151	0	#DIV/0!	#DIV/0!
Ref.series-0.5a	0	0	833		0.736208151	0.5	1.472416303	215.86
Ref.series-1a	0	0	833		0.736208151	1	0.736208151	107.93
Ref.series-2.5a	0	0	833		0.736208151	2.5	0.294483261	43.17
Ref.series- 5a	117016	140.475	833		5.848107715	5	1.169621543	171.47
Ref.series-10a	212415	255	833		10.01568413	10	1.001568413	146.83
Ref.series-12.5a	212415	255	833		10.01568413	12.5	0.801254731	117.46
Ref.series-25a	212415	255	833		10.01568413	25	0.400627365	58.73
Ref.series-50a	212415	255	833		10.01568413	50	0.200313683	29.37
Ref.series-75a	212415	255	833		10.01568413	75	0.133542455	19.58
Ref.series-100a	212415	255	833		10.01568413	100	0.100156841	14.68
Ref.series-150a	212415	255	833		10.01568413	150	0.066771228	9.79
Ref.series-200a	212415	255	833		10.01568413	200	0.050078421	7.34
Phaeocystis sp. (UVA+PAR) Batch 1	0	0	833		0.736208151	135	0.005453394	0.00
Phaeocystis sp. (UVA+PAR) Batch 1	0	0	833		0.736208151	135	0.005453394	0.00
Phaeocystis sp. (UVA+PAR) Batch 2	0	0	833		0.736208151	71	0.010369129	0.00
Phaeocystis sp. (UVA+PAR) Batch 2	0	0	833		0.736208151	71	0.010369129	0.00
Phaeocystis sp. (UVB+UVA+PAR) Batch 1	76493	91.828	833		4.077838428	91	0.044811411	6.57
Phaeocystis sp. (UVB+UVA+PAR) Batch 1	49920	59.928	833		2.916994178	91	0.032054881	4.70
Phaeocystis sp. (UVB+UVA+PAR) Batch 2	9253	11.108	833		1.140429403	155	0.007357609	1.08
Phaeocystis sp. (UVB+UVA+PAR) Batch 2	0	0	833		0.736208151	155	0.00474973	0.00
Phaeocystis sp. (PAR) Batch 1	0	0	833		0.736208151	135	0.005453394	0.00
Phaeocystis sp. (PAR) Batch 1	0	0	833		0.736208151	135	0.005453394	0.00
Phaeocystis sp. (PAR) Batch 2	0	0	833		0.736208151	128	0.005751626	0.00
Phaeocystis sp. (PAR) Batch 2	0	0	833		0.736208151	128	0.005751626	0.00
Gymnodinium sp. (UVA+PAR) Batch 1	0	0	833		0.736208151	164	0.004489074	0.00
Gymnodinium sp. (UVA+PAR) Batch 1	0	0	833		0.736208151	164	0.004489074	0.00
Gymnodinium sp. (UVA+PAR) Batch 2	0	0	833		0.736208151	155	0.00474973	0.00
Gymnodinium sp. (UVA+PAR) Batch 2	0	0	833		0.736208151	155	0.00474973	0.00
Gymnodinium sp. (UVB+UVA+PAR) Batch 1	9	0.011	833		0.736608443	133	0.005538409	0.81
Gymnodinium sp. (UVB+UVA+PAR) Batch 1	7	0.008	833		0.736499272	133	0.005537589	0.81
Gymnodinium sp. (UVB+UVA+PAR) Batch 2	212328	254.896	833		10.01189956	177	0.056564404	8.29
Gymnodinium sp. (UVB+UVA+PAR) Batch 2	212250	254.802	833		10.00847889	177	0.056545078	8.29
Gymnodinium sp. (PAR) Batch 1	0	0	833		0.736208151	154	0.004780572	0.00
Gymnodinium sp. (PAR) Batch 1	0	0	833		0.736208151	154	0.004780572	0.00
Gymnodinium sp. (PAR) Batch 2	0	0	833		0.736208151	68	0.01082659	0.00
Gymnodinium sp. (PAR) Batch 2	0	0	833		0.736208151	68	0.01082659	0.00
F.cylindrus (UVA+PAR) Batch 1	0	0	833		0.736208151	87	0.008462163	0.00
F.cylindrus (UVA+PAR) Batch 1	0	0	833		0.736208151	87	0.008462163	0.00
F.cylindrus (UVA+PAR) Batch 2	0	0	833		0.736208151	175	0.004206904	0.00
F.cylindrus (UVA+PAR) Batch 2	67	0.08	833		0.73911936	175	0.004223539	0.62
F.cylindrus (UVB+UVA+PAR) Batch 1	0	0	833		0.736208151	101	0.00728919	0.00
F.cylindrus (UVB+UVA+PAR) Batch 1	6	0.007	833		0.736462882	101	0.007291712	1.07
F.cylindrus (UVB+UVA+PAR) Batch 2	0	0	833		0.736208151	235	0.003132801	0.00
F.cylindrus (UVB+UVA+PAR) Batch 2	0	0	833		0.736208151	235	0.003132801	0.00
F.cylindrus (PAR) Batch 1	0	0	833		0.736208151	72	0.010225113	0.00
F.cylindrus (PAR) Batch 1	0	0	833		0.736208151	72	0.010225113	0.00
F.cylindrus (PAR) Batch 2	0	0	833		0.736208151	173	0.004255538	0.00
F.cylindrus (PAR) Batch 2	0	0	833		0.736208151	173	0.004255538	0.00
blanco-1	0	0	833		0.736208151	0	#DIV/0!	#DIV/0!
blanco-2	0	0	833		0.736208151	0	#DIV/0!	#DIV/0!
blanco-3	0	0	833		0.736208151	0	#DIV/0!	#DIV/0!
blanco-4	17453	20.952	833		1.498653566	0	#DIV/0!	#DIV/0!
blanco-5	0	0	833		0.736208151	0	#DIV/0!	#DIV/0!
blanco-6	0	0	833		0.736208151	0	#DIV/0!	#DIV/0!
blanco-7	0	0	833		0.736208151	0	#DIV/0!	#DIV/0!
blanco-8	0	0	833		0.736208151	0	#DIV/0!	#DIV/0!
blanco-9	0	0	833		0.736208151	0	#DIV/0!	#DIV/0!
blanco-10	0	0	833		0.736208151	0	#DIV/0!	#DIV/0!
blanco-11	0	0	833		0.736208151	0	#DIV/0!	#DIV/0!

2nd Blot (Batch 1,2 *Phaeocystis*, *Gymnodinium*, Fragilariopsis; 2nd illumination)

Sample	Volume	Average	Area	ngijkdna/sample	opgebracht	ngijk/ngsample	T<>T/10^6n.
Ref.series-0	0	0	880	0.058425877	0	#DIV/0!	#DIV/0!
Ref.series-1	0	0	880	0.058425877	1	0.058425877	8.57
Ref.series-2.5	25	0.028	880	0.060893707	2.5	0.024357483	3.57
Ref.series- 5	20495	23.29	880	2.111131676	5	0.422226335	61.90
Ref.series-10	146386	166.348	880	14.71980434	10	1.471980434	215.79
Ref.series-12.5	171494	194.88	880	17.23452318	12.5	1.378761854	202.13
Ref.series-25	217234	246.857	880	21.81560903	25	0.872624361	127.93
Ref.series-50	220500	250.568	880	22.14268465	50	0.442853693	64.92
Ref.series-75	220724	250.823	880	22.16515953	75	0.29553546	43.33
Ref.series-100	221138	251.293	880	22.20658382	100	0.222065838	32.55
Ref.series-150	221023	251.162	880	22.1950379	150	0.147966919	21.69
Ref.series-200	221595	251.813	880	22.25241495	200	0.111262075	16.31
Ref.series-0a	0	0	880	0.058425877	0	#DIV/0!	#DIV/0!
Ref.series-0.5a	0	0	880	0.058425877	0.5	0.116851754	17.13
Ref.series-1a	0	0	880	0.058425877	1	0.058425877	8.57
Ref.series-2.5a	0	0	880	0.058425877	2.5	0.023370351	3.43
Ref.series- 5a	29820	33.886	880	3.045029085	5	0.609005817	89.28
Ref.series-10a	136265	154.847	880	13.70614313	10	1.370614313	200.93
Ref.series-12.5a	174869	198.715	880	17.57252776	12.5	1.405802221	206.09
Ref.series-25a	213519	242.635	880	21.44349551	25	0.85773982	125.74
Ref.series-50a	220300	250.341	880	22.1226776	50	0.442453552	64.86
Ref.series-75a	220774	250.88	880	22.17018332	75	0.295602444	43.34
Ref.series-100a	220907	251.031	880	22.18349198	100	0.22183492	32.52
Ref.series-150a	221088	251.236	880	22.20156002	150	0.1480104	21.70
Ref.series-200a	221593	251.81	880	22.25215054	200	0.111260753	16.31
Phaeocystis sp. (UVA+PAR) Batch 1	0	0	880	0.058425877	135	0.000432784	0.00
Phaeocystis sp. (UVA+PAR) Batch 1	0	0	880	0.058425877	135	0.000432784	0.00
Phaeocystis sp. (UVA+PAR) Batch 2	0	0	880	0.058425877	71	0.0008229	0.00
Phaeocystis sp. (UVA+PAR) Batch 2	0	0	880	0.058425877	71	0.0008229	0.00
Phaeocystis sp. (UVB+UVA+PAR) Batch 1	1681	1.91	880	0.226767143	91	0.002491947	0.37
Phaeocystis sp. (UVB+UVA+PAR) Batch 1	27	0.031	880	0.061158117	91	0.000672067	0.10
Phaeocystis sp. (UVB+UVA+PAR) Batch 2	9	0.01	880	0.059307245	155	0.000382627	0.06
Phaeocystis sp. (UVB+UVA+PAR) Batch 2	0	0	880	0.058425877	155	0.000376941	0.00
Phaeocystis sp. (PAR) Batch 1	0	0	880	0.058425877	135	0.000432784	0.00
Phaeocystis sp. (PAR) Batch 1	0	0	880	0.058425877	135	0.000432784	0.00
Phaeocystis sp. (PAR) Batch 2	0	0	880	0.058425877	128	0.000456452	0.00
Phaeocystis sp. (PAR) Batch 2	0	0	880	0.058425877	128	0.000456452	0.00
Gymnodinium sp. (UVA+PAR) Batch 1	0	0	880	0.058425877	164	0.000356255	0.00
Gymnodinium sp. (UVA+PAR) Batch 1	0	0	880	0.058425877	164	0.000356255	0.00
Gymnodinium sp. (UVA+PAR) Batch 2	0	0	880	0.058425877	155	0.000376941	0.00
Gymnodinium sp. (UVA+PAR) Batch 2	54	0.061	880	0.063802221	155	0.000411627	0.06
Gymnodinium sp. (UVB+UVA+PAR) Batch 1	0	0	880	0.058425877	133	0.000439292	0.00
Gymnodinium sp. (UVB+UVA+PAR) Batch 1	0	0	880	0.058425877	133	0.000439292	0.00
Gymnodinium sp. (UVB+UVA+PAR) Batch 2	131546	149.484	880	13.23346554	177	0.074765342	10.96
Gymnodinium sp. (UVB+UVA+PAR) Batch 2	128794	146.357	880	12.9578618	177	0.073208259	10.73
Gymnodinium sp. (PAR) Batch 1	0	0	880	0.058425877	154	0.000379389	0.00
Gymnodinium sp. (PAR) Batch 1	0	0	880	0.058425877	154	0.000379389	0.00
Gymnodinium sp. (PAR) Batch 2	0	0	880	0.058425877	68	0.000859204	0.00
Gymnodinium sp. (PAR) Batch 2	0	0	880	0.058425877	68	0.000859204	0.00
F.cylindrus (UVA+PAR) Batch 1	0	0	880	0.058425877	87	0.000671562	0.00
F.cylindrus (UVA+PAR) Batch 1	0	0	880	0.058425877	87	0.000671562	0.00
F.cylindrus (UVA+PAR) Batch 2	0	0	880	0.058425877	175	0.000333862	0.00
F.cylindrus (UVA+PAR) Batch 2	0	0	880	0.058425877	175	0.000333862	0.00
F.cylindrus (UVB+UVA+PAR) Batch 1	0	0	880	0.058425877	101	0.000578474	0.00
F.cylindrus (UVB+UVA+PAR) Batch 1	0	0	880	0.058425877	101	0.000578474	0.00
F.cylindrus (UVB+UVA+PAR) Batch 2	0	0	880	0.058425877	235	0.000248621	0.00
F.cylindrus (UVB+UVA+PAR) Batch 2	0	0	880	0.058425877	235	0.000248621	0.00
F.cylindrus (PAR) Batch 1	0	0	880	0.058425877	72	0.000811471	0.00
F.cylindrus (PAR) Batch 1	0	0	880	0.058425877	72	0.000811471	0.00
F.cylindrus (PAR) Batch 2	8	0.009	880	0.059219108	173	0.000342307	0.05
F.cylindrus (PAR) Batch 2	4	0.005	880	0.058866561	173	0.000340269	0.05
blanco-1	3870	4.398	880	0.446051472	0	#DIV/0!	#DIV/0!
blanco-2	53	0.06	880	0.063714084	0	#DIV/0!	#DIV/0!
blanco-3	0	0	880	0.058425877	0	#DIV/0!	#DIV/0!
blanco-4	4671	5.308	880	0.526255949	0	#DIV/0!	#DIV/0!
blanco-5	0	0	880	0.058425877	0	#DIV/0!	#DIV/0!
blanco-6	0	0	880	0.058425877	0	#DIV/0!	#DIV/0!
blanco-7	0	0	880	0.058425877	0	#DIV/0!	#DIV/0!
blanco-8	0	0	880	0.058425877	0	#DIV/0!	#DIV/0!
blanco-9	0	0	880	0.058425877	0	#DIV/0!	#DIV/0!
blanco-10	0	0	880	0.058425877	0	#DIV/0!	#DIV/0!
blanco-11	5	0.006	880	0.058954698	0	#DIV/0!	#DIV/0!

3d Blot (all species)

Name	Volume	Average	Area	average-blanc	ngljkdna/sample	opgebracht	ngljk/ngsample	T<>T/10^6n.
Ref.series-0	0	0	1049	-0.2895	28.95051928	0	#DIV/0!	#DIV/0!
Ref.series-0.5	48	0.046	1049	-0.2435	28.9809486	0.5	57.9618972	8553.36
Ref.series-1	0	0	1049	-0.2895	28.95051928	1	28.95051928	4272.22
Ref.series-2.5	0	0	1049	-0.2895	28.95051928	2.5	11.58020771	1708.89
Ref.series- 5	0	0	1049	-0.2895	28.95051928	5	5.790103857	854.44
Ref.series-10	0	0	1049	-0.2895	28.95051928	10	2.895051928	427.22
Ref.series-12.5	0	0	1049	-0.2895	28.95051928	12.5	2.316041543	341.78
Ref.series-25	18	0.017	1049	-0.2725	28.9617649	25	1.158470596	170.95
Ref.series-50	0	0	1049	-0.2895	28.95051928	50	0.579010386	85.44
Ref.series-75	1340	1.277	1049	0.9875	29.79526361	75	0.397270181	58.61
Ref.series-100	196245	187.078	1049	186.7885	152.7039095	100	1.527039095	224.14
Ref.series-150	243032	231.68	1049	231.3905	182.2084408	150	1.214722939	178.27
Ref.series-200	265936	253.514	1049	253.2245	196.6517828	200	0.983258914	144.29
Ref.series-0a	607	0.579	1049	0.2895	29.33353179	0	#DIV/0!	#DIV/0!
Ref.series-0.5a	0	0	1049	-0.2895	28.95051928	0.5	57.90103857	8544.44
Ref.series-1a	503	0.48	1049	0.1905	29.2680426	1	29.2680426	4318.77
Ref.series-2.5a	144	0.137	1049	-0.1525	29.04114573	2.5	11.61645829	1714.20
Ref.series- 5a	0	0	1049	-0.2895	28.95051928	5	5.790103857	854.44
Ref.series-10a	0	0	1049	-0.2895	28.95051928	10	2.895051928	427.22
Ref.series-12.5a	0	0	1049	-0.2895	28.95051928	12.5	2.316041543	341.78
Ref.series-25a	30641	29.21	1049	28.9205	48.2731362	25	1.930925448	284.20
Ref.series-50a	39557	37.709	1049	37.4195	53.89528346	50	1.077905669	158.58
Ref.series-75a	78334	74.675	1049	74.3855	78.34854799	75	1.044647307	153.52
Ref.series-100a	164587	156.899	1049	156.6095	132.7402924	100	1.327402924	194.88
Ref.series-150a	262902	250.622	1049	250.3325	194.7387048	150	1.298258032	190.51
C.brevis (UVA+PAR) Batch 1	0	0	1049	-0.2895	28.95051928	227	0.127535327	0.00
C.brevis (UVA+PAR) Batch 1	0	0	1049	-0.2895	28.95051928	227	0.127535327	0.00
C.brevis (UVA+PAR) Batch 2	0	0	1049	-0.2895	28.95051928	182	0.159068787	0.00
C.brevis (UVA+PAR) Batch 2	0	0	1049	-0.2895	28.95051928	182	0.159068787	0.00
C.brevis (UVB+UVA+PAR) Batch 1	0	0	1049	-0.2895	28.95051928	187	0.154815611	0.00
C.brevis (UVB+UVA+PAR) Batch 1	0	0	1049	-0.2895	28.95051928	187	0.154815611	0.00
C.brevis (UVB+UVA+PAR) Batch 2	0	0	1049	-0.2895	28.95051928	151	0.191725293	0.00
C.brevis (UVB+UVA+PAR) Batch 2	0	0	1049	-0.2895	28.95051928	151	0.191725293	0.00
C.brevis ("PAR") Batch 1	0	0	1049	-0.2895	28.95051928	179	0.161734745	0.00
C.brevis ("PAR") Batch 1	0	0	1049	-0.2895	28.95051928	179	0.161734745	0.00
C.brevis (PAR) Batch 2	0	0	1049	-0.2895	28.95051928	178	0.162643367	0.00
C.brevis (PAR) Batch 2	0	0	1049	-0.2895	28.95051928	178	0.162643367	0.00
P.glacialis (UVA+PAR) Batch 1	0	0	1049	-0.2895	28.95051928	195	0.148464201	0.00
P.glacialis (UVA+PAR) Batch 1	6	0.006	1049	-0.2835	28.95448832	195	0.148484556	21.91
P.glacialis (UVA+PAR) Batch 2	1718	1.638	1049	1.3485	30.03406761	128	0.234641153	34.62
P.glacialis (UVA+PAR) Batch 2	0	0	1049	-0.2895	28.95051928	128	0.226175932	0.00
P.glacialis (UVB+UVA+PAR) Batch 1	0	0	1049	-0.2895	28.95051928	236	0.122671692	0.00
P.glacialis (UVB+UVA+PAR) Batch 1	0	0	1049	-0.2895	28.95051928	236	0.122671692	0.00
P.glacialis (UVB+UVA+PAR) Batch 2	2	0.002	1049	-0.2875	28.9518423	161	0.179825107	26.54
P.glacialis (UVB+UVA+PAR) Batch 2	0	0	1049	-0.2895	28.95051928	161	0.17981689	0.00
P.glacialis ("PAR") Batch 1	0	0	1049	-0.2895	28.95051928	174	0.166382295	0.00
P.glacialis ("PAR") Batch 1	0	0	1049	-0.2895	28.95051928	174	0.166382295	0.00
P.glacialis (PAR) Batch 2	0	0	1049	-0.2895	28.95051928	242	0.119630245	0.00
P.glacialis (PAR) Batch 2	0	0	1049	-0.2895	28.95051928	242	0.119630245	0.00
Pyramimonas sp. (UVA+PAR) Batch 1	0	0	1049	-0.2895	28.95051928	275	0.105274616	0.00
Pyramimonas sp. (UVA+PAR) Batch 1	28	0.027	1049	-0.2625	28.96837997	275	0.105339564	15.54
Pyramimonas sp. (UVA+PAR) Batch 2	0	0	1049	-0.2895	28.95051928	175	0.165431539	0.00
Pyramimonas sp. (UVA+PAR) Batch 2	0	0	1049	-0.2895	28.95051928	175	0.165431539	0.00
Pyramimonas sp. (UVB+UVA+PAR) Batch	405	0.386	1049	0.0965	29.20586095	234	0.124811372	18.42
Pyramimonas sp. (UVB+UVA+PAR) Batch	0	0	1049	-0.2895	28.95051928	234	0.123720168	0.00
Pyramimonas sp. (UVB+UVA+PAR) Batch	8675	8.27	1049	7.9805	34.42118145	162	0.212476429	31.32
Pyramimonas sp. (UVB+UVA+PAR) Batch	534	0.509	1049	0.2195	29.2872263	162	0.180785348	26.68
Pyramimonas sp. ("PAR") Batch 1	0	0	1049	-0.2895	28.95051928	257	0.112647935	0.00
Pyramimonas sp. ("PAR") Batch 1	0	0	1049	-0.2895	28.95051928	257	0.112647935	0.00
Pyramimonas sp. (PAR) Batch 2	0	0	1049	-0.2895	28.95051928	201	0.144032434	0.00
Pyramimonas sp. (PAR) Batch 2	0	0	1049	-0.2895	28.95051928	201	0.144032434	0.00

3d Blot (all species, continued)

Name	Volume	Average	Area	average-blanco	ngijkdna/sample	opgebracht	ngijk/ngsample	T<>T/10^6n.
Phaeocystis sp. (UVA+PAR) Batch 1	0	0	1049	-0.2895	28.95051928	131	0.22099633	0.00
Phaeocystis sp. (UVA+PAR) Batch 1	0	0	1049	-0.2895	28.95051928	131	0.22099633	0.00
Phaeocystis sp. (UVA+PAR) Batch 2	0	0	1049	-0.2895	28.95051928	128	0.226175932	0.00
Phaeocystis sp. (UVA+PAR) Batch 2	0	0	1049	-0.2895	28.95051928	128	0.226175932	0.00
Phaeocystis sp. (UVB+UVA+PAR) Batch 1	73	0.07	1049	-0.2195	28.99682477	142	0.204202991	30.13
Phaeocystis sp. (UVB+UVA+PAR) Batch 1	36090	34.404	1049	34.1145	51.70900311	142	0.364147909	53.58
Phaeocystis sp. (UVB+UVA+PAR) Batch 2	0	0	1049	-0.2895	28.95051928	45	0.643344873	0.00
Phaeocystis sp. (PAR) Batch 1	0	0	1049	-0.2895	28.95051928	146	0.198291228	0.00
Phaeocystis sp. (PAR) Batch 1	0	0	1049	-0.2895	28.95051928	146	0.198291228	0.00
Phaeocystis sp. (PAR) Batch 2	0	0	1049	-0.2895	28.95051928	119	0.243281675	0.00
Phaeocystis sp. (PAR) Batch 2	0	0	1049	-0.2895	28.95051928	119	0.243281675	0.00
Gymnodinium sp. (UVA+PAR) Batch 1	14	0.013	1049	-0.2765	28.95911887	178	0.162691679	24.01
Gymnodinium sp. (UVA+PAR) Batch 1	296	0.282	1049	-0.0075	29.13706423	178	0.163691372	24.15
Gymnodinium sp. (UVA+PAR) Batch 2	55	0.052	1049	-0.2375	28.98491764	144	0.20128415	29.70
Gymnodinium sp. (UVA+PAR) Batch 2	88	0.084	1049	-0.2055	29.00608586	144	0.201431152	29.72
Gymnodinium sp. (UVB+UVA+PAR) Batch 1	0	0	1049	-0.2895	28.95051928	186	0.155647953	0.00
Gymnodinium sp. (UVB+UVA+PAR) Batch 1	89	0.085	1049	-0.2045	29.00674737	186	0.155950255	23.01
Gymnodinium sp. (UVB+UVA+PAR) Batch 2	4363	4.159	1049	3.8695	31.70172653	116	0.273290746	40.31
Gymnodinium sp. (UVB+UVA+PAR) Batch 2	16509	15.738	1049	15.4485	39.36131508	116	0.339321682	49.99
Gymnodinium sp. (PAR) Batch 1	0	0	1049	-0.2895	28.95051928	206	0.140536501	0.00
Gymnodinium sp. (PAR) Batch 1	130	0.124	1049	-0.1655	29.03254614	206	0.14093469	20.80
Gymnodinium sp. (PAR) Batch 2	0	0	1049	-0.2895	28.95051928	104	0.278370378	0.00
Gymnodinium sp. (PAR) Batch 2	0	0	1049	-0.2895	28.95051928	104	0.278370378	0.00
F.cylindrus (UVA+PAR) Batch 1	0	0	1049	-0.2895	28.95051928	111	0.260815489	0.00
F.cylindrus (UVA+PAR) Batch 1	13	0.012	1049	-0.2775	28.95845737	111	0.260887003	38.50
F.cylindrus (UVA+PAR) Batch 2	15	0.014	1049	-0.2755	28.95978038	167	0.173411859	25.59
F.cylindrus (UVA+PAR) Batch 2	0	0	1049	-0.2895	28.95051928	167	0.173356403	0.00
F.cylindrus (UVB+UVA+PAR) Batch 1	620	0.591	1049	0.3015	29.34146987	155	0.189299806	27.93
F.cylindrus (UVB+UVA+PAR) Batch 1	51	0.049	1049	-0.2405	28.98293312	155	0.186986665	27.59
F.cylindrus (UVB+UVA+PAR) Batch 2	0	0	1049	-0.2895	28.95051928	164	0.176527557	0.00
F.cylindrus (UVB+UVA+PAR) Batch 2	0	0	1049	-0.2895	28.95051928	164	0.176527557	0.00
F.cylindrus (PAR) Batch 1	0	0	1049	-0.2895	28.95051928	193	0.150002691	0.00
F.cylindrus (PAR) Batch 1	0	0	1049	-0.2895	28.95051928	193	0.150002691	0.00
F.cylindrus (PAR) Batch 2	0	0	1049	-0.2895	28.95051928	168	0.17232452	0.00
F.cylindrus (PAR) Batch 2	0	0	1049	-0.2895	28.95051928	168	0.17232452	0.00

IJK-0	0
IJK-0b	0.579
avèrage blanco=	0.2895

4th Blot (Rerun samples of all species; 3rd illumination)

Name	Volume	Average	Area	average - blanco	ngijkdna/sample	opgebracht	ngljk/ngsample	T<>T/10^6n.
Ref.series-0	6	0.001	10496	0.0005	2.293278937	0	#DIV/0!	#DIV/0!
Ref.series-1	0	0	10496	-0.0005	2.293194818	1	2.293194818	336.18
Ref.series-2.5	0	0	10512	-0.0005	2.293194818	2.5	0.917277927	134.47
Ref.series- 5	8050	0.766	10509	0.7655	2.357629542	5	0.471525908	69.13
Ref.series-10	1E+06	113.146	10506	113.1455	11.81085969	10	1.181085969	173.15
Ref.series-12.5	2E+06	179.532	10515	179.5315	17.39514637	12.5	1.391611709	204.01
Ref.series-25	3E+06	251.336	10515	251.3355	23.43518674	25	0.93740747	137.42
Ref.series-50	3E+06	246.053	10512	246.0525	22.99078903	50	0.459815781	67.41
Ref.series-75	3E+06	250.178	10518	250.1775	23.33777759	75	0.311170368	45.62
Ref.series-100	3E+06	255	10496	254.9995	23.7433967	100	0.237433967	34.81
Ref.series-0a	4	0	10496	-0.0005	2.293194818	0	#DIV/0!	#DIV/0!
Ref.series-1a	200	0.019	10496	0.0185	2.294793069	1	2.294793069	336.42
Ref.series-2.5a	364	0.035	10496	0.0345	2.296138964	2.5	0.918455585	134.65
Ref.series- 5a	84	0.008	10496	0.0075	2.293867766	5	0.458773553	67.26
Ref.series-10a	988359	94.165	10496	94.1645	10.2142076	10	1.02142076	149.74
Ref.series-12.5a	2E+06	144.744	10496	144.7435	14.46883412	12.5	1.157506729	169.69
Ref.series-25a	3E+06	251.291	10496	251.2905	23.43140141	25	0.937256057	137.40
Ref.series-50a	3E+06	245.906	10496	245.9055	22.97842362	50	0.459568472	67.37
Ref.series-75a	3E+06	254.848	10496	254.8475	23.7306107	75	0.316408143	46.39
Ref.series-100a	3E+06	254.749	10496	254.7485	23.72228297	100	0.23722283	34.78
C.brevis (UVA+PAR) Batch 1	0	0	10496	-0.0005	2.293194818	344	0.006666264	0.00
C.brevis (UVA+PAR) Batch 1	0	0	10496	-0.0005	2.293194818	344	0.006666264	0.00
C.brevis (UVB+UVA+PAR) Batch 1	0	0	10496	-0.0005	2.293194818	227	0.01010218	0.00
C.brevis (UVB+UVA+PAR) Batch 1	12	0.001	10496	0.0005	2.293278937	204.3	0.011225056	1.65
C.brevis ("PAR") Batch 1	0	0	10496	-0.0005	2.293194818	169	0.0135692	0.00
C.brevis ("PAR") Batch 1	0	0	10496	-0.0005	2.293194818	169	0.0135692	0.00
C.brevis (PAR) Batch 2	11	0.001	10496	0.0005	2.293278937	221	0.010376828	1.52
C.brevis (PAR) Batch 2	0	0	10496	-0.0005	2.293194818	221	0.010376447	0.00
P.glacialis (UVA+PAR) Batch 1	376	0.036	10496	0.0355	2.296223082	261	0.00879779	1.29
P.glacialis (UVA+PAR) Batch 1	0	0	10496	-0.0005	2.293194818	261	0.008786187	1.29
P.glacialis (UVB+UVA+PAR) Batch 1	13	0.001	10496	0.0005	2.293278937	342.9	0.006687894	0.98
P.glacialis (UVB+UVA+PAR) Batch 1	0	0	10496	-0.0005	2.293194818	361.95	0.006335667	0.00
P.glacialis ("PAR") Batch 1	26	0.002	10496	0.0015	2.293363055	256	0.008958449	1.31
P.glacialis ("PAR") Batch 1	0	0	10496	-0.0005	2.293194818	256	0.008957792	0.00
P.glacialis (PAR) Batch 2	11	0.001	10496	0.0005	2.293278937	463	0.004953086	0.73
P.glacialis (PAR) Batch 2	1	0	10496	-0.0005	2.293194818	439.85	0.005213584	0.76
Pyramimonas sp. (UVA+PAR) Batch 1	62	0.006	10496	0.0055	2.293699529	367	0.006249862	0.92
Pyramimonas sp. (UVA+PAR) Batch 1	50	0.005	10496	0.0045	2.29361541	367	0.006249633	0.92
Pyramimonas sp. (UVB+UVA+PAR) Batch 1	9606	0.915	10496	0.9145	2.37016319	279	0.008495209	1.25
Pyramimonas sp. (UVB+UVA+PAR) Batch 1	12632	1.204	10496	1.2035	2.394473419	279	0.008582342	1.26
Pyramimonas sp. (UVB+UVA+PAR) Batch 2	960902	91.549	10496	91.5485	9.994153769	209	0.047818918	7.01
Pyramimonas sp. (UVB+UVA+PAR) Batch 2	699132	66.609	10496	66.6085	7.896239906	209	0.037781052	5.54
Pyramimonas sp. ("PAR") Batch 1	103	0.01	10496	0.0095	2.294036003	292	0.007856288	1.15
Pyramimonas sp. ("PAR") Batch 1	0	0	10496	-0.0005	2.293194818	277.4	0.008266744	1.21
Pyramimonas sp. (PAR) Batch 2	0	0	10496	-0.0005	2.293194818	138	0.016617354	2.44
Pyramimonas sp. (PAR) Batch 2	0	0	10496	-0.0005	2.293194818	138	0.016617354	2.44
Phaeocystis sp. (UVB+UVA+PAR) Batch 1	11	0.001	10496	0.0005	2.293278937	136	0.016862345	2.47
Phaeocystis sp. (UVB+UVA+PAR) Batch 1	21	0.002	10496	0.0015	2.293363055	136	0.016862964	2.47
Phaeocystis sp. (UVB+UVA+PAR) Batch 1	0	0	10496	-0.0005	2.293194818	234	0.009799978	0.00
Phaeocystis sp. (UVB+UVA+PAR) Batch 1	286	0.027	10496	0.0265	2.295466016	234	0.009809684	1.44
Phaeocystis sp. (UVB+UVA+PAR) Batch 2	0	0	10496	-0.0005	2.293194818	111	0.020659413	0.00
Phaeocystis sp. (UVB+UVA+PAR) Batch 2	0	0	10496	-0.0005	2.293194818	111	0.020659413	0.00
Gymnodinium sp. (UVB+UVA+PAR) Batch 2	0	0	10496	-0.0005	2.293194818	424	0.005408478	0.00
Gymnodinium sp. (UVB+UVA+PAR) Batch 2	280	0.027	10496	0.0265	2.295466016	424	0.005413835	0.79
Gymnodinium sp. (UVB+UVA+PAR) Batch 2	8481	0.808	10496	0.8075	2.361162517	496	0.004760408	0.70
Gymnodinium sp. (UVB+UVA+PAR) Batch 2	700	0.067	10496	0.0665	2.298830754	446.4	0.00514971	0.75

IJKA-0	0.001
IJKB-0	0
Average blanco =	0.0005

4th Blot (Reruri samples of all species; 4th illumination)

Name	Volume	Average	Area	average - blanco	ngijkdna/sample	opgebrach	ngljk/ngsa	T<>T/10^6n.
Ref.series-0	1563	0.149	10496	0.148	1.446314066	0	#DIV/0!	#DIV/0!
Ref.series-1	312056	29.731	10496	29.73	2.791623084	1	2.791623	409.25
Ref.series-2.5	647	0.062	10496	0.061	1.442357542	2.5	0.576943	84.58
Ref.series- 5	570238	54.329	10496	54.328	3.910273318	5	0.782055	114.65
Ref.series-10	2302785	219.396	10496	219.395	11.41707217	10	1.141707	167.37
Ref.series-12.5	2568362	244.699	10496	244.698	12.56778389	12.5	1.005423	147.39
Ref.series-25	2676108	254.965	10496	254.964	13.03465369	25	0.521386	76.44
Ref.series-50	2674071	254.77	10496	254.769	13.02578562	50	0.260516	38.19
Ref.series-75	2675265	254.884	10496	254.883	13.03097003	75	0.173746	25.47
Ref.series-100	2676273	254.98	10496	254.979	13.03533585	100	0.130353	19.11
Ref.series-0a	25	0.002	10496	0.001	1.439628905	0	#DIV/0!	#DIV/0!
Ref.series-1a	3	0	10496	-0.001	1.439537951	1	1.439538	211.04
Ref.series-2.5a	2706	0.258	10496	0.257	1.45127109	2.5	0.580508	85.10
Ref.series- 5a	438928	41.819	10496	41.818	3.341352494	5	0.66827	97.97
Ref.series-10a	2165808	206.346	10496	206.345	10.82359361	10	1.082359	158.67
Ref.series-12.5a	2474393	235.746	10496	235.745	12.16062577	12.5	0.97285	142.62
Ref.series-25a	2676480	255	10496	254.999	13.0362454	25	0.52145	76.44
Ref.series-50a	2675182	254.876	10496	254.875	13.03060621	50	0.260612	38.21
Ref.series-75a	2676473	254.999	10496	254.998	13.03619992	75	0.173816	25.48
Ref.series-100a	2676480	255	10496	254.999	13.0362454	100	0.130362	19.11
C.brevis (UVA+PAR) Batch 1	1	0	10496	-0.001	1.439537951	344	0.004185	0.61
C.brevis (UVA+PAR) Batch 1	0	0	10496	-0.001	1.439537951	344	0.004185	0.00
C.brevis (UVB+UVA+PAR) Batch 1	16	0.002	10496	0.001	1.439628905	227	0.006342	0.93
C.brevis (UVB+UVA+PAR) Batch 1	108	0.01	10496	0.009	1.439992724	204.3	0.007048	1.03
C.brevis ("PAR") Batch 1	1241	0.118	10496	0.117	1.44490427	169	0.00855	1.25
C.brevis ("PAR") Batch 1	288	0.027	10496	0.026	1.440765837	169	0.008525	1.25
C.brevis (PAR) Batch 2	36	0.003	10496	0.002	1.439674383	221	0.006514	0.96
C.brevis (PAR) Batch 2	7	0.001	10496	0	1.439583428	221	0.006514	0.95
P.glacialis (UVA+PAR) Batch 1	1646	0.157	10496	0.156	1.446677884	261	0.005543	0.81
P.glacialis (UVA+PAR) Batch 1	81	0.008	10496	0.007	1.439901769	261	0.005517	0.81
P.glacialis (UVB+UVA+PAR) Batch 1	11	0.001	10496	0	1.439583428	342.9	0.004198	0.62
P.glacialis (UVB+UVA+PAR) Batch 1	542	0.052	10496	0.051	1.44190277	361.95	0.003984	0.58
P.glacialis ("PAR") Batch 1	0	0	10496	-0.001	1.439537951	256	0.005623	0.00
P.glacialis ("PAR") Batch 1	4	0	10496	-0.001	1.439537951	256	0.005623	0.82
P.glacialis (PAR) Batch 2	0	0	10496	-0.001	1.439537951	463	0.003109	0.00
P.glacialis (PAR) Batch 2	0	0	10496	-0.001	1.439537951	439.85	0.003273	0.00
Pyramimonas sp. (UVA+PAR) Batch 1	966	0.092	10496	0.091	1.443721861	367	0.003934	0.58
Pyramimonas sp. (UVA+PAR) Batch 1	210	0.02	10496	0.019	1.440447496	367	0.003925	0.58
Pyramimonas sp. (UVB+UVA+PAR) Batch 1	606529	57.787	10496	57.786	4.067533767	279	0.014579	2.14
Pyramimonas sp. (UVB+UVA+PAR) Batch 1	583307	55.574	10496	55.573	3.966892537	279	0.014218	2.08
Pyramimonas sp. (UVB+UVA+PAR) Batch 2	1963580	187.079	10496	187.078	9.947382782	209	0.047595	6.98
Pyramimonas sp. (UVB+UVA+PAR) Batch 2	1676805	159.757	10496	159.756	8.704852426	209	0.04165	6.11
Pyramimonas sp. ("PAR") Batch 1	2011	0.192	10496	0.191	1.448269589	292	0.00496	0.73
Pyramimonas sp. ("PAR") Batch 1	351	0.033	10496	0.032	1.441038701	277.4	0.005195	0.76
Pyramimonas sp. (PAR) Batch 2	0	0	10496	-0.001	1.439537951	138	0.010431	0.00
Pyramimonas sp. (PAR) Batch 2	80	0.008	10496	0.007	1.439901769	138	0.010434	1.53
Phaeocystis sp. (UVB+UVA+PAR) Batch 1	256	0.024	10496	0.023	1.440629406	136	0.010593	1.55
Phaeocystis sp. (UVB+UVA+PAR) Batch 1	358	0.034	10496	0.033	1.441084178	136	0.010596	1.55
Phaeocystis sp. (UVB+UVA+PAR) Batch 1	119	0.011	10496	0.01	1.440038201	234	0.006154	0.90
Phaeocystis sp. (UVB+UVA+PAR) Batch 1	1	0	10496	-0.001	1.439537951	234	0.006152	0.00
Phaeocystis sp. (UVB+UVA+PAR) Batch 2	24	0.002	10496	0.001	1.439628905	111	0.01297	1.90
Phaeocystis sp. (UVB+UVA+PAR) Batch 2	0	0	10496	-0.001	1.439537951	111	0.012969	0.00
Gymnodinium sp. (UVB+UVA+PAR) Batch 2	104873	9.992	10496	9.991	1.893946973	424	0.004467	0.65
Gymnodinium sp. (UVB+UVA+PAR) Batch 2	42746	4.073	10496	4.072	1.624766929	424	0.003832	0.56
Gymnodinium sp. (UVB+UVA+PAR) Batch 2	289147	27.548	10496	27.547	2.692346173	496	0.005428	0.80
Gymnodinium sp. (UVB+UVA+PAR) Batch 2	542421	51.679	10496	51.678	3.789758516	446.4	0.00849	1.24

IJKB-0	0.002
IJKB-1	0
Average blanco =	0.001

4th Blot (Rerun samples of all species; 5th illumination)

Name	Volume	Average	Area	average - blanco	ngIjkdna/sample	opgebracht	ngijk/ngsample	T<>T/10^6n
Ref.series-0	21157	2.016	10496	0.987	0.092536908	0	#DIV/0!	#DIV/0!
Ref.series-1	316868	30.189	10496	29.16	1.36837243	1	1.36837243	200.60
Ref.series-2.5	362297	34.518	10496	33.489	1.564414455	2.5	0.625765782	91.74
Ref.series- 5	1362918	129.851	10496	128.822	5.881641156	5	1.176328231	172.45
Ref.series-10	2554124	243.343	10496	242.314	11.02121185	10	1.102121185	161.57
Ref.series-12.5	2640762	251.597	10496	250.568	11.39500045	12.5	0.911600036	133.64
Ref.series-25	2676377	254.99	10496	253.961	11.54865501	25	0.461946201	67.72
Ref.series-50	2676138	254.967	10496	253.938	11.54761344	50	0.230952269	33.86
Ref.series-75	2676480	255	10496	253.971	11.54910787	75	0.153988105	22.57
Ref.series-100	2676480	255	10496	253.971	11.54910787	100	0.115491079	16.93
Ref.series-0a	436	0.042	10496	-0.987	0.003142831	0	#DIV/0!	#DIV/0!
Ref.series-1a	2435	0.232	10496	-0.797	0.011747124	1	0.011747124	1.72
Ref.series-2.5a	443580	42.262	10496	41.233	1.915107327	2.5	0.766042931	112.30
Ref.series- 5a	1327251	126.453	10496	125.424	5.727760167	5	1.145552033	167.94
Ref.series-10a	2540781	242.071	10496	241.042	10.96360837	10	1.096360837	160.73
Ref.series-12.5a	2663930	253.804	10496	252.775	11.49494611	12.5	0.919595689	134.81
Ref.series-25a	2676480	255	10496	253.971	11.54910787	25	0.461964315	67.72
Ref.series-50a	2675621	254.918	10496	253.889	11.54539444	50	0.230907889	33.85
Ref.series-75a	2676480	255	10496	253.971	11.54910787	75	0.153988105	22.57
Ref.series-100a	2676480	255	10496	253.971	11.54910787	100	0.115491079	16.93
C.brevis (UVA+PAR) Batch 1	265468	25.292	10496	24.263	1.146608097	344	0.003333163	0.49
C.brevis (UVA+PAR) Batch 1	228549	21.775	10496	20.746	0.987338103	344	0.002870169	0.42
C.brevis (UVB+UVA+PAR) Batch 1	93970	8.953	10496	7.924	0.406684177	227	0.00179156	0.26
C.brevis (UVB+UVA+PAR) Batch 1	135751	12.934	10496	11.905	0.58696676	204.3	0.002873063	0.42
C.brevis ("PAR") Batch 1	44999	4.287	10496	3.258	0.195380853	169	0.0011561	0.17
C.brevis ("PAR") Batch 1	17235	1.642	10496	0.613	0.075600036	169	0.000447337	0.07
C.brevis (PAR) Batch 2	58259	5.551	10496	4.522	0.252622045	221	0.001143086	0.17
C.brevis (PAR) Batch 2	2962	0.282	10496	-0.747	0.014011412	221	6.34001E-05	0.01
P.glacialis (UVA+PAR) Batch 1	385469	36.725	10496	35.696	1.664360112	261	0.006376859	0.93
P.glacialis (UVA+PAR) Batch 1	332579	31.686	10496	30.657	1.436165202	261	0.005502549	0.81
P.glacialis (UVB+UVA+PAR) Batch 1	418910	39.911	10496	38.882	1.808640522	342.9	0.005274542	0.77
P.glacialis (UVB+UVA+PAR) Batch 1	483214	46.038	10496	45.009	2.086106331	361.95	0.005763521	0.84
P.glacialis ("PAR") Batch 1	271828	25.898	10496	24.869	1.174051263	256	0.004586138	0.67
P.glacialis ("PAR") Batch 1	46949	4.473	10496	3.444	0.203804003	256	0.000796109	0.12
P.glacialis (PAR) Batch 2	9392	0.895	10496	-0.134	0.041771579	463	9.02194E-05	0.01
P.glacialis (PAR) Batch 2	63785	6.077	10496	5.048	0.276442351	439.85	0.000628492	0.09
Pyramimonas sp. (UVA+PAR) Batch 1	707809	67.436	10496	66.407	3.055130876	367	0.008324607	1.22
Pyramimonas sp. (UVA+PAR) Batch 1	610542	58.169	10496	57.14	2.635467802	367	0.007181111	1.05
Pyramimonas sp. (UVB+UVA+PAR) Batch 1	1642058	156.446	10496	155.417	7.086015759	279	0.025397906	3.72
Pyramimonas sp. (UVB+UVA+PAR) Batch 1	1590751	151.558	10496	150.529	6.864658998	279	0.024604513	3.61
Pyramimonas sp. (UVB+UVA+PAR) Batch 2	2523270	240.403	10496	239.374	10.88807173	209	0.052096037	7.64
Pyramimonas sp. (UVB+UVA+PAR) Batch 2	2403981	229.038	10496	228.009	10.37339915	209	0.049633489	7.28
Pyramimonas sp. ("PAR") Batch 1	358414	34.148	10496	33.119	1.547658727	292	0.005300201	0.78
Pyramimonas sp. ("PAR") Batch 1	90661	8.638	10496	7.609	0.392419165	277.4	0.001414633	0.21
Pyramimonas sp. (PAR) Batch 2	193590	18.444	10496	17.415	0.83649126	138	0.006061531	0.89
Pyramimonas sp. (PAR) Batch 2	288924	27.527	10496	26.498	1.247821755	138	0.009042187	1.33
Phaeocystis sp. (UVB+UVA+PAR) Batch 1	453698	43.226	10496	42.197	1.958762793	136	0.014402668	2.11
Phaeocystis sp. (UVB+UVA+PAR) Batch 1	303515	28.917	10496	27.888	1.310768952	136	0.009638007	1.41
Phaeocystis sp. (UVB+UVA+PAR) Batch 1	385578	36.736	10496	35.707	1.664858256	234	0.007114779	1.04
Phaeocystis sp. (UVB+UVA+PAR) Batch 1	310404	29.574	10496	28.545	1.340521692	234	0.005728725	0.84
Phaeocystis sp. (UVB+UVA+PAR) Batch 2	57617	5.489	10496	4.46	0.249814328	111	0.00225058	0.33
Phaeocystis sp. (UVB+UVA+PAR) Batch 2	101496	9.67	10496	8.641	0.439154062	111	0.003956343	0.58
Gymnodinium sp. (UVB+UVA+PAR) Batch 2	1171914	111.653	10496	110.624	5.057531021	424	0.011928139	1.75
Gymnodinium sp. (UVB+UVA+PAR) Batch 2	979177	93.29	10496	92.261	4.225948737	424	0.00996686	1.46
Gymnodinium sp. (UVB+UVA+PAR) Batch 2	1241554	118.288	10496	117.259	5.358001993	496	0.010802423	1.58
Gymnodinium sp. (UVB+UVA+PAR) Batch 2	1283257	122.262	10496	121.233	5.537967575	446.4	0.012405841	1.82

IJKA-0	2.016
IJKB-0	0.042
Average blanco =	1.029