STELLINGEN

1. UVB-straling kan een positief effect hebben op algen.
   Dit proefschrift

2. Door de huidige luchtvervuiling hoeven wij ons in Nederland geen zorgen te maken over de voorspelde afname van de ozonconcentratie in de stratosfeer.
   Dit proefschrift

3. Aangezien elk organisme een specifieke respons op UVB-stress vertoont en elke onderzoeker een andere respons belangrijk vindt, is het onmogelijk een algemene biologische weegfunctie te ontwikkelen om de golflengte-afhankelijkheid van UVB-effecten te beschrijven.
   Dit proefschrift

4. Het waterbeheer in Nederland, dat voornamelijk is gericht op het weer helder maken van oppervlaktewater, kan een negatief effect hebben op het ecosysteem door het toenemen van de UVB-stress.
   Dit proefschrift

5. Het bewijs dat Müller-Navarra geeft dat de concentratie van het vetzuur eicosapentaenoic acid (EPA, C20:5ω3) in het voedsel essentieel is voor de groei van *Daphnia* wordt weerlegd door de waarneming dat *Daphnia* een hogere groeisnelheid heeft op een dieet van algen waarin dit essentiële vetzuur niet was aangetoond, dan op een dieet van algen waarin het wel voorkwam.
   Müller-Navarra D. (1995) Evidence that a highly unsaturated fatty acid limits Daphnia *growth in nature.* Archiv für Hydrobiologie, *132, 297-307.*
   Dit proefschrift

6. Het onderzoek naar de gevolgen van ozonafname is exponentieel toegenomen sinds UVB-straling nauwkeurig kan worden gemeten.

7. Het is frustrerend om in Nederland afhankelijk te zijn van mooi weer voor het uitvoeren van veldwerk.

8. Honderd kilometer roeien verschaft diepere inzichten in de beperkingen van het menselijk en aquatisch systeem.

9. Een overheid die Nederland als kennisland wil stimuleren maar tegelijkertijd blijft bezuinigen op het hoger onderwijs en onderzoek, voert een beleid dat niet duurzaam is.

10. Als je op schouders van reuzen staat is het wel zaak de goede kant uit te kijken.
vrij naar Newton

11. Het getuigt van weinig fantasie om door twee punten een rechte lijn te trekken.

12. Als mensen zeuren hebben ze niets te klagen.

13. Het succes van de parlementaire enquête naar de Bijlmerramp is illustratief voor de onmacht die de Tweede Kamer gewoonlijk heeft.

14. Als een leerstoelgroep geen eerste-geldstroom promotieonderzoek meer uitvoert, maar ervoor kiest afhankelijk te zijn van de derde-geldstroom, leidt zij geen onafhankelijke onderzoekers meer op maar projectmanagers.

Stellingen behorende bij het proefschrift:
"Effects of ultraviolet-B radiation on phytoplankton-zooplankton interactions"
H.J. de Lange, Wageningen, 24 maart 1999.

Effects of Ultraviolet-B Radiation on Phytoplankton-Zooplankton Interactions

Effecten van ultraviolet-B straling op interacties tussen fytoplankton en zoöplankton

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Effects of Ultraviolet-B Radiation on Phytoplankton-Zooplankton Interactions

Hendrika Joacomina de Lange

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van de Landbouwuniversiteit Wageningen,
dr. C.M. Karssen,
in het openbaar te verdedigen
op woensdag 24 maart 1999
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aan mijn ouders

VOORWOORD

Promotieonderzoek doe je niet zomaar, en zeker niet alleen. Het voorwoord bij een proefschrift is een mooie plaats om van dat alles verslag te doen. Vanaf het eerste practicum aquatische oecologie dat ik deed wilde ik AiO aquatische oecologie worden. Veldwerk doen, rondsjouwen in een waadbroek met schepnet, onderzoek doen met water en alles wat groeit en bloeit en ons telkens weer boeit. En 5 jaar na dat eerste practicum werd ik ook AiO en deed ik ook (ongeveer) al die dingen. Dat eerste practicum lijkt kort geleden, maar nu is het al bijna 9 jaar later en heb ik mijn promotieonderzoek alweer afgerond.

In de afgelopen 4 jaar heb ik veel geleerd, zowel op persoonlijk als op wetenschappelijk vlak. Terugkijkend is alles redelijk soepel gegaan. Het onderzoek doen vond en vind ik nog steeds erg leuk. Natuurlijk ging niet alles makkelijk, zoals veldwerk met slecht weer terwijl je de zon nodig hebt, problemen met de spectroradiometer, cosmonderzoek waar veel niet volgens planning ging. Aankomende motivatiedipjes heb ik gemakshalve altijd maar genegeerd en het kwam altijd wel weer goed. Uiteindelijk blijven alleen de goede herinneringen over, de keren dat de zon wél scheen.

De werkomstandigheden bij de vakgroep waren dynamisch, er waren veel veranderingen in korte tijd. De naamsveranderingen alleen al geven daarvan een (psychedelische?) indruk. (What's in a name: vakgroep of leerstoelgroep? oecologie of ecologie? en welke bloedgroep wordt als eerste genoemd? of zijn we inmiddels al één groep geworden? en waarom heten we niet gewoon limnologie?). De veranderingen zijn nog steeds bezig, bij de groep en bij de hele universiteit. Gelukkig is er nu weer een nieuwe lichting AiO's die voor leven in de brouwerij kan zorgen.

En nu de persoonlijke bedankjes, als eerste mijn promotor. Wim, we hebben niet heel vaak overleg gehad, een halve dag in de week professor in Wageningen was niet veel. En daarna was de geografische verwijdering daaraan debet. Maar de kwaliteit van overleg maakte veel goed, en per telefoon, e-mail of fax lukt overleg ook prima. Zeker in de laatste fase was je er nauw bij betrokken en je snelheid van lezen en becommentariëren is ongeëvenaard. Mede daardoor kan ik nu gepromoveerd naar de V.S. vertrekken.

Ellen, als dagelijkse begeleider heb je meer invloed en sturing gehad op de richting en uitvoering van mijn onderzoek. Je hebt me heel veel vrijheid gegund, waarvan ik veel heb geleerd. Ook jij vond op een gegeven moment een nieuwe baan, bij het NIOO in Nieuwersluis, maar ook hier geldt dat je telefonisch even makkelijk (soms beter) te bereiken was.

Op deze plaats past ook een woord van dank voor het snelle lezen van de leden van de leescommissie, prof. Admiraal, prof. Scheffer en dr. Gieskes.

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Veel studenten hebben me geholpen met het uitvoeren van dit onderzoek, in willekeurige volgorde: Karen Smit, Rob Exalto, Roy Geerts, Petra Kwakman, Anthony Verschoor, Arjan Huurnink, Sonja Huggers, Caroline Moermond, Remko Rosenboom, Paul van Reeuwijk, Henk Tamerus, Arjan Luiten en Harold Veldmaat. Hun werk is in meer of mindere mate terug te vinden in dit proefschrift.

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Naast het onderzoek heb ik gelukkig nog veel andere dingen gedaan. In verschillende commissies en besturen binnen en buiten de universiteit, met als trefwoord 'solidariteit', heb ik het nut gezocht dat ik soms in mijn onderzoek heb gemist. Verder hebben vrienden en familie me verhinderd een totale vakidioot te worden. Met name wil ik daarvoor de volgende personen bedanken: de Ranzige Anja's van het eerste uur (Anne, Annelieke, Annemiek, Ingrid, Jacoline) voor de culinaire en muzikale afleiding, Ingrid voor de vakanties, Janine en Roos voor de goede wandelingen, Hinke en Arjan zonder nadere toelichting.

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Lieve papa en mama, bedankt voor alle liefde, zorg en ondersteuning, belangstelling voor mijn studie en werk, het geven van een stabiele basis en altijd een thuis om op terug te vallen.

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

"Would you tell me, please, which way I ought to walk from here?"
"That depends a great deal on where you want to get to," said the Cat.
"I don't much care where," said Alice.
"Then it doesn't matter which way you walk," said the Cat.
"- so long as I get somewhere," Alice added.
"Oh, you're sure to do that," said the Cat, "if you only walk long enough!"

(Lewis Carroll - Alice in Wonderland)

1.1 OZONE AND UVB RADIATION

The decline in stratospheric ozone concentration and the resulting increase in ultraviolet-B (UVB) radiation (wavelengths 280-320 nm) are regarded world-wide as serious environmental problems. UVB radiation is harmful for organisms, and therefore scientific research into UVB effects on terrestrial, marine and freshwater organisms, ranging from bacteria to humans to complete ecosystems, receives great interest (*e.g.* Kessler & Björn 1995, Weiler & Penhale 1994, Williamson & Zagarese 1994, Young *et al.* 1993).

The United Nations Environmental Program (UNEP) has organized several international meetings to develop agreements to control the emission of ozone deteriorating substances (Vienna Convention 1985, Montreal Protocol 1987, London 1990, Copenhagen 1992). If the agreements made in Montreal will hold, expectations are that the stratospheric ozone concentration will recover within the next 50 years. A brief description of ozone chemistry is given in Appendix 1.1.

Since the early 1970s, predictions have been made that human activities will lead to a diminishing of the earth's protective ozone layer, those human activities included exhausts of supersonic transport (Johnston 1971), and emission of chlorofluorocarbons (Molina & Rowland 1974). Evidence of downward trends in the thickness of the ozone layer was found with the discovery of the Antarctic ozone hole in 1985 (Farman *et al.* 1985). Ozone decreases during the Antarctic spring are now well documented (*e.g.* Crutzen 1992, Downey *et al.* 1996, Johnson *et al.* 1995, Jones & Shanklin 1995). Ozone decreases outside the Antarctic have been reported at southern mid-latitudes (Kirchhoff *et al.* 1997), northern mid-latitudes (Madronich *et al.* 1995), and Arctic regions (Jokela *et al.* 1995, Müller *et al.* 1997). It has now been established that there is an apparent downward trend in the total column amount of ozone over mid-latitude areas of the Northern hemisphere in all seasons (*e.g.* Stolarski *et al.* 1992).

The decrease in stratospheric ozone concentration has received wide attention because the ozone layer protects the earth from harmful ultraviolet radiation. There is a direct link between observed decreases in ozone concentration, and increases in UVB radiation reaching the earth's surface (Jokela *et al.* 1995, Kerr & McElroy 1993, Kirchhoff *et al.* 1997, Madronich *et al.* 1995, Orce & Helbling 1997, Seckmeyer *et al.* 1997, Smith *et al.* 1992).

Solar radiation is usually divided in visible radiation (wavelengths of 400 to 700 nm), infrared radiation (wavelengths >700 nm), and ultraviolet radiation (wavelengths <400 nm). Ultraviolet radiation is then further divided in UVA (320-400 nm), UVB (280-320 nm) and UVC (<280 nm). These are arbitrary boundaries, but they have some practical meaning. UVC radiation is extremely harmful to life, but is completely absorbed by the atmosphere. UVB radiation reaches the earth's surface in low intensities, and is harmful to organisms. UVA radiation is less harmful than UVB, and can also be beneficial. The visible part of the solar spectrum is usually referred to as PAR, photosynthetically active radiation, because these

wavelengths are used in photosynthesis processes. Since ozone specifically absorbs UVB radiation, decreases in stratospheric ozone concentration will lead to increases in UVB, but not in longer wavelengths.

Stratospheric ozone concentration is not the only determinant of the intensity of UVB radiation reaching the earth's surface, other important factors are solar zenith angle, altitude, cloud cover, and tropospheric conditions. The solar zenith angle is the angle between the local vertical direction and the direction of the centre of the solar disk. Time of day, season, and geographic location (latitude and longitude) determine the solar zenith angle (see Kirk 1994 for calculations of zenith angle). This determines the pathlength of radiation through the atmosphere. Altitude also determines the pathlength. The effect of a longer pathlength on irradiance is wavelength specific, shorter wavelengths are absorbed more quickly in the atmosphere, a longer pathlength thus results in a smaller proportion of shorter wavelengths. The magnitude of this pathlength effect was shown for different altitudes, UVB increased with 19% per 1000 m altitude increase, and UVA with 11% (Blumthaler *et al.* 1992).

Clouds play an important role in modulating UVB radiation reaching the earth's surface. A first approximation is that cloudy skies provide the same attenuation for all wavelengths. However, when tropospheric ozone concentration is low, less attenuation occurs in the UVA than in the PAR region. For wavelengths in the UVB region, clouds in polluted air are absorbers as well as scatterers of radiation, and the situation becomes far more complicated (*e.g.* Booth *et al.* 1997, Frederick & Steele 1995, Gautier *et al.* 1994).

Tropospheric conditions, such as humidity, haze, smog and aerosols, influence the UVB radiation reaching the earth's surface (Brühl & Crutzen 1989, Dickerson *et al.* 1997, Estupiñán *et al.* 1996, Németh *et al.* 1996). An increase in tropospheric ozone pollution may cancel the effect of an increase of UVB radiation due to stratospheric ozone depletion (Ma & Guicherit 1997).

1.2 UVB Penetration in Aquatic Systems

For aquatic organisms the exposure to UVB is not only dependent on ozone layer, cloud cover, zenith angle, and tropospheric conditions, but also on the water properties defining the underwater light field. Fig. 1.1 illustrates the wavelength dependency of intensity *vs.* depth. UVB is attenuated more rapidly than wavelengths in the green and yellow part of the spectrum (500-600 nm).

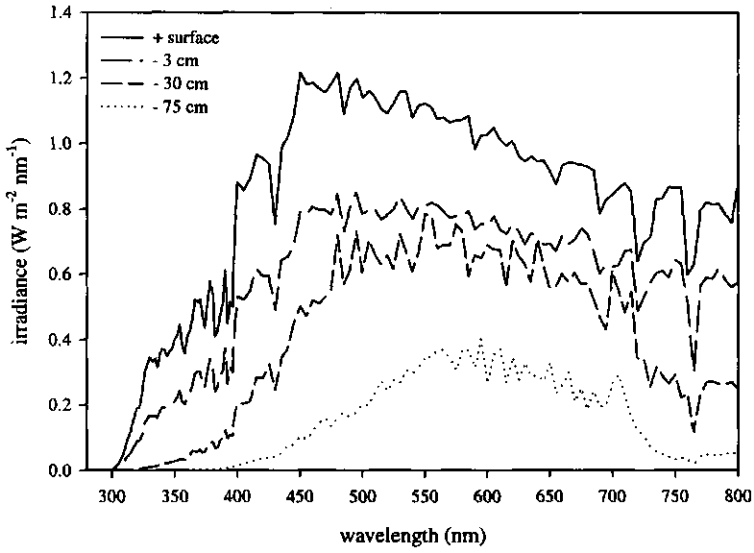

Figure 1.1: Solar irradiance at the water surface and at 3 depths, measured in Lake Blauwe Kamer, 11 May 1998 (see Chapter 2 for full description).

Exposure of an aquatic organism to UVB radiation is highly dependent on the vertical positioning in the water column. For planktonic organisms with limited mobility, mixing processes will be important in determining the position in the water column, and therefore UVB exposure. In clear, colourless, oligotrophic lakes UVB can penetrate to depths of more than 10 m (Kirk 1994). However, in most inland waters, UVB penetration is limited to less than 1 m. This is mainly caused by a higher concentration of dissolved humic substances. Another important factor in the attenuation of light is phytoplankton biomass. In eutrophic systems with high phytoplankton biomass, radiation of all wavelengths is quickly absorbed. The attenuation of UVB, UVA and PAR radiation will be discussed in more detail in Chapter 2.

Because of the importance of humic substances in attenuating UVB radiation, UVB exposure for a system is linked with several other environmental issues. Both climate warming and acidification processes can cause a decline in dissolved organic carbon (DOC). Declines in lake DOC in North American boreal lakes were explained by the reduced transport of DOC from the terrestrial and wetland watershed to a lake, as drought caused streamflows and water tables to decline (Schindler *et al.* 1996). Lower water tables will also increase the oxidation processes in exposed areas, which will result in a lower pH when the

water table fills again. This acidification process is expected to lower the DOC concentration. Increases in aluminium concentration, a common consequence of acidification, can also lower DOC concentrations (Yan *et al.* 1996).

For clear, oligotrophic systems, decreases in DOC concentrations might have a larger effect on the exposure of UVB of a system than declines in ozone concentration. The reason for this is that the relation between ozone decrease and UVB increase is approximately linear, and the relation between DOC decrease and UVB penetration depth is exponential for DOC concentrations below 5 mg l^{-1}.

1.3 UVB Effects on Aquatic Organisms and Ecosystems

1.3.1 UVB effects on phytoplankton

UVB radiation is harmful for aquatic organisms. Already in 1964, Steemann-Nielsen found that UV radiation reduced photochemical and enzymatical processes in photosynthesis of plankton algae. Lorenzen (1979) found that UV inhibited the ^{14}C incorporation by natural phytoplankton populations. The major effect was restricted to the upper part of the euphotic zone.

UVB effects on freshwater phytoplankton have been reviewed by Karentz *et al.* (1994). The most important effects are DNA damage, photosynthesis inhibition, and decreased growth rate. UVB wavelengths are strongly absorbed by DNA, causing structural changes in these molecules (dimers) that can interfere with vital cellular processes of growth and reproduction (Karentz & Lutze 1990). DNA damage can be repaired in three cellular repair mechanisms. In photoreactivation (light repair), a single-enzyme system, cyclobutane dimers are recognized and monomerized. Long-wave UV (310-400 nm) and/or short-wave visible light (400-480 nm) is needed. In excision repair (dark repair), a series of enzymes recognize DNA damage, remove the damaged portion of the molecule, and resynthesize that segment of DNA. Postreplication (recombination) repair is a complex process that occurs after DNA replication and corrects the DNA defect on the basis of information in the undamaged strand. The net effect of UVB on DNA is the resultant of the balance between damage and repair processes (Mitchell & Karentz 1993, Karentz 1994).

UVB contributes significantly to the photoinhibition of primary production (*e.g.* Holm-Hansen *et al.* 1993, Vincent & Roy 1993). Field measurements made in springtime in the Antarctic marginal ice zone clearly showed that as the ozone layer thinned, UVB intensities increased, and UVB photosynthesis inhibition increased. This photoinhibition was estimated to reduce primary production by 6 to 12% (Smith *et al.* 1992). UVB can interfere with many aspects of the photosynthesis process, especially photosystem II, electron transport, and destruction of D1 protein. Repair mechanisms do exist for the UV-damaged

photosynthetic apparatus. If these repair processes can balance damage, no net reduction of photosynthesis will be observed. This does not imply, however, that there is no detrimental effect. The energy reserves needed to resynthesize proteins and/or pigments may affect long-term survival and fitness (Cullen & Neale 1994). Recovery depends on longer wavelengths for direct repair and energy conversion, the ratio of UVB, UVA and PAR determines the energy balance between photosynthesis and recovery (*e.g.* Smith *et al.* 1992).

UVB can affect cell morphology and biochemical composition. Cell volume may increase, possibly caused by a stagnation of cells in G2 phase as result of DNA damage (Buma *et al.* 1995, Karentz *et al.* 1991). Cell wall structure may alter as well; Van Donk & Hessen (1995) found a thickened cell wall for UV-stressed *Selenastrum capricornutum*, resulting in a decreased digestibility for *Daphnia*. Biochemical composition may alter, generally protein and lipid content decrease and carbohydrate content increases, which will affect the nutritional quality for herbivorous zooplankton. These aspects are discussed further in Chapter 3.

Phytoplankton organisms generally have several strategies to cope with UVB stress, these can be categorized as avoidance or adaptation strategies (flight or fight). Avoidance strategies minimize the exposure of the organism to UVB radiation, through behavioural patterns or habitat strategies, and by morphological and structural features (Karentz 1994).

Migration implies an ability to detect UVB radiation (or longer wavelengths that are proportional to UVB), and an ability to move. Motile phytoplankton generally exhibit a negative phototaxis at high light intensities. UV impairs both orientation and motility in flagellates, this would impair escape from UVB under some conditions. In negatively buoyant cells, loss of motility and subsequent sinking would reduce the UVB exposure (Häder 1993). Some cyanobacteria react to UVB radiation by migrating into deeper strata of mat communities (Quesada & Vincent 1997).

Producing sunscreen pigments to protect cellular organs from UVB radiation can be regarded as a second avoidance strategy. Cyanobacteria can produce an extra-cellular sheath pigment, scytonemin, with an absorption band of 280-450 nm. It is primarily considered to be a UVA protectant, but it also absorbs UVB (Garcia-Pichel & Castenholz 1991). Many marine organisms contain mycosporine-like amino acid compounds (MAAs), with a range of absorption maxima from 310 to 360 nm (Carreto *et al.* 1990, Karentz *et al.* 1991). These MAAs are recently also identified in freshwater phytoplankton (Sommaruga & Psenner 1997, Xiong *et al.* 1997). Carotenoid pigments have an absorption spectrum that tails in the ultraviolet part of the spectrum, and may serve as protective pigment (Gala & Giesy 1991). UVB irradiance at low intensities can result in increased levels of cellular carotenoid pigments (*e.g.* Buma *et al.* 1996b, Döhler 1995). Other studies show a decreased pigment synthesis as result of UVB irradiance (*e.g.* Döhler 1984, Lohmann *et al.* 1998).

Adaptation mechanisms include defence against radicals and photoproducts, and repair of damage. The defences against radicals and active forms of oxygen produced in photochemical reactions include various biochemical antioxidants, quenchers, scavengers and enzyme systems. Carotenoids are known to inhibit free radical reactions, quench singlet

oxygen and photosensitized macromolecules, and protect cellular systems from photooxidations induced by visible and ultraviolet radiation. The radical scavenging role of carotenoid pigments is probably more important than the UVB absorbing properties of carotenoids (Gala & Giesy 1991). Another mechanism against radicals is the superoxide dismutase enzyme system that reacts with and neutralizes reactive oxygen species (Vincent & Quesada 1994).

A second adaptation mechanism is the variety of repair mechanisms and photoreactivation processes available to restore cellular components after UV damage. Such mechanisms include DNA repair mechanisms, and photosynthesis repair mechanisms, as described above. The ability to repair damage is species specific, and will also depend on other environmental factors as temperature, nutrient concentrations, and PAR light.

Responses of phytoplankton to UVB radiation are taxon specific. Diatoms are typically more sensitive to UVB radiation than other types of algae, while blue-green algae are relatively resistant (Gala & Giesy 1991). Motile dinoflagellates appear to be more sensitive than diatoms (Cullen *et al.* 1992, Ekelund 1990). Sensitivity appears to be size dependent, picoplankton is more sensitive than nanoplankton (Garcia-Pichel 1994, Helbling *et al.* 1992). An explanation for this size-dependent sensitivity may be that intracellular sunscreens are not possible for picoplankters due to physical and physiological constraints, but are feasible for nanoplankters (Garcia-Pichel 1994). These differences in UVB sensitivity may result in shifts in species composition under UVB stress.

1.3.2 UVB effects on zooplankton

UVB effects on zooplankton have been reviewed by Siebeck *et al.* (1994). Mechanisms of damage and repair processes on cellular level are similar for phytoplankton and zooplankton. An important consequence of zooplankton having specialized cell types is the possibility of differential damage and repair in different tissues (Siebeck *et al.* 1994).

UVB effects on organism level can be lethal, depending on the UVB intensity and exposure duration. UVB induced mortality in single-species assays was shown for cladocerans (*e.g.* Siebeck 1978, Zagarese *et al.* 1994), and copepods (*e.g.* Ringelberg *et al.* 1984, Zagarese *et al.* 1997). Short term *in situ* incubations with a natural population of zooplankton showed different sensitivity between taxa, the copepod *Diaptomus* showed a strong response to UVB radiation, and the rotifer *Keratella* showed no response to UVB radiation (Williamson *et al.* 1994). Short term incubations in a clear lake showed that UVB could induce mortality of *Daphnia* until depths of 1.5 m. Similar incubations in 3 eutrophic lakes did not show significant mortality below 20 cm depth (Zagarese *et al.* 1994). But even if short-term incubations do not show mortality, sub-lethal doses may negatively affect fecundity, as shown for copepods (*e.g.* Karanas *et al.* 1981, Williamson *et al.* 1994).

Survival strategies for zooplankton can be divided in avoidance and adaptation. Since adaptation processes on cellular level are similar to the processes in phytoplankton described

in the previous paragraph, only avoidance strategies will be described here. The well-known phenomenon of diel vertical migration (DVM) of zooplankton (see review by Lampert 1989) offers the potential for UVB avoidance. Visible light may act as a controlling, an initiating and an orientating cue during migration. Endogenous rhythms are important for some species but for others migration appears to result from responses to changes in exogenous environmental factors (Forward 1988).

Daphnia is able to detect UV radiation (Smith & Macagno 1990) and responds with an immediate downward migration (Hessen 1994). The UV receptor of *Daphnia* has a peak sensitivity at 348 nm. Comparison of migrating and non-migrating *Daphnia* showed that the former species was more sensitive to UVB than the latter one (Siebeck & Böhm 1994).

The occurrence of photoprotective compounds in zooplankton is well-known. Three major types occur in planktonic crustaceans: carotenoid pigments, cuticular melanin, and mycosporine-like amino acids (MAAs). In general, copepods have carotenoid pigments, and cladocerans have cuticular melanin. MAAs have not yet been detected in freshwater zooplankton, but they are widely distributed in marine Antarctic organisms (*e.g.* Karentz *et al.* 1991). Carotenoid pigmentation plays an important role in protecting copepods from short-wave radiation (*e.g.* Hairston 1976, Ringelberg *et al.* 1984). Carotenoids are less important for cladocerans, but various genera possess the ability of carapace melanization. Melanized clones of *Daphnia* almost uniquely occur in alpine or Arctic locations, within these regions melanic populations inhabit clearwater lakes and ponds, while transparent populations are found in ponds with slightly humic water (Hebert & Emery 1990, Hessen & Sörensen 1990, Hobæk & Wolf 1991). The adaptive significance of melanic pigmentation in cladocerans is linked to UVB exposure. Melanic morphs of *Daphnia* were more resistant to UVB, but had a lower growth rate than hyaline morphs, suggesting a trade-off between the metabolic cost of melanin synthesis for UVB protection and growth (Hessen 1996).

1.3.3 Importance of mixing on UVB effects

Mixing processes cause planktonic organisms to be exposed to fluctuating radiation levels, that may vary over a range of several orders of magnitude (Smith 1989). Consider two organisms with the same mean depth averaged over time, one fixed at constant depth, and the other with vertical movement. The radiation that they are exposed to differs in three important respects: 1) Because radiation attenuates exponentially with depth, a vertically moving organism will be exposed to higher doses of any given wavelength. 2) Because of the wavelength dependent attenuation, a vertically moving organism will be exposed to relatively shorter wavelengths. 3) A vertically moving organism will be exposed to pulses of radiation many times higher than the mean, followed by periods of very low intensity (Zagarese *et al.* 1998a).

The net effect of UVB on an organism is the result of damage and repair processes. When using static incubations in field experiments, or constant radiation conditions in

laboratory experiments, a balance between damage and repair might be established (Buma *et al.* 1996a, Cullen & Lesser 1991). Under the fluctuating irradiance of vertical mixing conditions, such a steady state may be delayed. It is frequently observed that the law of reciprocity fails for UVB effects, effects are not only dependent on the dose, but also on the dose rate. The explanation is the presence of repair processes such as photoreactivation (Lesser *et al.* 1994). It seems that reciprocity holds for damage, but not for repair processes.

The photoprotection and photoreactivation processes described in the previous paragraphs differ amongst species, resulting in a different UVB sensitivity (*e.g.* Zagarese *et al.* 1997). Zagarese *et al.* (1998b) showed in field experiments that static incubations could accurately predict the survival of moving individuals for zooplankton species without photorecovery, but failed to do so for zooplankton species capable of photorecovery.

Neale *et al.* (1998) developed a model for the inhibition of photosynthesis by UVB radiation in Antarctic phytoplankton. The inhibition can be enhanced or decreased by vertical mixing, depending on the depth of the vertical mixing layer. Mixing within the euphotic zone causes increased inhibition. Mixing well below the euphotic zone lessens inhibition. Predicted inhibition is most severe when mixing is rapid, and extends to the lower part of the euphotic zone (Neale *et al.* 1998).

1.3.4 UVB effects on ecosystem interactions

Photolysis of organic molecules by UVB radiation is well known (*e.g.* De Haan 1993, Strome & Miller 1978). Low molecular weight DOC is considered to be easily utilized by bacterioplankton. Bacterial productivity was stimulated by the photodegradation of large, recalcitrant organic substrates into smaller molecules (*e.g.* Bertilsson & Tranvik 1998, Kieber *et al.* 1989, Lindell 1996, Wetzel *et al.* 1995). On the other hand, photodegradation may also release substances that cause negative effects on phytoplankton or zooplankton (*e.g.* Gjessing & Källqvist 1991, Hessen & Van Donk 1994). Humic substances absorb UVB radiation, and may thus protect organisms against UVB (*e.g.* Ekelund 1993). However, in the process of absorption, photodegradation of humic substances occurs, and this may result in a decrease in UVB attenuation (Morris & Hargreaves 1997).

The interaction between UVB radiation, large organic molecules, and planktonic organisms is complicated. High concentrations of DOC provide variable degrees of photoprotection to bacteria, phytoplankton and zooplankton (Karentz *et al.* 1994). Photolysis of large molecules can act positively, because it stimulates bacterial growth, but can act negatively because toxic substances may be released, and photodegradation will result in an increase in UVB penetration.

As described in the previous paragraph, the UVB exposure is partly determined by vertical mixing. For bacterioplankton, the net effect of UVB in a well mixed system may be positive, because mixing prevents damage, but UVB photolysis provides easily accessible substrates (*e.g.* Müller-Niklas *et al.* 1995).

Interactions between planktonic organisms may be affected by UVB radiation. A direct effect of UVB radiation can be inhibition of grazing, as found for heterotrophic nanoflagellates (Ochs 1997, Ochs & Eddy 1998, Sommaruga *et al.* 1996). An indirect effect is a reduced grazing rate as found for *Daphnia magna* feeding on UVB stressed *Selenastrum capricornutum* (Van Donk & Hessen 1995). Bothwell *et al.* (1994) found that in shallow outdoor experimental streams total algal biomass doubled in the UV exposed community as UV radiation selectively suppressed chironomid grazers.

Several multitrophic studies using microcosms or mesocosms have been published so far, with different results. The variable tolerances and responses of different species to UVB radiation is expected to result in changes in community structure of both phytoplankton and zooplankton. Worrest *et al.* (1978, 1981) showed that increased UVB irradiance caused a decrease in algal biomass, and a decreased community diversity. Experiments on large marine enclosures showed effects of UVB on the primary producers, and to a lesser extent on primary consumers. However, no effect was seen on the higher trophic levels (Keller *et al.* 1997). In an enclosure experiment in a high-altitude mountain lake in the Central Alps, no UVB effect was found on phytoplankton growth and species composition (Halac *et al.* 1997). In an outdoor mesocosm experiment in a high-altitude Andean lake the abundance of chlorophytes increased and the abundance of diatoms decreased in the UVB treatment (Cabrera *et al.* 1997).

Effects of UVB radiation vary between the experiments, various mechanisms may be responsible for the different effects. Williamson (1995) defined four hypotheses to describe the role of UVB radiation in freshwater ecosystems. 1) Solar ambush hypothesis: Aquatic organisms that cannot detect and respond to changes in UVB are especially vulnerable. Some organisms have photoreceptors for UVA, but not for UVB. If this is the case, increases due to ozone depletion of UVB but not of UVA may result in unexpectedly high UVB exposure. 2) Solar bottleneck hypothesis: Some small zooplankters show a reverse diel vertical migration, seeking refuge from large zooplankters in the epilimnion. In oligotrophic waters, these small zooplankters may then be exposed to damaging levels of UVB radiation. 3) Solar cascade hypothesis: In cascading trophic interactions, UVB can have a strong effect on organisms by influencing the abundance of their food resources or predators. 4) Acid transparency hypothesis: Anthropogenically acidified environments are harsher UVB environments than naturally acidified lakes.

Phytoplankton-zooplankton interactions receive great interest in aquatic ecology. UVB effects on individual species of phytoplankton or zooplankton are already widely studied (see previous paragraphs). It is widely hypothesized that UVB-induced changes in phytoplankton will affect herbivorous zooplankton. UVB effects on phytoplankton primary production, species composition, morphology, and biochemical composition are likely to affect herbivorous zooplankton. Van Donk & Hessen (1995) found a reduced grazing rate for *Daphnia magna* feeding on UVB stressed *Selenastrum capricornutum*. This was explained by a reduced digestibility as result of increased cell wall thickness. There is however little further

information to support the hypothesis that UVB will affect phytoplankton-zooplankton interactions. The aim of this thesis is to provide this information.

The underlying hypothesis in this thesis is that phytoplankton is directly affected by UVB radiation, since it needs PAR for photosynthesis. Zooplankton on the other hand is not dependent on light, and is able to move actively through the water column. This capability of vertical migration, and the possible ability to detect UV radiation may allow zooplankters to regulate their exposure to UVB. Therefore, indirect effects on zooplankton through changes in its food may play a more important role.

1.4 BIOLOGICAL WEIGHTING FUNCTIONS

The harmful effects of ultraviolet radiation are wavelength dependent, with shorter wavelengths causing more damage. Biological weighting functions (BWF, or action spectra) have been developed to define effects as function of wavelength. These weighting functions describe generalized wavelength dependent responses. However, every organism has its specific response to ultraviolet radiation, and every scientist has his/her specific response of interest. Therefore any weighting function will give an approximation of the biological effective dose.

The most commonly used action spectra used for higher plants are: 1) Photosynthesis Inhibition (PI) action spectrum (Jones & Kok 1966), derived from Hill reaction inhibition of isolated spinach chloroplasts by radiation between 260 and 560 nm. 2) Plant Action Spectrum (Caldwell 1971), derived from the damage spectra of several terrestrial plants for wavelengths below 313 nm. 3) DNA Action Spectrum (Setlow 1974), derived from photoproducts in DNA and the mutation rates and mortality of bacteria and phages at wavelengths between 250 and 370 nm.

The use of action spectra has been reviewed extensively in photobiology literature (*e.g.* Coohill 1989, Halldal 1967, Rundel 1983). With the increased interest in UVB effects on phytoplankton, the need for a general phytoplankton action spectrum increased. Recently, several action spectra have been developed for the photosynthesis inhibition of (marine) phytoplankton. Fig. 1.2 gives a comparison between the 'old' action spectra, and a selection of action spectra recently developed for phytoplankton.

Cullen and co-workers have constructed biological weighting functions combined with a P-I model, taking into consideration that photoinhibition is dependent on both absolute UV irradiance, and UV relative to PAR. Weighting functions were developed for natural phytoplankton populations in Antarctica (curve g in Fig. 1.2), and on laboratory cultures (*e.g. Phaeodactylum* sp., curve d in Fig. 1.2) (Cullen *et al.* 1992, Neale *et al.* 1994). Behrenfeld *et al.* (1993) developed a biological weighting function for the UVB induced inhibition of phytoplankton carbon fixation. Measurements were made on phytoplankton populations off the Washington state coast. Boucher & Prézelin (1996) developed a daily integrated biological

weighting function for inhibition of primary production. Measurements were made on a natural community of Antarctic diatoms under natural daylight conditions.

The main differences between the curves are that the PI biological weighting function (curve c) is very flat, and the DNA weighting function (curve b) is very steep compared with the other curves. The BWF developed for *Phaeodactylum* (curve d, Cullen *et al.* 1992) and the BWF developed for natural temperate phytoplankton community (curve f, Behrenfeld *et al.* 1993) are rather similar. The other curves are quite similar as well, however the BWF for natural Antarctic diatom populations is more sensitive to wavelengths below 300 nm (curve e, Boucher & Prézelin 1996). In contrast, the BWF for natural Antarctic phytoplankton populations studied by Neale *et al.* (1994) (curve g) is less sensitive to wavelengths below 300 nm.

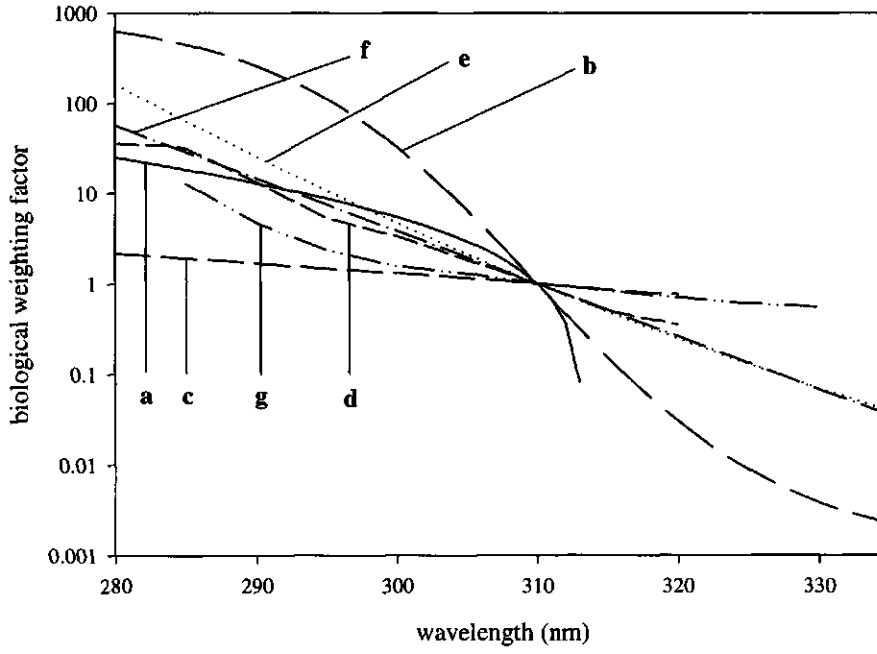

Figure 1.2: A comparison of different biological weighting functions. For clarity of the figure, the spectra are normalized to 1 at 310 nm, and the y-axis is on a log-scale. a = Caldwell (1971), b = Setlow (1974), c = Jones & Kok (1966), d = Cullen *et al.* (1992), e = Boucher & Prézelin (1996), f = Behrenfeld *et al.* (1993), g = Neale *et al.* (1994). See text for further explanation.

1.5 AIM OF THIS STUDY AND THESIS OUTLINE

The problem of ozone depletion, fluctuations in UVB radiation, and effects on terrestrial, marine and freshwater ecosystems has many aspects. The work in this thesis concentrates on the implications of UVB radiation for freshwater ecosystem functioning, especially the effects on phytoplankton and zooplankton. The underlying hypothesis in this thesis is that phytoplankton will be directly affected by UVB radiation, and that zooplankton will be indirectly affected by UVB through changes in its food. The results presented in this thesis can be divided into the following aspects:

- What is the effect of current intensities of UVB radiation at temperate latitude on phytoplankton-zooplankton interactions?
- What is the potential effect of UVB radiation on phytoplankton-zooplankton interactions?

This thesis is composed of 9 chapters. These mostly contain (parts of) papers published in or submitted to international scientific journals. The attenuation of solar radiation in aquatic systems in the Netherlands, and theoretical relations between water quality variables and attenuation properties are described in Chapter 2. Chapter 3 gives a review of UVB effects on phytoplankton cells, and implications for herbivorous zooplankton. Chapter 4 studies the (short-term) effects on grazing rates of different zooplankton species when grazing on UVB irradiated phytoplankton. Chapter 5 considers the (long-term) effects on survival, somatic growth and fecundity of 2 species of *Daphnia* when fed with UVB irradiated phytoplankton. Chapter 6 studies the effects of prolonged UVB exposure on indoor freshwater microcosm systems, with special attention for the phytoplankton-zooplankton interactions. Chapter 7 describes results of field experiments studying plankton interactions at different latitudes, in the Netherlands, Norway, and Spitsbergen (Norway). Chapter 8 attempts to link field seston quality and quantity variables (as food for *Daphnia*) with differences in light penetration of the sample site. Finally, concluding remarks are given in Chapter 9.

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APPENDIX 1.1: OZONE PRODUCTION AND DESTRUCTION REACTIONS

Ozone is continuously produced in the stratosphere. Radiation at wavelengths less than 240 nm dissociates molecular oxygen into atoms that attach themselves to O_2 to form ozone (O_3) (Box 1). The production of ozone peaks in the stratosphere; at higher altitudes the amount of oxygen diminishes. At lower altitudes (in the troposphere) no radiation < 240 nm is available as it has been absorbed by the overhead stratospheric O_2 and O_3. The amount of ozone in the stratosphere is determined in the dynamic balance of production and loss processes. The chemical destruction of ozone in the stratosphere is brought about by catalytic reactions of nitrogen, hydrogen, chlorine, and bromine oxides (Box 2). In these catalytic cycles the active NO_x, HO_x, Cl_x and Br_x are not lost, so the ozone-destroying steps can be repeated many times.

Observed reductions in ozone concentrations were much larger than could be explained by these gas phase reactions. Strong evidence exists to support the hypothesis that heterogeneous gas-solid reactions occur on the surface of polar stratospheric clouds (PSC). The dark polar winter stratosphere becomes loaded with gaseous Cl_2 and HOCl, and when the sun rises in spring, photolytic reactions liberate Cl atoms for the ozone destroying catalytic cycles described in Box 2 (Box 3) (Madronich 1993, Madronich 1994, Stolarski *et al.* 1992).

Temperatures in the lower stratosphere are closely coupled to ozone through dynamics and photochemistry. Extremely low temperatures (lower than -78 °C) contribute to the presence of polar stratospheric clouds (PSCs). PSCs enhance the production and lifetime of reactive chlorine, leading to ozone depletion in the presence of sunlight.

Depletion of ozone in the Antarctic generally starts in September, in the lower stratosphere (14 to 22 km), and continues until December. In this period there is limited exchange of air between the polar vortex and midlatitudes, and partial exposure of the vortex to sunlight. When the stratosphere warms up, and the polar vortex is broken, ozone-rich air from lower latitudes will mix and ozone concentrations increase (*e.g.* Solomon 1990, Stolarski *et al.* 1992). Chemical ozone loss in the Arctic is more difficult to predict. During cold winters, chemical ozone loss can be substantial. Ozone concentrations are more perturbed by atmospheric dynamics, therefore are patterns of ozone depletion in the Northern hemisphere less predictable (Müller *et al.* 1997). Observations show that ozone depletion occurs more rapidly over Scandinavia, than over most regions at corresponding latitudes (Björn *et al.* 1998).

Box 1: Production and destruction of ozone in pure-oxygen reactions (Chapman mechanism)

$O_2 + h\nu(\lambda<240\ nm) \rightarrow O + O$

$O + O_2 \rightarrow O_3$

$O_3 + h\nu(\lambda<240\ nm) \rightarrow O + O_2$

$\Downarrow$

net production: $3O_2 \rightarrow 2O_3$

net destruction: $2O_3 \rightarrow 3O_2$

Box 2: Chemical ozone destruction reactions in catalytic cycles

$O3 + X \rightarrow XO + O_2$

$O + XO \rightarrow O_2 + X$

$O3 + XO \rightarrow 2O_2 + X$

$\Downarrow$

net reaction: $2O_3 \rightarrow 3O_2$

where X may be NO, HO, Cl or Br

Box 3: Reactions occurring on the surface of polar stratospheric clouds

$ClONO_2\ (g) + HCl\ (s) \rightarrow Cl_2\ (g) + HNO_3\ (s)$

$ClONO_2\ (g) + H_2O\ (s) \rightarrow HOCl\ (g) + HNO_3\ (s)$

$Cl_2 + h\nu \rightarrow Cl + Cl$

$HOCl + h\nu \rightarrow OH + Cl$

CHAPTER 2

ULTRAVIOLET AND VISIBLE RADIATION IN THE NETHERLANDS: ASPECTS OF ATTENUATION PROCESSES IN DUTCH AQUATIC SYSTEMS

It's a beautiful day
The sun is shining
I feel good
And no-one's gonna stop me now, oh yeah.

(Queen - It's a beautiful day)

2.1 INTRODUCTION

2.1.1 Optical properties of water

The light climate is one of the most important factors determining biological processes in aquatic systems (*e.g.* Horne & Goldman 1994). It is therefore not surprising that optical properties of freshwater and marine systems have been studied widely (*e.g.* Bricaud *et al.* 1981, Højerslev 1978, Jerlov & Nielsen 1974, Kirk 1994a, Smith & Baker 1979, 1981).

Optical properties of water can be divided into two classes, the inherent and the apparent optical properties. Inherent optical properties can be considered as the (theoretical) interaction of incident radiation and the water constituents (inorganic and organic particles, humic substances, and water itself). The absorption coefficient (a, m^{-1}), the scattering coefficient (b, m^{-1}), and the total beam attenuation coefficient (c = a + b, m^{-1}) are inherent optical properties. Apparent optical properties are dependent on the radiance distribution and the inherent optical properties. Commonly measured optical properties as Secchi depth, light attenuation and reflectance are apparent optical properties (Kirk 1994a, Smith & Baker 1981).

Changes in cloud cover, stratospheric ozone concentration, atmospheric smog, etc., will influence the intensity of solar radiation reaching the earth's surface (see Chapter 1). Time of year and latitude will affect day length and zenith angle (Kirk 1994a). Zenith angle, and water surface properties (waves, whitecaps, or flat surface), will affect the transmission across the air-water interface (Kirk 1994a). These factors acting above the water surface will not be described in this chapter. This chapter will focus on how ultraviolet and PAR radiation acts within (Dutch) aquatic systems.

2.1.2 Absorption of light within the aquatic medium

In the water column, a photon can be either scattered or absorbed. Light absorption in natural waters is attributable to four components: water itself, dissolved yellow pigments (humic acids), organic particulate matter (phytoplankton), and inorganic particulate matter (tripton).

The light absorption by pure water is very low, and is therefore difficult to measure (Kirk 1994b). Pure water absorbs very weakly in the blue and green regions of the spectrum, absorption begins to rise above 500 nm, towards the red region (Kirk 1994a, Smith & Baker 1981).

Dissolved yellow pigments originate from the decomposition of aquatic or terrestrial biomass. Decomposition takes place by microbial action, most is converted to carbon dioxide, water and mineral salts, but a proportion ends up as a complex group of yellow-brown compounds, referred to as 'yellow substance', 'gelbstoff', 'gilvin', or 'humic acids' (Kirk 1994a). The absorption spectrum of these humic acids is very characteristic, and rises exponentially towards the ultraviolet end of the spectrum (Kirk 1994b). The concentration of dissolved

organic carbon (DOC) is a quantitative estimate of humic acids, disregarding the highly heterogeneous characteristics of this group.

The absorption of light by phytoplankton is mainly determined by the photosynthetic pigments: chlorophylls, carotenoids and biliproteins. Light absorption by algal cells grown in laboratory cultures has received much attention. The phytoplankton pigment composition is dependent on environmental variables as nutrient concentration, and history of light intensity and temperature. It is therefore difficult to derive information on light absorption properties of natural phytoplankton populations directly from laboratory measurements with single species (Kirk 1994a).

Light absorption by tripton is difficult to measure. At typical concentrations, the material does not absorb light strongly but scatters quite intensely (Kirk 1994a). Tripton absorption is low or absent at the red end of the spectrum, and rises steadily as wavelength decreases towards the blue and ultraviolet.

Scattering does not remove light, but affects the vertical penetration of light. This increases the pathlength and consequently increases the probability of absorption (Kirk 1994a). Measurement of scattering in water is difficult, because one cannot separate scattering from absorption processes, and therefore one measures the resulting beam attenuation (c). However, when also the absorption coefficient, a, is measured, the scattering coefficient, b, may be calculated (b=c-a).

The scattering and absorption processes cause that the intensity of light diminishes with depth in an approximately exponential way:

$$I_z = I_0 e^{-Kd*z}$$

where I_z and I_0 are the values of downward irradiance at z m and just below the surface, and K_d is the vertical attenuation coefficient for downward irradiance. K_d is wavelength dependent (see § 2.1.3). K_d is usually independent of depth, however sometimes in clear oceanic water it can be observed that blue and green light is attenuated more rapidly below 10 metres. This is attributed to the downward flux becoming more diffuse, with a consequent increase in attenuation (Kirk 1994a).

2.1.3 Attenuation of ultraviolet radiation in freshwater

The attenuation of UV radiation in marine and freshwater systems has gained considerable interest since the discovery of the thinning of the ozone layer. Long before that, Jerlov (1950) already investigated the penetration of ultraviolet radiation in the sea, and concluded that ultraviolet radiation could penetrate as far as 20 m depth. Recently, several field studies have been published that measured *in situ* UV penetration in freshwater systems and water quality variables, and described empirical relationships between water quality variables and UV attenuation (Hodoki & Watanabe 1998, Laurion *et al.* 1997, Morris *et al.* 1995, Scully & Lean 1994). Table 2.1 gives a review of the empirical relationships.

Table 2.1: Significant empirical relations between K_d values and DOC, Chlorophyll-*a* (Chl-*a*), or absorption coefficient (K_a). References: 1= Scully & Lean 1994, Table 5, study sites in North America; 2= Morris *et al.* 1995, Table 3, study sites in North and South America; 3=Laurion *et al.* 1997, calculated from Table 1, study sites in subarctic Canada; 4=Hodoki & Watanabe 1998, calculated from Table 1 & 2, study sites in Japan.

K_d	relation	r^2	n	reference
K_dUVB	$0.414*DOC^{1.86}$	0.97	20	1
K_d310	$0.237 + 1.229*K_a310$	0.98	20	1
K_dUVA	$0.299*DOC^{1.53}$	0.95	20	1
K_d305	$2.76*DOC^{1.23}$	0.96	44	2
K_d320	$2.09*DOC^{1.12}$	0.87	63	2
K_d340	$1.64*DOC^{1.13}$	0.89	63	2
K_d380	$0.83*DOC^{1.16}$	0.92	62	2
K_dPAR	-0.05 + 0.22*DOC + 0.07*Chl-*a*	0.68	63	2
K_d305	$-2.38 + 2.62*K_a305$	0.91	44	2
K_d320	$-0.12 + 1.51*K_a320$	0.84	63	2
K_d340	$-0.16 + 1.77*K_a340$	0.83	63	2
K_d380	$0.25 + 2.28*K_a380$	0.73	62	2
K_dPAR	$0.12 + 1.30*K_a440$	0.58	63	2
K_d305	$0.86*DOC^{1.50}$	0.82	22	3
K_d320	-6.94 + 3.89*DOC	0.90	22	3
K_d340	-5.21 + 2.96*DOC	0.90	22	3
K_d380	-3.04 + 1.68*DOC	0.88	22	3
K_dUVB	$2.46*DOC^{1.47}$	0.52	21	4
K_dUVB	3.26 + 0.17*Chl-*a*	0.79	22	4
K_dUVA	$1.47*DOC^{1.54}$	0.47	21	4
K_dUVA	1.84 + 0.12*Chl-*a*	0.81	22	4
K_dPAR	$0.67*DOC^{1.39}$	0.59	21	4
K_dPAR	0.92 + 0.04*Chl-*a*	0.77	22	4

In three of these field studies, the DOC concentration is a good predictor of K_dUVB, with r^2 values above 0.8. In one study DOC is a weak predictor of K_dUVB (Hodoki & Watanabe 1998). In all 4 studies, the best relation to describe DOC concentration *vs.* K_dUVB is a power function, meaning that UVB penetration increases exponentially as DOC decreases. Two factors can be responsible for this: i) the proportion of colourless DOC increases exponentially as DOC decreases; ii) photobleaching and photodegradation of coloured DOC compounds increase as a function of residence time (Schindler *et al.* 1996). Other relations are also possible to describe the DOC *vs.* K_d relation. Vincent *et al.* (1998) find the best fit of DOC versus $1/K_d$ in a log-log linear regression: $\log(1/K_d320) = 0.42 - 1.98*\log(DOC)$, $r^2 = 0.95$, n=20. Study sites were 4 Antarctic freshwater lakes, combined with data from Laurion *et al.* (1997).

As expected from theoretical relations (Kirk 1994b), the importance of DOC concentration, as a measure of humic acids, in attenuating ultraviolet radiation is confirmed by these field studies. This is especially true for water systems with low algal biomass. In

eutrophic systems the role of algal biomass (measured as chlorophyll-*a* concentration) seems to become more important than DOC in the attenuation of ultraviolet radiation.

This chapter describes the results of PAR, UVA and UVB irradiance measurements in different aquatic systems in the Netherlands. The objective of this field measurement program was 3-fold:

1. How deep does UVB irradiance penetrate into Dutch aquatic systems?
2. Which water or seston variable explains the measured K_d values for PAR, UVA and UVB best? (theoretical approach).
3. What is the easiest way to predict K_dUVB? (pragmatic approach).

Complementary to these *in situ* measurements, theoretical relations between water properties and light attenuation were tested in laboratory experiments. These relations were used to develop a model to predict K_d values.

2.2 MATERIALS AND METHODS

2.2.1 *In situ* attenuation measurements

Measurements of the attenuation of solar irradiance in different aquatic systems were made in spring 1998, at 19 different locations in the Netherlands (Fig. 2.1, Table 2.2). The selection of the sample locations was based on expected chlorophyll-*a* and DOC concentrations. To have a comparable zenith angle, radiation measurements were made within 3 hours of local noon, and within 2 months of the summer solstice.

Measurements were made with a scanning spectroradiometer (Macam SR9910), with a side-view cosine sensor, at 6 different depths. Measurements of Wadden Sea water were made in a white container, further described in § 2.2.2. Scans were made from 280 to 320 nm with 1 nm interval, from 320 to 400 nm with 2 nm interval, and from 400 to 800 nm with 5 nm interval. In cases of shallow water depth, fewer depths were measured. UVB irradiance was integrated from 280 to 320 nm, UVA irradiance was integrated from 320 to 400 nm, and PAR was integrated from 400 to 700 nm. Vertical attenuation coefficients (K_d) were calculated by plotting the natural log of the irradiance versus depth (m), and calculating a linear regression line, where the slope of the regression line is the vertical attenuation coefficient (m^{-1}). In these cases where there were not enough points to calculate a linear regression, K_d was calculated as

$$(\ln(I_{z1}/I_{z2}))/z$$

where I_{z1} and I_{z2} is the irradiance measured at 2 different depths, and z the interval between those 2 depths (m) (Kirk 1994a).

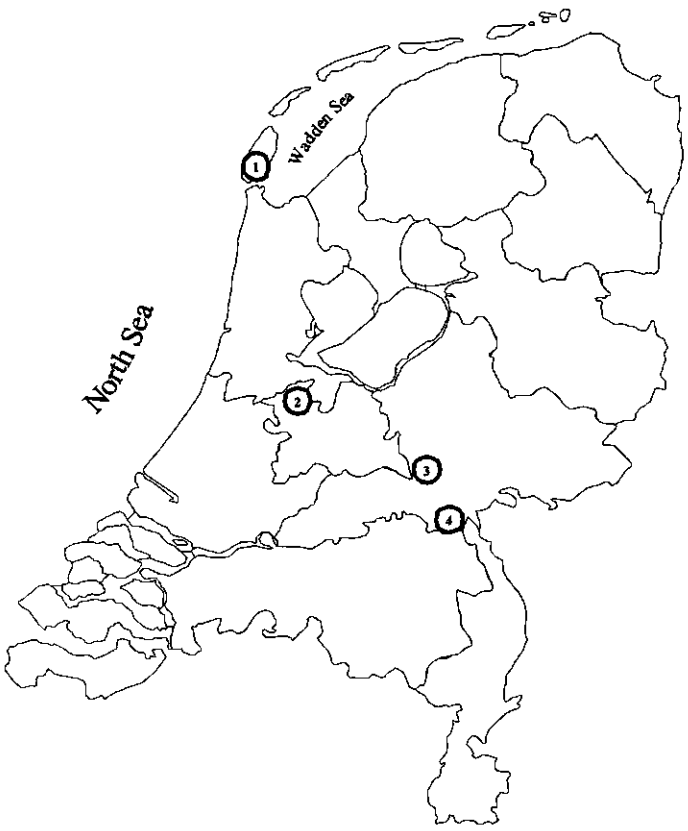

Figure 2.1: Locations of the sample sites in the Netherlands. 1: Pool Waal en Burgerdijk, Lake Oude Schans, Ditch Oude Schans, Ditch Hendrikpolder, Wadden Sea. 2: Lake Zwemlust, Lake Loosdrecht, Lake Maarsseveen. 3: Sinderhoeve, Veenkampen, Pond Dreijen, Argo harbour, Wageningen canal, Grift canal, Lake Blauwe Kamer. 4: Uiversnest fen.

Table 2.2: Latitude and longitude of the sample locations, with estimated surface area and estimated maximum depth.

Location	Latitude (N)	Longitude (E)	Surface area (ha)	Depth (m)
Pool Waal en Burgerdijk	53°06'	04°48'	< 0.1	0.6
Lake Oude Schans	53°02'	04°50'	< 1	0.6
Ditch Oude Schans	53°02'	04°50'	< 0.1	0.2
Ditch Hendrikpolder	53°01'	04°48'	< 0.1	1.5
Wadden Sea	53°00'	04°50'	-	-
Lake Zwemlust	52°12'	05°00'	1.5	1.5
Lake Loosdrecht (Stille Plas)	52°11'	05°05'	150	1.5
Lake Maarsseveen	52°09'	05°05'	70	30
Sinderhoeve, ditch 7, 9, 13	52°00'	05°45'	< 0.1	0.5
Veenkampen, ditch I	51°59'	05°37'	< 0.1	0.6
Veenkampen, ditch II	51°59'	05°37'	< 0.1	0.2
Pond Dreijen	51°58'	05°41'	< 1	1
Argo harbour	51°58'	05°39'	< 1	1.5
Wageningen canal	51°58'	05°40'	< 1	1
Grift canal	51°58'	05°37'	< 0.1	1
Lake Blauwe Kamer	51°57'	05°37'	1	1
Uiversnest fen	51°48'	05°48'	2	< 1

At each location the following measurements were made *in situ*: dissolved oxygen (WTW OXI 320 oximeter), pH (WTW pH 320 pH-meter), conductivity (WTW LF 92 EGV-meter), temperature, turbidity (HACH 2100 P turbidimeter), and Secchi depth. Additional water samples were taken and transported back to the laboratory for the following measurements:

The particulate matter of a known volume (v in litres) of water was collected on preweighed (m_0 in milligrammes) GF/F filters (Whatmann), dried (105 °C, 1 hour), weighed (m_d), combusted (550 °C, 3 hours) and weighed (m_c). Two control filters (c) were processed similarly with each measurement. Dry weight (DW), ash weight (AW) and ash-free dry weight (AFDW) were calculated with the following equations:

$$DW\ (mg\ l^{-1}) = ((m_d-m_0) - (c_d-c_0))/v$$

$$AFDW\ (mg\ l^{-1}) = ((m_d-m_c) - (c_d-c_c))/v$$

$$AW\ (mg\ l^{-1}) = ((m_c-m_0) - (c_c-c_0))/v$$

Ash-free dry weight is a measure for total organic particulate matter, ash weight is a measure for total inorganic particulate matter.

Samples for chlorophyll-*a* were filtered on GF/C filters (Schleicher & Schuell), chlorophyll-*a* was extracted with ethanol at 70 °C and analyzed spectrophotometrically at 750 and 665 nm, with correction for phaeopigments (Moed & Hallegraeff 1978).

Total organic carbon (TOC) and total inorganic carbon (TIC) were measured on unfiltered samples, dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) were measured on filtered (0.2 µm membrane filter, Schleicher & Schuell) samples. Carbon

was measured with a TOC analyzer (O.I. Analytical, model 700) following persulphate oxidation at 100 °C, and detection of CO_2 gas with an infrared detector.

Total nitrogen (N) and total phosphorus (P) were measured on unfiltered samples, phosphate, ammonium and nitrite+nitrate were measured on filtered (0.2 µm membrane filter, Schleicher & Schuell) samples with a Skalar SA40 autoanalyzer. Alkalinity estimation was done by titration of 100.0 ml of water sample with 0.05 N HCl to pH 4.4.

Absorbance at 250, 300, 365, 380 and 440 nm was measured in 5 cm cuvettes with a Beckman DU-64 spectrophotometer of filtered (0.2 µm membrane, Schleicher & Schuell), and unfiltered samples. Selection of these wavelengths was based on literature. Absorbance at 250 and 365 nm is used by De Haan & De Boer (1987), absorbance at 380 nm is used in a model by Buiteveld (1995), absorbance at 440 nm is used by Kirk (1994a). Absorbance at 300 nm was chosen because of expected relationship with K_dUVB. The absorption coefficient at specific wavelengths can be obtained from the absorbance values using:

$K_a = 2.303 * D/r$

where D is the absorbance value, r is the pathlength (m) through the system, and K_a is the absorption coefficient (m^{-1}) (Kirk 1994a).

Variables as nutrient concentration, alkalinity, conductivity, pH, temperature and oxygen were measured to give additional information on the type of system, like trophic state or vulnerability to acidification.

DOC, chlorophyll-*a*, and particulate matter (dry weight, ash-free dry weight and ash weight) were expected to have a direct (theoretical) relationship with the K_d values. Product-moment correlation coefficients between these variables and K_d were calculated. Further, linear and power functions were fitted to obtain the relation between these variables and K_d (theoretical approach, see § 2.1.3).

Absorbance at various wavelengths, turbidity, and Secchi depth were expected to be correlated with K_d. Product-moment correlation coefficients were calculated between these variables and K_d values. Linear regressions were calculated to obtain the relation between these variables and K_d (pragmatic approach).

Multiple linear regression models with K_d as response variable were selected with an iterative procedure in Genstat (Genstat 1993). In this procedure all possible models are calculated, and the best models are selected on criteria of minimal number of predictor variables, and minimal residual mean sum of squares ($MS_{residual}$) (Montgomery and Peck 1982).

2.2.2 Laboratory experiments

The relation between different water variables and light attenuation was tested in laboratory experiments. Experiments were done in a white container, with a diameter of 48 cm at the bottom, 60 cm at the surface, and a water depth of 30 cm. Reflection from the walls was

assumed to be comparable with the natural situation. Artificial radiation was provided by one high pressure metal halide lamp (Philips HPI-T, 400 W), and 2 UVB fluorescent tubes (Philips TL12, 20 W), positioned 55 cm above the water surface. Surface intensity of UVB was 1.34 W m^{-2}, UVA was 3.13 W m^{-2}, and PAR was 17.31 W m^{-2}. Irradiation measurements were made at 6 depths at 5 cm intervals with a scanning spectroradiometer (Macam SR9910), with a side-view cosine sensor. Scans were made from 260 to 800 nm with 1 nm interval. Calculation of the attenuation coefficients was done as described in § 2.2.1.

Measurements were made with artificial humic acids, algal biomass, and suspended clay at different concentrations. Each concentration series started with demineralized water. Increasing concentrations were obtained by adding the respective substance. At least 7 concentrations were used.

Artificial humic acid was obtained from Aldrich (H1,675-2). Humic acid was measured as DOC concentration, and absorption at the same wavelengths as in the field study. Algal biomass was tested in three separate experiments with the following species from different taxonomic groups: *Scenedesmus acutus* Meyen (Chlorophyceae, strain from the culture collection Max Planck Institut für Limnologie, Plön, Germany); *Cryptomonas pyrenoidifera* Geitler (Cryptophyceae, strain from Norwegian Institute for Water Research, NIVA-2/81); *Microcystis aeruginosa* Kützing (Bacillariophyceae, strain NIVA-CYA 43). Algal biomass was measured as chlorophyll-*a*, DW and AFDW. For control DOC and TOC was measured at 3 concentrations. Suspended clay was obtained from a brick factory (Doorwerth, the Netherlands). Suspended clay was measured as DW and AW. For control DOC and TOC was measured at 3 concentrations. The relation between the concentration of each variable and K_d was calculated with linear regression.

2.3 RESULTS

2.3.1 *In situ* PAR and UV attenuation

A full report of the field measurements is given in Appendix 2.1. Table 2.3 gives the depth of 1% surface intensity (calculated as $4.61/K_d$). This depth for PAR is regarded as the euphotic zone. The mean depth of 1% UVB penetration is 0.28 m, 1% UVA is 0.57 m, and 1% PAR is 2.43 m. Lake Maarsseveen is the clearest system, UVB penetrates to a depth of 0.51 m, and PAR penetrates to a depth of 5.55 m. Other clear systems are Veenkampen ditch I, and Argo harbour. Turbid systems are Sinderhoeve ditch 13 and Ditch Hendrikpolder, with a PAR penetration depth of less than 1 m, and a UVB penetration depth of less than 0.1 m.

Table 2.4 gives the correlation matrix of the variables that are expected to be related or correlated with K_d values. It is clear that the K_a and K_d values are closely correlated. Considering independent variables, the strongest correlation of K_dUVB is with TOC ($r=0.53$, $p=0.020$) and with DOC ($r=0.53$, $p=0.020$). The strongest correlation of K_dUVA is with TOC ($r=0.51$, $p=0.026$). K_dPAR is most strongly correlated with chlorophyll-*a* ($r=0.58$, $p=0.008$).

Table 2.3: Depth (m) of 1% surface intensity of UVB, UVA and PAR irradiance.

Location	1% UVB	1% UVA	1% PAR
Pool Waal en Burgerdijk	0.29	0.73	2.97
Lake Oude Schans	0.09	0.28	1.91
Ditch Oude Schans	0.12	0.28	1.48
Ditch Hendrikpolder	0.08	0.20	0.95
Wadden Sea	0.49	1.05	3.24
Lake Zwemlust	0.38	0.61	2.77
Lake Loosdrecht (Stille Plas)	0.16	0.26	1.17
Lake Maarsseveen	0.51	1.25	5.55
Sinderhoeve, ditch 7	0.33	0.61	3.24
Sinderhoeve, ditch 9	0.35	0.64	3.36
Sinderhoeve, ditch 13	0.07	0.15	0.85
Veenkampen, ditch I	0.46	1.14	4.08
Veenkampen, ditch II	0.08	0.19	1.18
Pond Dreijen	0.21	0.34	0.94
Argo harbour	0.49	1.03	4.22
Wageningen canal	0.19	0.28	0.84
Grift canal	0.46	0.95	3.54
Lake Blauwe Kamer	0.37	0.75	3.05
Uiversnest fen	0.12	0.16	0.72
mean depth	0.28	0.57	2.43
median depth	0.30	0.61	2.77

Table 2.4: Product-moment correlation coefficients, bold figures indicate a significance level of $\alpha<0.01$ (threshold value of r=0.576 at n=19). turb = turbidity, Chl-*a* = Chlorophyll-*a*.

DW	**0.60**													
AFDW	**0.82**	**0.87**												
AW	0.28	**0.91**	**0.58**											
Chl-*a*	**0.60**	0.52	**0.60**	0.34										
DOC	0.41	0.27	0.36	0.14	0.28									
TOC	**0.61**	0.39	**0.61**	0.12	0.42	**0.77**								
K_d250	0.24	0.29	0.34	0.20	0.06	**0.58**	**0.59**							
K_d300	0.19	0.27	0.31	0.19	0.06	0.49	0.52	**0.99**						
K_d365	0.11	0.21	0.25	0.14	0.03	0.31	0.37	**0.92**	**0.97**					
K_d380	0.10	0.21	0.25	0.13	0.03	0.27	0.34	**0.89**	**0.95**	**1.00**				
K_d440	0.10	0.15	0.26	0.04	0.00	0.15	0.26	**0.76**	**0.84**	**0.94**	**0.96**			
K_dUVB	0.19	0.36	0.28	0.35	0.19	0.53	0.53	**0.87**	**0.87**	**0.80**	**0.77**	**0.59**		
K_dUVA	0.26	0.29	0.30	0.23	0.27	0.40	0.51	**0.87**	**0.91**	**0.90**	**0.89**	**0.79**	**0.91**	
K_dPAR	0.39	0.37	0.43	0.24	**0.58**	0.31	0.52	**0.68**	**0.73**	**0.76**	**0.76**	**0.72**	**0.71**	**0.90**
	turb	DW	AF DW	AW	Chl-*a*	DOC	TOC	K_d 250	K_d 300	K_d 365	K_d 380	K_d 440	K_d UVB	K_d UVA

Fig. 2.2 gives the chlorophyll-*a* concentration and the DOC concentration *vs.* K_d. There is considerable scatter in the plots, with an indication of a positive relation. The positive relationship of chlorophyll-*a* is most evident in the plot of K_dPAR. The positive relationship of DOC *vs.* K_dUVB is present in the upper right panel, though with considerable scatter and not as evident as known from other field studies (*e.g.* Scully & Lean 1994).

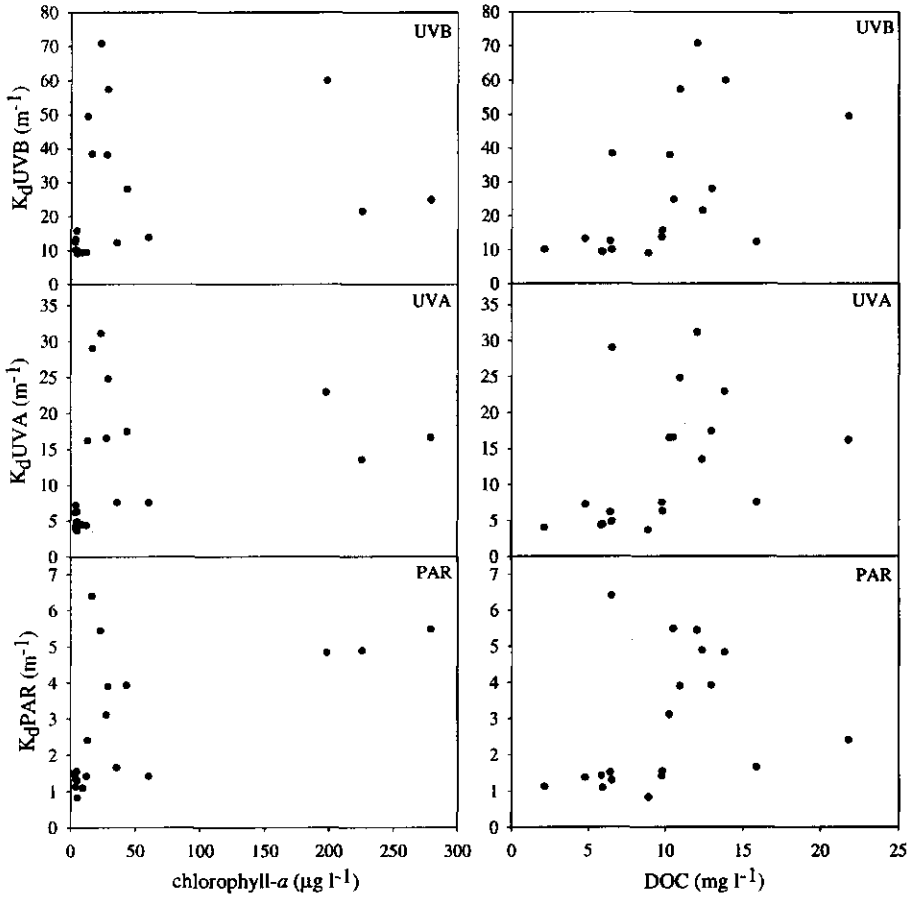

Figure 2.2: Chlorophyll-*a* concentration (left panels) and DOC concentration (right panels) *versus* K_d, upper panels are K_dUVB, middle panels are K_dUVA, and lower panels are K_dPAR.

To describe the empirical relations between K_d values and water variables, linear and power functions were calculated (Table 2.5). The best function to explain K_dUVB is a power function with TOC. The predictive power is however quite low (38% of the variation explained). For K_dUVA, the best function is a power function with TOC. For K_dPAR the best function is a power function with chlorophyll-*a*. The r^2 values are much lower than the r^2 values from other field studies (see § 2.1.3). The large variation between systems in this dataset might be the explanation for the low r^2 values.

Table 2.5: Empirical relations between K_d (UVB, UVA and PAR), and independent water variables, calculated from the field data (n=19, α<0.05).

K_d	relation	r^2
K_dUVB	$3.80*TOC^{0.80}$	0.38
K_dUVB	$3.62*DOC^{0.80}$	0.33
K_dUVB	$9.73*Chl\text{-}a^{0.25}$	0.25
K_dUVB	3.58 + 2.33*DOC	0.24
K_dUVB	6.04 + 2.13*TOC	0.24
K_dUVA	$1.86*TOC^{0.80}$	0.38
K_dUVA	$4.05*Chl\text{-}a^{0.31}$	0.37
K_dUVA	$1.84*DOC^{0.78}$	0.31
K_dUVA	4.05 + 0.92*TOC	0.21
K_dPAR	$0.85*Chl\text{-}a^{0.33}$	0.51
K_dPAR	$0.48*TOC^{0.74}$	0.38
K_dPAR	2.15 + 0.013*Chl-*a*	0.30
K_dPAR	$1.32*AFDW^{0.32}$	0.27
K_dPAR	0.98 + 0.19*TOC	0.23

The results of the multiple linear regressions are given in Table 2.6. In the model predicting K_dUVB by selecting only the theoretical related variables, ash weight and DOC were the best predictors. When also correlated variables were selected, the best model had chlorophyll-*a* and K_a300 as predictor variables.

There were no significant theoretical related variables to predict K_dUVA. When all variables could be selected, the best model had chlorophyll-*a* and K_a365 as predictor variables.

In the model predicting K_dPAR by selecting only the theoretical related variables, chlorophyll-*a* and DOC were the best predictors. When also correlated variables were selected, the best model had chlorophyll-*a* and K_a440 as predictor variables.

Table 2.6: Multiple linear regression models

K_d	relation	r^2
K_dUVB	1.23 + 0.475*AW + 2.156*DOC	0.28
K_dUVB	1.94 + 0.0342*Chl-*a* + 0.90*K_a300	0.76
K_dUVA	1.97 + 0.0263*Chl-*a* + 1.10*K_a365	0.86
K_dPAR	1.58 + 0.0119*Chl-*a* + 0.063*DOC	0.28
K_dPAR	0.61 + 0.0123*Chl-*a* + 0.23*K_a380	0.88

The explaining power of the multiple linear models with the theoretically related variables is below the r^2 values of the power functions (Table 2.5). Comparing the results from the power functions (Table 2.5), and the multiple linear regressions (Table 2.6), DOC concentration seems to be the most important variable for K_dUVB. This is in agreement with

other field studies. For K_dUVA and K_dPAR chlorophyll-*a* concentration seems to be the most important variable. However, the predictive power is much lower than obtained in similar field studies as described in § 2.1.3.

When also correlated variables as absorption are taken into account, the predictive power increases considerably. With chlorophyll-*a* concentration and absorption coefficient at specific wavelengths, the K_d value can be predicted with over 75% of the variation explained.

When one is only interested in a reliable prediction of K_d without theoretical background, absorption coefficients can give a good prediction of K_d values (Table 2.7). This is especially true for K_dUVB (r^2=0.75) and K_dUVA (r^2=0.81). For K_dPAR the relation with absorption is less strong (r^2=0.55). This indicates that scattering processes play a larger role for longer wavelengths. The K_d values for PAR, UVA and UVB are correlated (see Table 2.4). With simple linear regression, a good prediction for K_dUVB or K_dUVA can also be obtained from K_dPAR. When using inverse K_d values, the relations have even higher predictive power.

Table 2.7: Pragmatic predictions of K_d.

K_d	relation	r^2
K_dUVB	0.38 + 2.03*K_dUVA	0.82
KdUVB	3.51 + 0.91*K_a300	0.75
K_dUVB	4.73 + 7.71*K_dPAR	0.47
1/K_dUVB	0.0078 + 0.4194*(1/K_dUVA)	0.92
1/K_dUVB	0.0058 + 0.1028*(1/K_dPAR)	0.82
KdUVA	2.14 + 0.42*K_a300	0.81
K_dUVA	0.48 + 4.38*K_dPAR	0.80
1/K_dUVA	-0.0055 + 0.2469*(1/K_dPAR)	0.90
KdPAR	1.22 + 0.24*K_a380	0.55

2.3.2 Results laboratory experiments

The linear relations between K_d and the measured variables are given in Appendix 2.2. Three-dimensional plots of wavelength and concentration *vs.* K_d are given in Appendix 2.3. Fig. 2.3 gives the scatter plots for dry weight or chlorophyll-*a* *vs.* K_d with linear regression lines. The slopes of the regression line of clay are significantly different from the slopes of all three phytoplankton species. The regression line for chlorophyll-*a* of *Scenedesmus* for K_dPAR, K_dUVA and K_dUVB, is significantly different from the other two phytoplankton species.

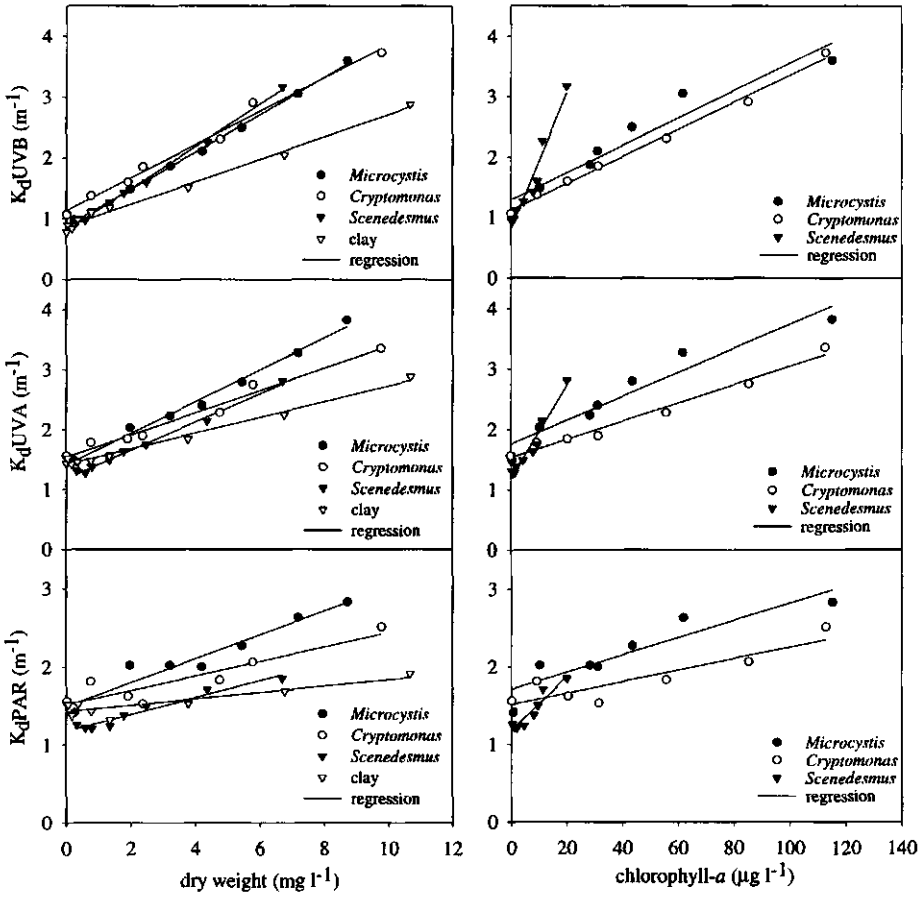

Figure 2.3: Scatter plots of dry weight (left side panels) and chlorophyll-*a* (right side panels) *versus* K_dUVB (upper panel), K_dUVA (middle panel) and K_dPAR (lower panel). Linear regression lines are all significant.

2.3.3 Development of a model

The attenuation of light in natural waters is caused by four components (see § 2.1.2). The relations between these components and K_d values that were obtained in the laboratory experiments are used in a model to predict K_d values measured in the field study. The following relations, an addition of the laboratory derived relations, are used in the model:

$$K_dUVB = 0.949 + 0.604*K_a250 + 0.023*Chl\text{-}a + 0.204*AW$$
$$K_dUVA = 1.410 + 1.058*K_a365 + 0.020*Chl\text{-}a + 0.146*AW$$
$$K_dPAR = 1.406 + 0.407*K_a440 + 0.009*Chl\text{-}a + 0.043*AW$$

The intercept of each relation is the average intercept of the clay and phytoplankton regressions, and represents the attenuation by water itself. The factor with K_a was obtained in the humic acid experiment and represents the attenuation by dissolved humic substances. The factor with chlorophyll-*a* was the average from the *Microcystis* and *Cryptomonas* regressions and represents the attenuation by organic particulate matter. The factor with ash weight was obtained in the suspended clay experiment and represents the attenuation by inorganic particulate matter.

K_a is chosen instead of DOC content to describe the influence of dissolved humic substances (or colour), because DOC is a quantitative measure that may have different colour properties. K_a at specific wavelengths is both quantitative and qualitative, and therefore more informative. Chlorophyll-*a* is chosen instead of ash-free dry weight to describe the particulate organic matter, because it resulted in a better prediction.

The relations are used to predict the K_d values from the field study with the measured ash weight, chlorophyll-*a* and absorption coefficient. Fig. 2.4 gives the comparison of predicted K_d and observed K_d values. A linear regression is fitted through the origin to estimate the accuracy of the predicted K_d. For K_dUVB, the prediction underestimates the observed value, with an r^2 of 0.80. The prediction for K_dUVA is accurate, with an r^2 of 0.85. The K_dPAR is underestimated by the prediction, with an r^2 of 0.76.

The relations obtained in the laboratory result in predictions of K_d values that are in agreement with the observed attenuation properties. Even though the conditions in the laboratory and range of concentrations were different from the field conditions.

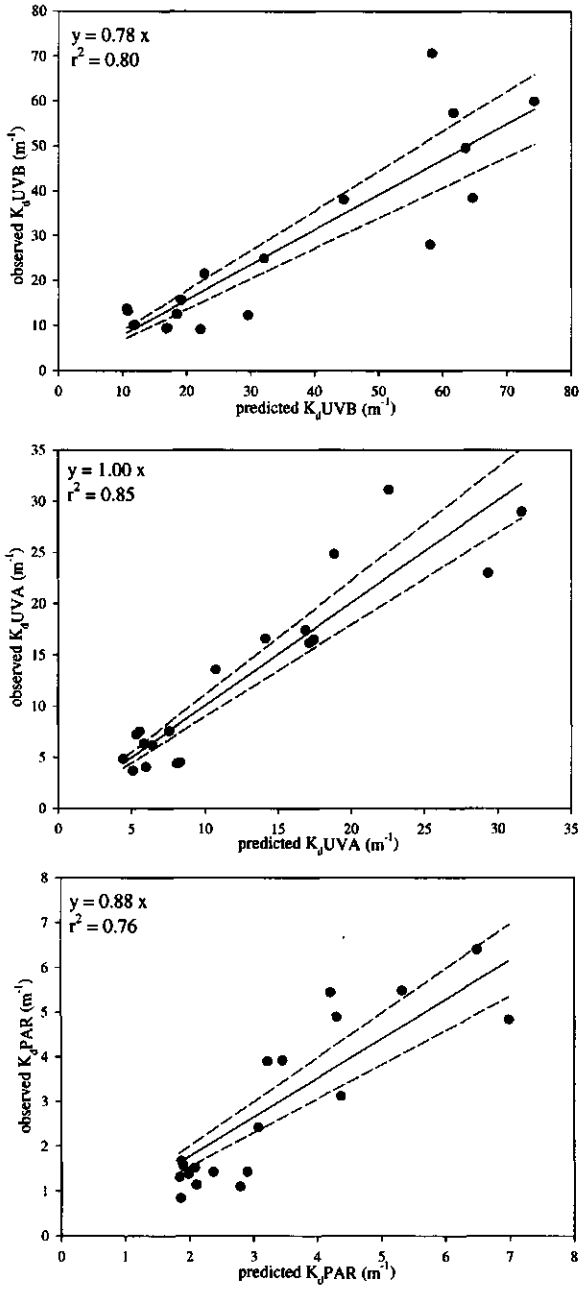

Figure 2.4: Predicted versus observed K_dUVB (upper panel), K_dUVA (middle panel), and K_dPAR (lower panel). Linear regression lines with 95% confidence intervals.

2.4 DISCUSSION

In the 19 systems studied the attenuation coefficients vary considerably. The K_dPAR values vary from 0.8 to 6.4 (m^{-1}), with an average value of 2.8 (m^{-1}). These values are well in the range found in other Dutch studies (Gons *et al.* 1998, Malthus & Dekker 1990). K_dUVA values in this study vary from 3.7 to 31.1 (m^{-1}), with an average value of 12.9 (m^{-1}). K_dUVB values vary from 9.1 to 70.7 (m^{-1}), with an average value of 26.5 (m^{-1}).

Compared with other studies, the K_dUVB measured in aquatic systems in the Netherlands is quite high. Scully & Lean (1994) find an average K_dUVB value of 6.54 (m^{-1}) (range 0.62 to 21.73 m^{-1}). Morris *et al.* (1995) find an average value for K_d305 of 10.15 (m^{-1}) (range 0.17 to 132.9 m^{-1}). Laurion *et al.* (1997) find an average K_d305 value of 9.28 (m^{-1}) (range 0.92 to 43.17 m^{-1}). Hodoki & Watanabe (1998) find an average K_dUVB value of 6.84 (m^{-1}) (range 1.96 to 15.4 m^{-1}).

The high K_dUVB value found in Dutch systems is either because of high humic acids (DOC) concentration, or because of high algal biomass. UVB radiation does not penetrate deep in those systems, less than 0.1 m. However, some of these systems with high K_dUVB are quite shallow, the maximum depth of 2 of the ditches was only 0.2 m. The impact of the UVB irradiation may still be considerable in such shallow systems. The overall impact of UVB on a system does not only depend on the attenuation of UVB, but also on depth (mean and maximum) and mixing processes (mixing depth). There is only one other (preliminary) study in the Netherlands measuring K_dUVB with spectroradiometer measurements. In Lake Veerse Meer (located in the south-west of the Netherlands, measurements made in May 1998), a K_dUVB of 19.8, K_dUVA of 7.8 and K_dPAR of 1.2 (m^{-1}) was measured (Brouwer unpubl.). These K_d values are well in the range of values measured in this study.

The empirical relations between K_dUVB with water and seston variables are not as strong as known from other field studies. An explanation may be the variation between the studied systems. This study does not reject the concept that DOC is the most important factor in attenuating UVB radiation. However, in eutrophic systems other factors as total organic carbon and chlorophyll-*a* concentration are also important. This is comparable with the results from eutrophic systems in Japan (Hodoki & Watanabe 1998). The best pragmatic relation of K_dUVB is an inverse linear relationship with K_dUVA.

The laboratory experiments showed significant linear relations between particulate matter and K_d, and between absorption and K_d. The slopes of the regression lines of chlorophyll-*a* versus K_d were significantly different between the phytoplankton species. The ratio chlorophyll-*a* to dry weight was also different between the species. This is a caution that chlorophyll-*a* concentration may not be the most accurate prediction of phytoplankton biomass. However, the relation obtained in the lab with only 3 phytoplankton species was applicable to the field data set with a phytoplankton population.

The laboratory experiment with artificial humic acid resulted in significant linear relations between humic acids and K_d. Humic acids are highly heterogeneous, and its

characteristics may vary from system to system, and within a season. Consequently, attenuation properties, especially K_dUVB, may be different at similar humic acids (or DOC) concentrations (see § 2.1.3). The linear relation obtained in the laboratory experiment (with only 1 humic acid) between K_a and K_d is applicable to the field data set (with unknown humic acid composition). Comparing the linear relations obtained in the monitoring (Table 2.6), and in the laboratory humic acids experiment (Appendix 2.2), shows that the K_d versus K_a relations are comparable. The slopes of the linear regression for K_dUVB and for K_dPAR obtained in the field and in the laboratory experiment are not significantly different. Only the slope for K_dUVA was significantly different ($p<0.01$).

The additive linear model developed from the laboratory-derived relations was a good predictor for the K_d values measured in this field study. The addition of linear relations has a theoretical basis. Although the laboratory experiments were simplifications of the natural situation, it did not limit the predictive power of the model.

2.5 CONCLUSIONS

The attenuation properties of Dutch systems vary. In most systems the penetration of UVB radiation is limited to the upper decimetres. Lake Maarsseveen is the clearest system in this study, with K_dUVB of 9.1 (m^{-1}). This corresponds to a 1% UVB penetration depth of 0.5 m. The relations obtained in the laboratory experiments can be used to give a good prediction of K_d values, based on 3 measurements (chlorophyll-*a*, ash weight, and absorption).

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APPENDIX 2.1: RESULTS FIELD MEASUREMENTS 1998

A: T is temperature, Cond. is conductivity, Alkal. is alkalinity, DW is dry weight, AW is ash weight, AFDW is ash free dry weight, Chl-*a* is chlorophyll-*a*, mv is missing value.

Location	Date	O_2 mg l^{-1}	T °C	pH -	Cond. µS cm^{-1}	Alkal. meq l^{-1}	DW mg l^{-1}	AFDW mg l^{-1}	AW mg l^{-1}	Chl-*a* µg l^{-1}
Waal en Burgerdijk	19 May	9.0	21.4	5.7	195	0.09	2.5	1.2	1.3	4.5
Lake Oude Schans	18 May	10.8	16.8	8.1	7920	2.72	9.7	4.5	5.2	13.1
Ditch Oude Schans	18 May	9.7	20.2	7.9	37700	4.38	49.6	13.6	36.0	27.5
Ditch Hendrikpolder	19 May	27.6	22.5	8.5	31100	6.00	75.4	37.1	38.3	197.9
Wadden Sea	19 May	mv	mv	8.3	42200	2.77	35.9	8.6	27.3	12.1
Lake Zwemlust	23 April	14.1	16.0	10.1	111	0.67	28.7	25.5	3.2	35.4
Lake Loosdrecht	22 July	7.9	22.3	8.2	374	2.12	22.8	19.4	3.4	43.3
Lake Maarsseveen	22 July	8.9	19.8	8.5	449	3.21	3.9	2.0	1.9	5.0
Sinderhoeve, ditch 7	13 May	14.1	25.7	9.1	138	1.25	4.5	3.8	0.7	60.0
Sinderhoeve, ditch 9	13 May	12.7	25.3	9.1	141	1.20	3.5	2.3	1.2	3.9
Sinderhoeve, ditch 13	13 May	5.0	26.2	6.9	122	3.01	16.2	6.4	9.8	23.1
Veenkampen, ditch I	29 April	9.9	15.2	9.2	416	3.35	11.4	10.1	1.3	3.8
Veenkampen, ditch II	29 April	7.0	15.5	7.7	168	1.65	4.0	1.6	2.4	28.9
Pond Dreijen	4 May	16.5	15.9	7.2	410	4.44	12.0	7.2	4.8	225.6
Argo harbour	11 May	mv	mv	8.1	769	1.39	4.3	2.0	2.3	9.1
Wageningen canal	15 May	15.1	19.3	8.9	535	1.07	37.9	23.9	14.0	279.1
Grift canal	22 June	7.9	18.1	7.3	679	4.57	6.6	1.9	4.7	4.8
Lake Blauwe Kamer	11 May	mv	mv	9.1	327	1.13	6.1	1.6	4.6	3.5
Uiversnest fen	27 July	6.0	25.1	4.2	53	0.08	8.4	8.1	0.3	16.3

B: Turb. is turbidity, NTU is nephalometric turbidity units, DOC is dissolved organic carbon, DIC is dissolved inorganic carbon, TOC is total organic carbon, TIC is total inorganic carbon.

Location	Turb. NTU	DOC mg l^{-1}	DIC mg l^{-1}	TOC mg l^{-1}	TIC mg l^{-1}	total N mgN l^{-1}	total P mgP l^{-1}	PO_4^{3-} µgP l^{-1}	NH_4^+ mgN l^{-1}	NO_{2+3}^- mgN l^{-1}
Waal en Burgerdijk	3	9.8	0.5	7.8	0.3	0.48	0.19	11.69	0.03	0.02
Lake Oude Schans	6	21.8	30.8	14.0	24.5	1.00	0.15	48.72	0.12	0.48
Ditch Oude Schans	7	10.2	39.3	7.9	41.4	0.66	0.28	438.77	0.30	0.41
Ditch Hendrikpolder	14	13.8	42.8	15.0	48.0	1.12	0.07	194.23	0.31	0.54
Wadden Sea	2	5.8	21.9	4.6	24.6	0.60	0.07	263.26	0.43	0.54
Lake Zwemlust	16	15.9	22.5	18.3	24.0	1.39	0.09	6.77	0.05	0.03
Lake Loosdrecht	14	13.0	21.2	17.7	21.6	1.78	0.02	2.90	0.01	0.01
Lake Maarsseveen	2	8.9	29.1	7.6	31.4	1.07	0.02	10.21	0.03	0.21
Sinderhoeve, ditch 7	6	9.8	9.2	4.2	11.3	0.90	0.11	23.81	0.17	0.04
Sinderhoeve, ditch 9	3	4.8	10.4	3.9	11.3	0.56	0.03	10.94	0.39	0.04
Sinderhoeve, ditch 13	8	12.0	10.2	15.0	11.7	1.59	0.41	124.67	0.46	0.56
Veenkampen, ditch I	5	2.2	15.4	7.4	14.0	1.77	0.18	36.23	0.04	0.03
Veenkampen, ditch II	3	10.9	40.2	11.6	41.3	0.39	0.04	48.51	0.75	0.23
Pond Dreijen	3	12.3	4.2	14.5	4.0	0.99	0.06	26.66	0.05	0.04
Argo harbour	4	5.9	23.0	3.6	26.5	3.09	0.10	32.35	0.06	2.91
Wageningen canal	22	10.5	22.4	12.1	25.9	3.19	0.19	97.87	0.26	1.93
Grift canal	5	6.5	33.4	3.7	32.4	4.60	0.06	11.64	0.07	4.60
Lake Blauwe Kamer	6	6.4	11.2	7.1	11.0	0.85	0.06	10.54	0.07	0.09
Uiversnest fen	3	6.5	0.2	6.4	0.3	0.68	0.03	21.75	0.09	0.08

C: Absorption coefficients; f indicates filtered sample, t indicates total sample, mv is missing value.

Location	K_a250f m^{-1}	K_a300f m^{-1}	K_a365f m^{-1}	K_a380f m^{-1}	K_a440f m^{-1}	K_a250t m^{-1}	K_a300t m^{-1}	K_a365t m^{-1}	K_a380t m^{-1}	K_a440t m^{-1}
Waal en Burgerdijk	12.8	5.7	1.7	1.3	0.4	12.8	6.1	2.3	1.8	0.8
Lake Oude Schans	44.0	21.3	6.1	4.5	1.4	46.2	23.0	7.6	5.9	2.5
Ditch Oude Schans	25.6	12.8	44.2	3.3	1.2	33.7	18.8	8.0	6.6	3.4
Ditch Hendrikpolder	43.8	22.9	7.8	6.0	2.2	51.0	29.1	13.1	11.2	7.1
Wadden Sea	7.3	3.5	1.0	0.8	0.2	9.6	5.1	2.1	1.8	1.2
Lake Zwemlust	19.5	8.1	2.1	1.6	mv	24.1	11.4	4.4	3.7	mv
Lake Loosdrecht	39.9	18.9	5.8	4.4	1.6	51.0	28.1	13.5	11.9	8.8
Lake Maarsseveen	14.9	5.8	1.4	0.9	0.4	16.1	6.7	2.1	1.6	0.9
Sinderhoeve, ditch 7	5.9	3.2	1.2	1.0	0.4	10.2	6.1	3.1	2.6	1.3
Sinderhoeve, ditch 9	6.8	3.8	1.5	1.2	0.5	11.0	6.8	3.6	3.0	1.6
Sinderhoeve, ditch 13	39.5	21.8	7.9	6.0	2.3	68.8	47.2	19.4	14.9	6.2
Veenkampen, ditch I	7.6	4.3	1.8	1.4	0.7	9.2	5.5	2.5	2.1	1.0
Veenkampen, ditch II	42.9	21.4	6.8	5.0	1.5	54.6	30.4	11.7	9.0	3.6
Pond Dreijen	11.3	5.7	1.9	1.5	0.6	19.9	13.0	8.3	7.7	6.5
Argo harbour	10.9	5.9	2.6	2.2	1.3	10.6	5.7	2.8	2.3	1.5
Wageningen canal	15.7	7.8	2.4	1.8	0.8	26.1	16.1	9.1	8.4	6.5
Grift canal	7.0	3.4	0.9	0.6	0.2	9.9	5.8	2.8	2.4	1.6
Lake Blauwe Kamer	11.9	5.2	1.8	1.3	0.5	15.1	7.6	3.5	2.9	1.5
Uiversnest fen	45.5	27.7	12.3	10.0	5.3	51.3	32.0	15.2	12.6	7.2

D: b indicates bottom depth

Location	K_dUVB m^{-1}	K_dUVA m^{-1}	K_dPAR m^{-1}	Secchi m
Waal en Burgerdijk	15.6	6.4	1.6	0.5b
Lake Oude Schans	49.5	16.2	2.4	0.6b
Ditch Oude Schans	38.1	16.5	3.1	0.2b
Ditch Hendrikpolder	60.0	22.9	4.8	0.4
Wadden Sea	9.4	4.4	1.4	mv
Lake Zwemlust	12.3	7.6	1.7	0.9
Lake Loosdrecht	28.0	17.4	3.9	0.4
Lake Maarsseveen	9.1	3.7	0.8	1.3b
Sinderhoeve, ditch 7	13.8	7.5	1.4	0.5b
Sinderhoeve, ditch 9	13.2	7.2	1.4	0.5b
Sinderhoeve, ditch 13	70.7	31.1	5.4	0.2
Veenkampen, ditch I	10.1	4.0	1.1	0.7b
Veenkampen, ditch II	57.4	24.8	3.9	0.2b
Pond Dreijen	21.5	13.5	4.9	0.4
Argo harbour	9.3	4.5	1.1	0.4b
Wageningen canal	24.9	16.6	5.5	0.4
Grift canal	10.0	4.9	1.3	0.6b
Lake Blauwe Kamer	12.5	6.2	1.5	0.75b
Uiversnest fen	38.4	29.0	6.4	b

APPENDIX 2.2: RELATIONSHIPS BETWEEN WATER VARIABLES AND K_D FOR PAR, UVA AND UVB, OBTAINED IN THE LABORATORY EXPERIMENTS

A: Dry weight relations, differences in slopes of the regression lines was tested separately for K_dPAR, K_dUVA and K_dUV, according to Fowler & Cohen (1990). N=8 for *Scenedesmus*, n=7 for *Microcystis*, n=7 for *Cryptomonas*. Significant differences are indicated by small letters ($p<0.05$).

K_d	experiment	relation		r^2
K_dPAR	*Scenedesmus*	1.17 + 0.108*DW	ab	0.939
	Cryptomonas	1.45 + 0.093*DW	a	0.953
	Microcystis	1.34 + 0.153*DW	b	0.992
	clay	1.45 + 0.041*DW	c	0.833
K_dUVA	*Scenedesmus*	1.18 + 0.237*DW	a	0.992
	Cryptomonas	1.53 + 0.186*DW	b	0.971
	Microcystis	1.40 + 0.270*DW	a	0.997
	clay	1.41 + 0.132*DW	c	0.986
K_dUVB	*Scenedesmus*	0.79 + 0.348*DW	a	0.997
	Cryptomonas	1.13 + 0.274*DW	b	0.983
	Microcystis	0.86 + 0.307*DW	b	0.997
	clay	0.86 + 0.185*DW	c	0.990

B: Ash-free dry weight relations, differences in slopes of the regression lines was tested separately for K_dPAR, K_dUVA and K_dUV, according to Fowler & Cohen (1990). N=8 for *Scenedesmus*, n=7 for *Microcystis*, n=7 for *Cryptomonas*. Significant differences are indicated by small letters ($p<0.05$).

K_d	experiment	relation		r^2
K_dPAR	*Scenedesmus*	1.18 + 0.141*AFDW	a	0.773
	Cryptomonas	1.50 + 0.112*AFDW	a	0.638
	Microcystis	1.53 + 0.161*AFDW	b	0.894
K_dUVA	*Scenedesmus*	1.17 + 0.324*AFDW	a	0.916
	Cryptomonas	1.47 + 0.236*AFDW	a	0.892
	Microcystis	1.46 + 0.280*AFDW	a	0.964
K_dUVB	*Scenedesmus*	0.79 + 0.472*AFDW	a	0.897
	Cryptomonas	1.01 + 0.353*AFDW	a	0.948
	Microcystis	0.93 + 0.324*AFDW	a	0.981

C: Ash weight relations, n=9.

K_d	experiment	relation	r^2
K_dPAR	clay	1.46 + 0.043*AW	0.843
K_dUVA	clay	1.43 + 0.146*AW	0.988
K_dUVB	clay	0.88 + 0.204*AW	0.989

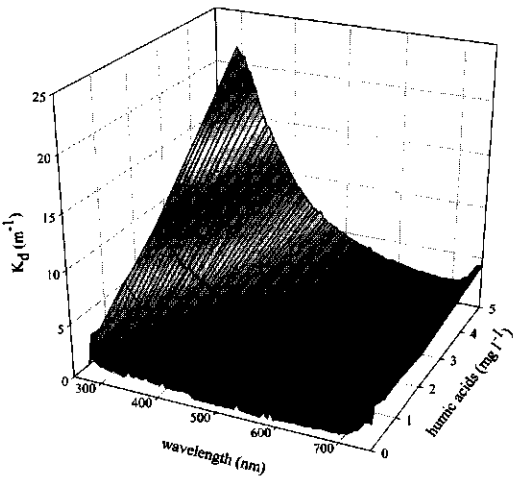
D: Chlorophyll-*a* relations, differences in slopes of the regression lines was tested separately for K_dPAR, K_dUVA and K_dUV, according to Fowler & Cohen (1990). N=8 for *Scenedesmus*, n=7 for *Microcystis*, n=7 for *Cryptomonas*. Significant differences are indicated by small letters ($p<0.05$).

K_d	experiment	relation		r^2
K_dPAR	*Scenedesmus*	1.17 + 0.035*Chl-*a*	a	0.884
	Cryptomonas	1.51 + 0.007*Chl-*a*	b	0.758
	Microcystis	1.71 + 0.011*Chl-*a*	b	0.797
K_dUVA	*Scenedesmus*	1.18 + 0.077*Chl-*a*	a	0.949
	Cryptomonas	1.53 + 0.015*Chl-*a*	b	0.973
	Microcystis	1.75 + 0.020*Chl-*a*	b	0.918
K_dUVB	*Scenedesmus*	0.79 + 0.113*Chl-*a*	a	0.938
	Cryptomonas	1.11 + 0.022*Chl-*a*	b	0.993
	Microcystis	1.28 + 0.023*Chl-*a*	b	0.904

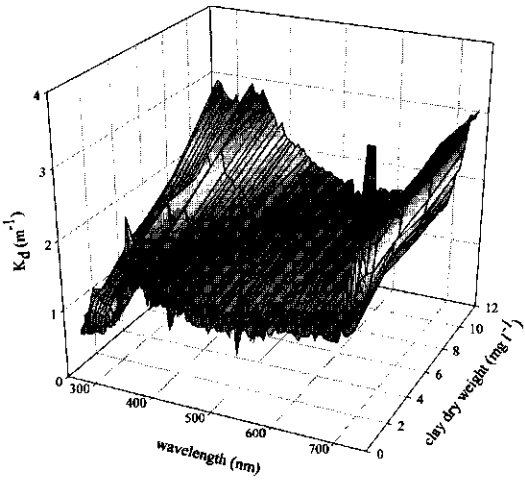
E: Results humic acids experiment, HA is the added humic acid concentration in the experiment (n=7 for K_dUVB, n=9 for K_dUVA, n=10 for K_dPAR).

Relation K_dPAR	r^2	relation K_dUVA	r^2	relation K_dUVB	r^2
-1.85 + 1.469*DOC	0.977	-13.82 + 7.358*DOC	0.954	-33.04 + 14.619*DOC	0.764
0.33 + 1.205*TOC	0.972	-2.59 + 6.016*TOC	0.955	-17.73 + 13.986*TOC	0.955
1.31 + 0.076*K_a250	0.986	0.90 + 0.414*K_a250	0.992	2.17 + 0.604*K_a250	0.980
1.40 + 0.104*K_a300	0.985	1.40 + 0.566*K_a300	0.993	2.92 + 0.824*K_a300	0.978
1.53 + 0.195*K_a365	0.984	2.05 + 1.058*K_a365	0.993	3.88 + 1.540*K_a365	0.977
1.52 + 0.228*K_a380	0.984	2.04 + 1.235*K_a380	0.993	3.87 + 1.797*K_a380	0.977
1.57 + 0.407*K_a440	0.983	2.31 + 2.215*K_a440	0.993	4.27 + 3.221*K_a440	0.976
1.48 + 0.377*HA	0.982	1.76 + 2.051*HA	0.995	3.51 + 0.377*HA	0.982

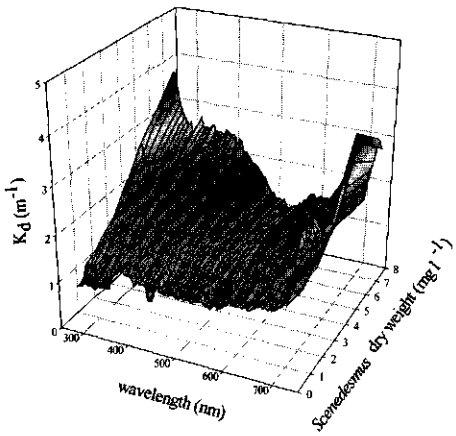
APPENDIX 2.3: VALUES OF K_D OBTAINED IN THE LABORATORY EXPERIMENTS AS FUNCTION OF WAVELENGTH AND CONCENTRATION



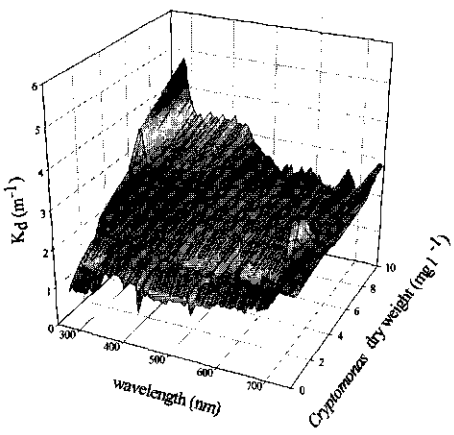
Humic acids attenuation



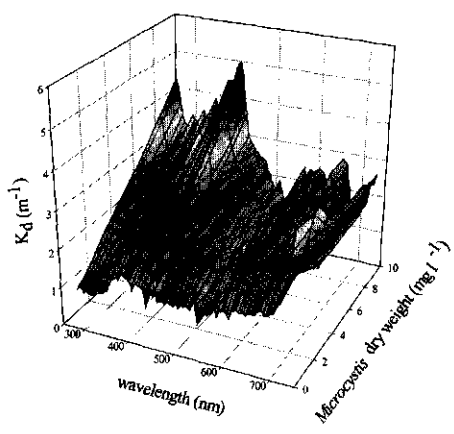
Clay attenuation



Scenedesmus attenuation



Cryptomonas attenuation



Microcystis attenuation

CHAPTER 3

UV-INDUCED CHANGES IN PHYTOPLANKTON CELLS

This chapter is based on:
Hessen D.O., De Lange H.J. & Van Donk E. (1997) UV-induced changes in phytoplankton cells and its effects on grazers. *Freshwater Biology*, **38**, 513-524.

De wereld is een speeltoneel,
Elk speelt zijn rol en krijgt zijn deel.

(Joost van den Vondel - Op het toneel)

For the drama of life is like a puppet show in which stage, scenery, actors and all are all made of the same stuff. The players, indeed, 'have their exits and their entrances,' but the exit is by way of translation into the substance of the stage; and each entrance is a transformation scene. So stage and players are bound together in the close partnership of an intimate comedy; and if we would catch the spirit of the piece, our attention must not all be absorbed in the characters alone, but must be extended also to the scene, of which they are born, on which they play their part, and with which, in a little while, they merge again.

(Alfred J. Lotka - Elements of Physical Biology)

3.1 INTRODUCTION

In aquatic environments with low primary production and low input of allochthonous carbon, shortwave radiation may penetrate to significant depths (Chapter 2). In these ecosystems, even present day UVA and UVB may be major ecological parameters affecting nutrient flow, biogeochemical cycles and food web structure (Karentz *et al.* 1994, Siebeck *et al.* 1994). Upward trends in UVB radiation owing to stratospheric ozone depletion (Kerr & McElroy 1993) may add to these effects. A major effect of UV radiation is decreased primary production in aquatic ecosystems (El Sayed 1988, Lorenzen 1979, Worrest *et al.* 1978), and there are concerns that this will reduce not only algal carbon fixation, but also the carbon supply for higher trophic levels (Häder 1993, Worrest *et al.* 1978).

In addition to the quantitative food web responses, there are a number of qualitative effects of UV-radiation on primary producers. There is an intimate interplay between light, nutrients and cell biochemistry in phytoplankton. Phytoplankton cells exposed to UV radiation undergo a series of physiological changes that affect both cell-volumes and intracellular morphology as well as biochemical pathways and products. These may in part rely on changes in photosynthesis or macromolecules associated with photosystems I and II, but may also be more direct effects of direct damage to other cell constituents. Among the most prominent changes are genomic damage that may affect a variety of cell processes and transcript products (Karentz *et al.* 1991). There are also reports of reduced uptake of inorganic nutrients (Döhler & Alt 1989, Döhler & Biermann 1987, 1994, Hessen *et al.* 1995), loss or immobilization of flagella (Häder 1993, Hessen *et al.* 1995), and increased cell volumes (Karentz *et al.* 1991, Behrenfeld *et al.* 1992, Van Donk & Hessen 1995, Buma *et al.* 1995) with concomitant accumulation of intracellular starch and lipids. The fatty acid (FA) composition is also affected (Goes *et al.* 1994, Wang & Chai 1994). These data indicate that long-chained poly-unsaturated fatty acids, notably ω3 FA, are particularly susceptible to UVA and UVB radiation. These FA have proven essential to a number of heterotrophs.

From the various cellular and biochemical changes, a set of hypothetical effects on zooplankton grazers may be deduced. Each of the affected properties of phytoplankton cells will influence digestibility and nutritional quality for the herbivores. Thus UV-radiation will not only influence secondary production owing to reduced primary productivity, but also due to phytoplankton community changes and cellular changes (Fig. 3.1).

This chapter summarizes current knowledge on UV-induced changes in phytoplankton focusing on qualitative effects, combined with new results on UV effects on the biochemical composition of phytoplankton. New links between the field of photobiology and more conventional ecology are posed, mediated by changes in food quality for planktonic grazers.

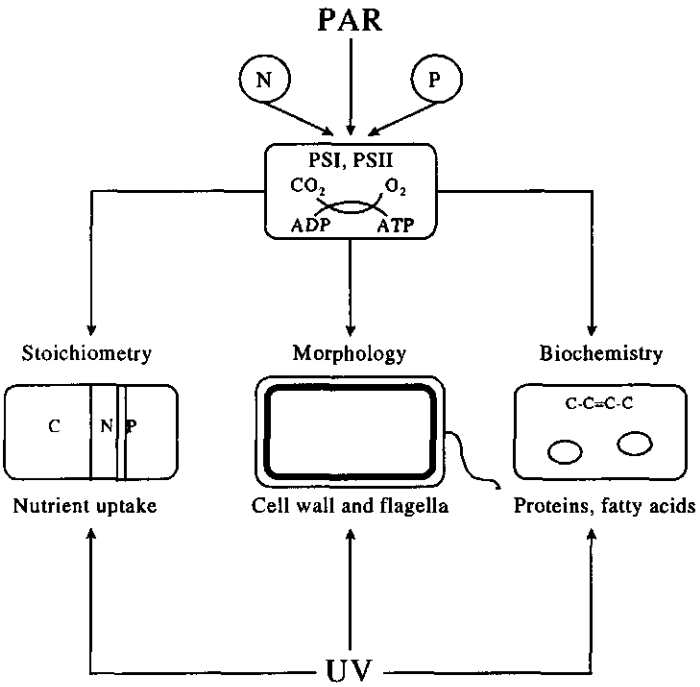


Figure 3.1: Conceptual outline of potential light/nutrient factors affecting phytoplankton cell morphology and biochemistry. On top, the photosynthetically active radiation (PAR, 400-700 nm) and nutrients that in combination influences cell stoichiometry, size, shape and cell wall properties as well as the basic biochemistry and macromolecules of the cell. From below, the harmful, short-wave light (UV, <400 nm) that has a negative influence on most of the same parameters, in particularly with regard to food quality for zooplankton.

3.2 Effects on Nutrient Uptake and Production

The effects of UVB radiation on photosynthesis has been the main focus of a number of reports demonstrating decreased gross and net primary production under UVB exposure. Since the trapping of light by chlorophyll causes formation of excited states, it is likely that singlet oxygen would be produced by UVB exposure. This has profound effects on the electron transport chain, and could block the photophosphorylation. The main sites of damage are the oxidizing side of PSII. The D1 reaction centre protein appears to be a specific target for UVB induced photoinhibition in photosystem II (Bornman 1989, Cullen & Neale 1994). Excluding UVB radiation from natural sunlight reduced the rate of D1 protein degradation by as much as 30% (Greenberg *et al.* 1989). Although plant cells have various methods to cope with intracellular oxidative stress, the resulting decrease in ATP levels and concurrently reduced CO_2-fixation seem to be common properties of UV-stressed cells.

Even present day UV radiation causes considerable stress for aquatic algae (Calkins &

Thordardottir 1980, El Sayed 1988, Lorenzen 1979, Steemann-Nielsen 1964, Worrest *et al.* 1978). Thus, screening off UVB invariably increases carbon fixation and net photosynthesis in upper water layers. In a study of natural phytoplankton assemblages in Antarctica, the reduction of natural UV, additions of artificial UV or measurements outside and inside the "ozone hole" resulted in up to a fourfold decrease in primary production when doubling the UV-dose (El Sayed *et al.* 1990) and about a 20% decrease in primary production in shallow layers caused by ozone anomalies (Smith *et al.* 1992). Moeller (1994) found up to 95 % UV-mediated reduction of photosynthesis in the upper layers of a clear lake, with UVA contributing strongly to the effects. He concluded that nutrient deficiency could have precluded effective photoadaptation. Gala & Giesy (1991) found reduced primary production in Lake Michigan down to 6 m depth, where UVB intensities were only 1% of surface values. They estimated a 13% loss in primary production over the total water column that could be directly linked to present UVB.

Various phytoplankton taxa and species have different susceptibilities to UV-radiation. For a diatom-dominated community studied in Antarctica, Boucher *et al.* (1995) found that exposure to very low ambient UVB in early spring resulted in a 34% reduction in daily averaged C-fixation. UVA and UVB apparently contributed equally to this inhibition. Some species appeared to have very low UVB sensitivity, probably owing to an UV-absorbing outer sheath. These changes at the base of the food web would undoubtedly affect the consumers as well, and thus the indirect effects on zooplankton grazers would not only relate to reduced primary production. Different susceptibility among algal species and size classes (*e.g.* Karentz *et al.* 1991) could induce changes at the community level that would add to the quantitative effects. A variety of changes in food quality could in fact be superimposed on these quantitative effects.

UV-induced effects on nutrient uptake and productivity would clearly bridge the area of photobiology and nutrient quality. There are, however few studies on direct UV-effects on nutrient uptake. Enzymes involved in the nitrogen metabolism of phytoplankton are affected by UV-radiation, and reduced uptake of ammonia and nitrate under ambient UV-radiation have been demonstrated for a number of marine diatoms (Döhler & Alt 1989, Döhler & Biermann 1987, Döhler & Kugel 1994). Interestingly, even the highly light adapted green algae *Haematococcus lacustris* and its activated aplanospores experienced reduced uptake of nitrogen under low additional doses of artificial UVB (Braune & Döhler 1994). The uptake of ^{15}N was reduced both during and after irradiation. Nutrient starved cells were less susceptible to UVB radiation than actively growing cells. A more subtle pattern was detected when applying artificial UVB together with monochromatic light (Döhler & Kugel 1994). Under blue and green light, the uptake of ^{15}N-ammonium in the marine diatom *Lithodesium variable* was stimulated by UVB, while under red and white light, UVB decreased the uptake of N. Conversely, UVB reduced chlorophyll levels more under blue and green light relative to red and white. This indicates a variable interplay between UVB and different qualities of PAR light.

Thus there are indications that uptake of both nitrate and ammonium is inhibited by

UV-radiation, and such effects may be anticipated also for P-uptake. Hessen *et al.* (1995) found an initial stimulation of P-uptake in the green algae *Chlamydomonas reinhardtii* at low UV-doses (1.2-3.6 kJ m^{-2} UV_{312}), but a severe inhibition at doses > 3.6 kJ m^{-2}. They reported most pronounced effects in P-starved cells. The initial stimulation was supposed to reflect a stress response with increased P-demands for photorepair. Reduced ATP-content in UV-stressed phytoplankton cells (Vosjan *et al.* 1990) also points towards effects on the intracellular P-metabolism. On the contrary, Döhler & Biermann (1994) found no effect of UVB radiation on ATP-content in a marine diatom.

There may be a multitude of reasons for reduced nutrient uptake, besides enzymatic damage. Membrane damage, flagellar inactivation and changed cell wall properties (see below) may put constraints on uptake capacities. Inhibition of photophosphorylation, DNA damage and protein synthesis would also imply reduced nutrient demands. It should also be stressed that there are inherent feedback mechanisms, as nutrient limitation in turn will affect the general UVB sensitivity (Lesser *et al.* 1994). The effects of short wave radiation on nutrient uptake and nutrient cycling is a matter of major interest, yet with few conclusive data so far.

Reduced nutrient uptake, reduced protein synthesis as well as intracellular accumulation of carbon-rich compounds will invariably skew the C:N:P-ratio. There are no direct data on UV effects on cell stoichiometry of algae, however. A preliminary study of the fresh-water species *Selenastrum capricornutum, Rhodomonas lacustris* and *Cyclotella* sp. did however show decreased, rather than increased C:P and N:P-ratios (Fig. 3.2) after two days of UV_{312} radiation. At low doses there may be an initial increase in P-uptake for photorepair purposes, that is overruled by membrane damage and decreased P-uptake at higher doses (*cf.* Hessen *et al.* 1995).

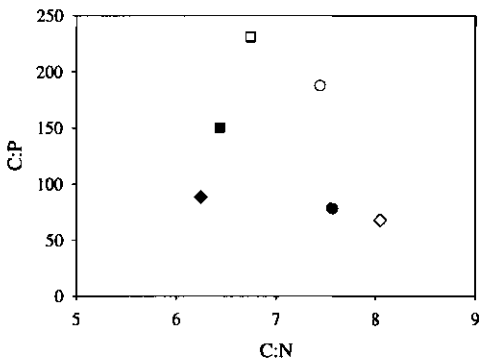


Figure 3.2: C:P and C:N weight ratio for *Selenastrum capricornutum* (squares), *Rhodomonas lacustris* (circles) and *Cyclotella* sp. (diamonds) exposed for two days with 4 h UVB (peak intensity at 312 nm), 14 kJ m^{-2} d^{-1} and continuous PAR-light, 70 µmol m^{-2} s^{-1}. Filled symbols are exposed cells (UVB + PAR), open symbols are controls (PAR only).

3.3 Effects on Carbohydrates, Proteins, Lipids, Amino-Acids and Fatty Acids

The observations on direct effects of UVB on intracellular biochemistry in phytoplankton are sparse, but give circumstantial evidence of effects that could be accredited to reduced uptake of inorganic nutrients. To the extent that UVB reduces nutrient uptake in cells, it will also have profound effects on cell biochemistry. These effects will also be manifest by direct effects on DNA, gene expression and the overall cellular machinery. Nutrient deficiency affects phytoplankton in numerous ways. A general pattern is intracellular accumulation of carbohydrates, notably as starch. Lipids also tend to accumulate while protein content decreases (Demadariaga & Joint 1992, Harrison *et al.* 1990, Shifrin & Chisholm 1981). Phospholipids tend to decrease both in absolute and relative terms (Lombardi & Wangersky 1991). As for a number of biochemical responses to both nutrient starvation and UVB stress, it must be stressed that responses may be highly species-specific. Reitan *et al.* (1994) noted increased lipid levels upon P-starvation in a Bacillariophyceae and a Prymnesiophyceae species, whereas specific lipid content decreased in two green flagellates. While phytoplankton cells in culture usually show an intimate linkage between availability of inorganic nutrient, production and lipid status, this is not always apparent in natural communities. Wainman & Lean (1992) and Wainman *et al.* (1993) studied lipid synthesis in a number of lakes, and concluded that nutrient deficiency does not govern lipid synthesis in natural aquatic ecosystems, but rather that physical factors such as light and daylength are the major determinants.

While some of the predominant nutrient-deficiency effects are seen also under UV-stress, this is by no means universal. Yet, in spite of reduced photosynthesis and CO_2-fixation via the Calvin cycle, carbon-rich compounds accumulate within the UV-stressed cells. Goes *et al.* (1996) studied natural marine phytoplankton assemblages and found that exposure to UVR resulted in a decrease of storage carbohydrates, and an increase in the structural or cell wall carbohydrates. The overall carbohydrate content had decreased under UV radiation. The increase in structural carbohydrates is in agreement with observed increased cell wall thickness in UV-stressed *Selenastrum* (see § 3.5, Van Donk & Hessen 1995).

There are apparent trade-offs between dose and the cell protein response. Buma *et al.* (1995) reported a strong increase (up to five-fold) in cell specific protein content at low and moderate UV-doses, but a reduction at higher doses. Veen *et al.* (1997) reported increased cell protein under UV-exposure. Strid *et al.* (1994) reviews early effects and responses of low UV-B doses on plants, and concludes that a general decrease in protein synthesis, and notably, a decrease of mRNA transcripts for proteins involved in chloroplasts and photosynthesis, are among the very early events of UVB damage. Protein synthesis in procaryotes also appears sensitive to UVB (El Adhami *et al.* 1994). In general, delayed cell division would induce the accumulation of various primary and secondary photoproducts, including protein, while on the other hand P-deficiency could reduce RNA and slow down the transcription process. Total

lipids may show contrasting patterns, but in general the synthesis of a number of essential fatty acids may be reduced by UVB, inducing also decreased levels of total lipids (Goes *et al.* 1994, Chapter 5). Skerratt *et al.* (1998) examined the UVB effects on 3 Antarctic phytoplankton species. UVB treatments induced species-specific changes in lipid class profiles. UVB induced a reduction of lipid content in *Odontella*. UVB induced an increase in cellular lipid content of *Chaetoceros*. Exposure of *Phaeocystis* to low UVB radiation resulted in a reduction of storage lipids and an increase in structural lipids, indicating enhanced cell growth and metabolism. High UVB radiation increased total lipid content, triacylglycerol and free fatty acid, indicating the colonial life stage of *Phaeocystis*.

In both laboratory and field studies, the allocation of photosynthates to lipid, protein and carbohydrates was affected by UVB radiation. However it was not possible to identify one aspect of photosynthate allocation as consistently diagnostic of UV stress because the results were species-specific (Arts & Rai 1997, Smith *et al.* 1998).

Effects of UV exposure on amino acid and fatty acid composition are subtle. P, N or Si limitation influences the amount and composition of phospholipids and free amino acids of various algae in different ways (Demadariaga & Joint 1992, Flynn *et al.* 1992, 1993, Harrison *et al.* 1990, Lombardi & Wangersky 1991). Demadariaga & Joint (1992) reported a six-fold increase in free amino-acids in *Pavlova lutheri*. Döhler & Biermann (1994) found a strong inhibitory effect of UVB on cell division, protein synthesis pigments and some fatty acids in *Ditylum brightwelli* during the light period, but no effect in the dark. They concluded that the cell cycle state was important for the effect of UVB.

Goes *et al.* (1995a, 1995b) found that UV radiation caused in marine phytoplankton a decline in the overall rate of carbon incorporated into amino acids and a reduction in the pool size of total cellular dissolved free amino acids. In contrast, UVR increased the absolute concentration of the intracellular dissolved free amino acid pool. They concluded that the UVR effects show similarity with phytoplankton synthesizing under nitrogen stress.

The evolution of photosynthesis and the subsequent accumulation of oxygen in the atmosphere over the next millions of year involves an interesting dichotomy. While this provided a UVC and partly a UVB shield owing to the establishment of a stratospheric ozone layer, it also provided an ever-present oxidative stress on all aerobic organisms. UV-radiation may strongly add to this ambient oxidative stress. It is a most powerful oxidant, and promotes the formation of an array of intracellular free radicals and other strong oxidants like peroxides (Halliwell & Gutteridge 1989). The joint action of high O_2-concentrations that frequently occur in surface waters, and UV-radiation could induce a severe stress situation. Rancidity is a well recognized problem for all fatty and oily compounds, and this lipid peroxidation is one of the most damaging effects of oxygen and UV on cells. The membranes of cells and organelles in particular continuously fight against the oxidative deterioration of polyunsaturated lipids. In contrast, fatty acids with zero, one or two double bonds are more resistant to oxidative attack. The peroxidation process can be initiated by any chemical species that abstracts a hydrogen atom from methylene. Hydroxyl radicals ($OH^{\cdot}$), superoxide radicals (O_2^{-}) or its protonated

form ($HO_2^{\cdot}$), or hydrogen peroxide (H_2O_2) are all capable of lipid peroxidiation. UV-radiation appears as one of the most powerful ways to induce cell peroxidation (Tyrrell 1991), as has been demonstrated for a number of higher plants exposed to both UVA (Joshi *et al.* 1994) and UVB (Saradhi *et al.* 1995). These oxidation processes invariably have detrimental effects on polyunsaturated fatty acids (PUFAs), and in fact this loss may be used as a direct measure on the lipid peroxidation process. Given the crucial role of fatty acid profiles for animal nutrition, UVB mediated changes of essential FA would induce qualitative food chain effects that could be superimposed on quantitative effects, like reduced primary production or phytoplankton biomass.

Because of its oxidative properties, UVB radiation can be predicted to cause a reduction in long-chain PUFAs in algal cells. Goes *et al.* (1994), in a thorough examination of UVB exposed *Tetraselmis* sp. (4 h exposure to UVB, 190 mW m^{-2}, *ca.* 2.7 kJ m^{-2}), reported an overall increase in saturated and monounsaturated FA, while PUFAs decreased by 50%. 16:4, but also 18:3 and 20:5 were highly susceptible, and in fact the entire FA profile changed upon radiation. The most stable FA were 16:0 and 18:1. At least for 16:0 FA, biosynthesis is probably independent of light (Pohl & Wagner 1972), and accumulation of short-chain saturated and monounsaturated FA as constituents of storage lipids seem largely unaffected by UVB (Goes *et al.* 1994). Wang & Chai (1994) reported a reduction of the ω3 fatty acids eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) in a number of marine diatoms exposed to UVB (standard integrated intensity 12 kJ m^{-2} d^{-1}, 4 d exposure), but with different susceptibility among the 8 tested species. In general, log-phase cultures were most sensitive to UVB, and N, P or Si starved cells were more susceptible than saturated cells. Visible light (PAR) strongly reduced the UVB damage on FA. The unsaturated:saturated FA ratio decreased with UVB exposure. There also seemed to be a general correlation between decreased growth rate and decreased levels of ω3 FA for the various algae.

In support of the above data, Hessen (unpubl.) exposed *Selenastrum capricornutum* and *Rhodomonas lacustris* for two days to UV_{312} (14.3 kJ m^{-2} d^{-1}, 1.0 W m^{-1}, 4 hours d^{-1}), and noted minor changes in the relative share of saturated, monounsaturated and polyunstaurated FA (Fig. 3.3), while there were pronounced changes in the entire FA profiles (Fig. 3.4).

The two species responded slightly different, however. In *Selenastrum*, most FA decreased after UV-radiation, while 20:1, 22:1, 18:3ω3 and 18:4ω3 increased, whereas in *Rhodomonas* a number of FA increased. For this species, there was an apparent "recovery" for some FA from day 1 to 2, in line with the observations of Goes *et al.* (1994). The two most vulnerable FA were 20:5ω3 (EPA) and 22:6ω3 (DHA) for both species, supporting the data of Wang & Chai (1994) for marine diatoms.

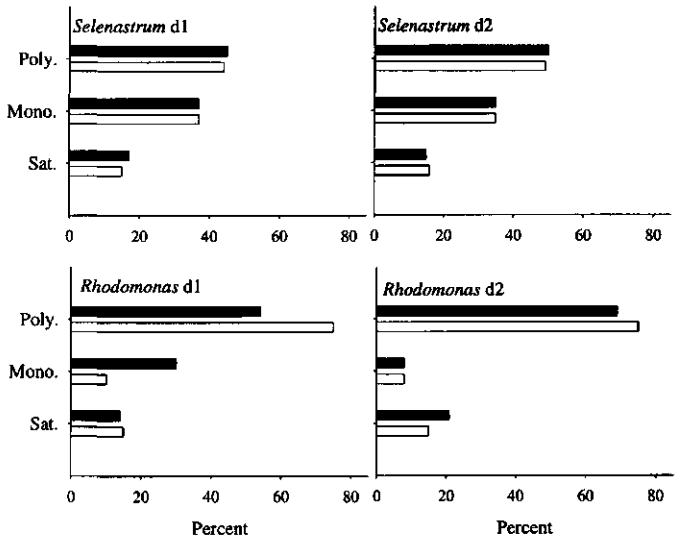


Figure 3.3: Composition of polyunsaturated, monounsaturated and saturated fatty acids in *Selenastrum capricornutum* and *Rhodomonas lacustris* after 1 and 2 days of either UVB+PAR (solid bars) or PAR only (open bars). Intensities as given in Fig. 3.2.

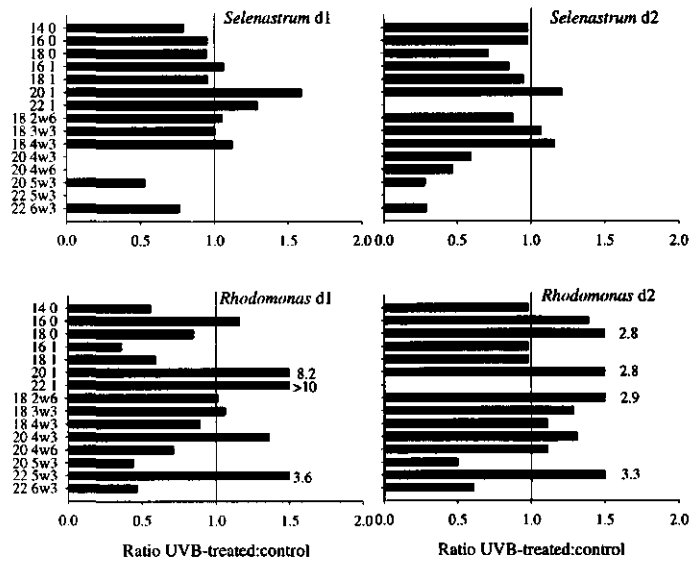


Figure 3.4: Fatty acid profiles in *Selenastrum capricornutum* and *Rhodomonas lacustris* after 1 and 2 days UVB + PAR irradiation given as deviation from control (vertical line). Intensities as given in Fig. 3.2.

De Lange (unpubl., Chapter 5) also provide evidence for UV-induced decline in EPA and DHA. The freshwater species *Stephanodiscus hantzschii*, *Rhodomonas lacustris* and *Synedra tenuis* all experienced major losses (both absolute and relative) in EPA and DHA after 3 d of UVB irradiation (Fig. 3.5). The results are not entirely consistent however, since *Cryptomonas pyrenoidifera* strongly increased its specific content of EPA, yet exhibited a remarkably strong (98%) decrease in DHA, following the same UVB treatment. No consistent trends in the ratio of saturated to unsaturated FA was seen in this study. In fact for all species except *Cryptomonas* there were also strong reductions in a number of short chained saturated FA like 16:0. These results are not entirely unifying. Döhler & Biermann (1994) found that the impact of UVB resulted in an increase of long chained fatty acids (C-18 and C-20), while a reduction of short chained fatty acids (C-14, C-16).

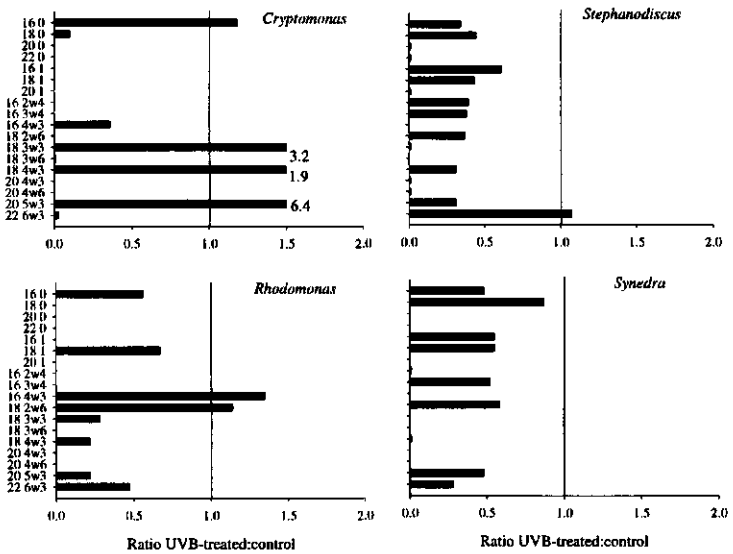


Figure 3.5: Fatty acid profiles in *Cryptomonas pyrenoidifera*, *Stephanodiscus hantzschii*, *Rhodomonas lacustris* and *Synedra tenuis* after 3 days of UVB radiation, 3 h d^{-1}, 3 kJ m^{-2} d^{-1} DNA weighted, given as deviation from control (vertical line).

While lipid-peroxidation is a likely explanation for decrease in unsaturated FA with UVB exposure, there are linkages to a number of other cellular mechanisms. Acetyl-cocarboxylase, an important enzyme in FA synthesis depends on ATP (Harwood 1988), and apparently the process of chain elongation and desaturation requires a large amount of ATP (Thompson *et al.* 1990). Nitrogen limitation may suppress the synthesis of 16:4 FA (Pohl & Wagner 1972). Goes *et al.* (1994) support the view that UVB inhibition of certain FA may be

caused by reduced nutrient uptake and ATP-production. If so, this poses yet another interesting linkage between UVB mediated blocking of nutrient uptake and vital cell biochemistry. Goes *et al.* (subm.) proposed a flow diagram how UVB may affect fatty acid synthesis (Fig. 3.6). UVB radiation allows chain elongation (solid lines), but not the desaturation processes (dotted lines). Chain elongation processes require adequate carbon supply. The synthesis of the neutral and phospholipids (left-hand side of the figure) require adequate supply of nitrogen and ATP. The synthesis of monogalactosyl diglyceride (MGDG), sulfoquinovosyl diglyceride (SQDG), and digalactosyl diglyceride (DGDG) (right-hand side of the figure) are dependent on the light quality and adequate supply of nitrogen. A decrease in storage compounds may impair the amino acid synthesis (Goes *et al.* subm.).

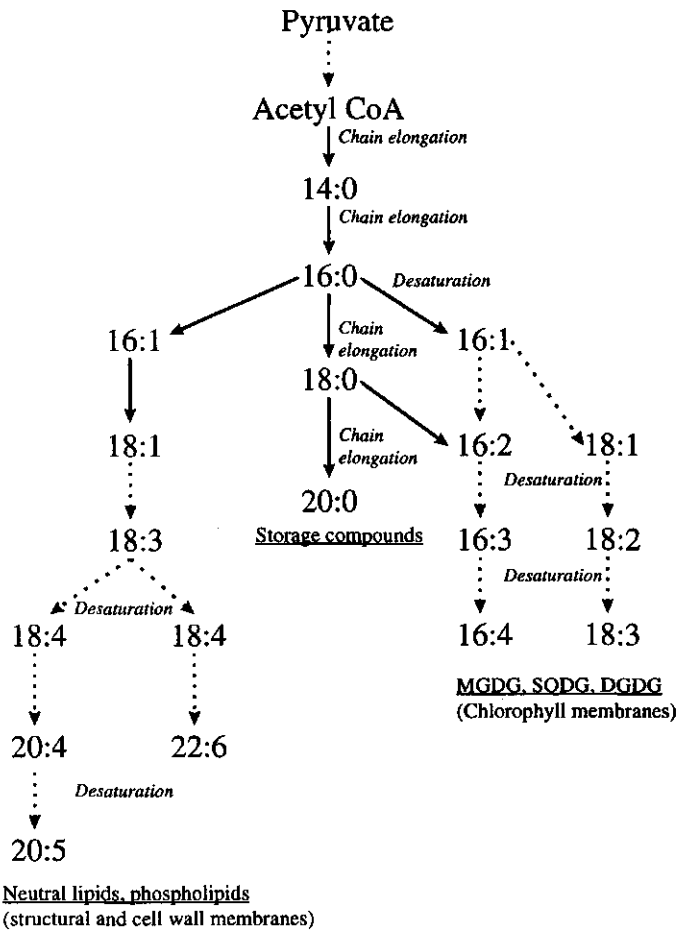


Figure 3.6: Schematic diagram showing the major pathways of fatty acid synthesis. Dotted lines indicate pathways possibly affected by UVB radiation. Further explanation can be found in the text. Figure was redrawn from Goes *et al.* (subm.).

The FA results should be interpreted with caution, owing to the limited number of tested species and possible dependency on spectral intensities and qualities. Yet it is clear that although there are pronounced species-specific responses, there is a general tendency for a relative reduction in polyunsaturated FA following UV-exposure, and 20:5ω3 and 22:6ω3 seem particularly susceptible, although these responses may not apply to all species or taxa. In general, fatty acid profiles seem more susceptible than overall production parameters like photosynthesis, and may be affected by shorter exposure times and smaller dose-rates. Probably FA composition may be affected by short exposure periods via peroxidation while total FA content depends on longer exposure periods and reduced photosynthesis. Given the crucial role of FA and PUFAs in particular for animal nutrition, these aspects of UV-radiation call for a further elaboration.

3.4 Effects on Motility and Orientation

This field was extensively reviewed previously (*e.g.* Häder 1993), and will thus only be briefly considered here. The motility and phototaxis of flagellated algae seem highly susceptible to even very low doses of UVB and UVA radiation. A number of taxa and species including *Chlamydomonas, Euglena, Cryptomonas, Gymnodinium, Gyrodinium* and *Peridinium* sp. are severely impaired by solar radiation or artificial UV-light, as evidenced by reduced swimming speed, loss of orientation and flagellar inactivation (Ekelund 1991, 1993, Ekelund & Häder 1988, Häder 1986, 1993, Hessen *et al.* 1995). The vertical migration and depth distribution of motile phytoplankton species are also influenced by UV-light (Häder 1993). The spectral distribution and the UV:PAR-ratio are probably major determinants of vertical distributions. The loss of flagella in *Chlamydomonas reinhardtii* was tightly coupled to UVB exposure in the laboratory (Hessen *et al.* 1995). While the flagellar sensitivity was confirmed by *in situ* incubations under sun-light, it is also evident that UVA radiation can induce flagellar inactivation (Van Donk & Hessen 1996). Hessen *et al.* (1995) showed that inactivation or loss of the flagella in *Chlamydomonas reinhardtii* was correlated with reduced P-uptake. While there are indications of a significant P-uptake by the flagella of this species, the causality remains speculative.

3.5 Effects on Cell Morphology

UV-light affects cell morphology in various ways. The mechanisms may include direct cell responses and probably some protective strategies, but may also reflect the secondary effects of changes in cell biochemistry. Decreased growth rates and increased cell volumes are common in diatoms under chronic UVB exposure (Karentz *et al.* 1991, Behrenfeld *et al.* 1992, Bothwell *et al.* 1993, Buma *et al.* 1995). These effects reflect a decoupling between cell

growth and cell division in UV-stressed algae. Karentz *et al.* (1991) observed elongated cells under UV-radiation for various Antarctic marine diatoms, caused by arrested cell division. Behrenfeld *et al.* (1992) found generally smaller cell volumes in the diatom *Phaeodactylum tricornutum* grown when ambient UVB was screened off, compared with cells exposed to ambient UVB. This response reached a maximum of a 15 to 20% cell volume decrease after 5-10 days incubation when solar radiation had a peak period, but levelled off to minor differences when ambient irradiation decreased. Additional UVB from lamps gave a further increase in cell sizes, at most 25% larger than the control (from 60 to 80 μm^3). While effects of PAR-light on cell sizes are also well known, these effects could only be attributed to changes in UVB. Cell volumes in the marine diatom *Cyclotella* sp. increased from an average of 900 to around 1200 μm^3 from controls to cells exposed to 370 J m^{-2} UVB over 5-10 days exposure period (Buma *et al.* 1995). Further increases in doses yielded no further increase in cell size.

Increases in cell volume in response to UVB are not restricted to the diatoms. Van Donk & Hessen (1995) found increased cell volumes in the green freshwater algae *Selenastrum capricornutum* following artificial UV_{312} exposure. Increased cell size was already seen after 8 h exposure (130 kJ m^{-2} UV_{312}), while after approximately one doubling time (24 h, 250 kJ m^{-2} UV_{312}), average cell volumes had increased from 30 to 55 μm^3. Parallel cultures without UV were grown under strong N or P-limitation. The N-limited cells increased their cell size from initial 31 μm^3 (nutrient satiated control) to 40 μm^3, while the P-limited cells reached an average volume of nearly 60 μm^3. Microscopic examination also revealed the symptoms of nutrient deficiency in the irradiated cells, including a granulated surface and large intracellular stores of carbohydrates. These effects were confirmed in later experiments with even lower doses. Hessen (unpubl.) found that *Selenastrum capricornutum* exhibited no cell volume response relative to the control after a two day treatment (14.3 kJ m^{-2} d^{-1}, 1.0 W m^{-2}, 4 hours d^{-1}) with UV_{312} exposure, but showed a small, but significant increase after 3 days. On the contrary, *Rhodomonas lacustris* initially increased the average cell volume with nearly 20% after 2 days exposure, but experienced massive leakage of cell cytoplasm and cell shrinkage after 3 d exposure. Thus, this species appears to be highly susceptible to UV exposure. A complete cell lysis of this species was recorded after 4 d irradiation with UVB at 3 kJ m^{-2} d^{-1} DNA weighted (De Lange unpubl.). Owing to the limited number of experiments, differences in light sources and cell status, generalizations are premature. However, for most phytoplankton species, UVB exposure seems to decouple the accumulation of photosynthetic products and cell division, ultimately leading to increased cell sizes.

Although it may be anticipated that UVB would affect the ultrastructure of phytoplankton cells, there are few reports on this subject. Buma *et al.* (1995) noted enlarged cell size accompanied with increased numbers of plastids and mitochondria after the UVB treatment of *Cyclotella*. No visual damage could be seen on outer membranes, nuclear envelope, mitochondria or thylakoids. In contrast, in higher plants, thylakoid damage has been

reported as an early sign of UVB damage (Joshi *et al.* 1994, Strid *et al.* 1994).

3.6 Implications of UVB Induced Changes For Aquatic Food Chains

An underlying prediction is that the array of qualitative UV-effects at the base of the food web will have profound effects, not only on the primary grazers, but on the entire food web. While there is every reason to stress that there is a fundamental lack of *in situ* confirmation of such effects, there are also several reasons to believe that such effects can be significant (see Chapters 4 and 5). The outcome of UVB in a food-web context may be rather complex and unexpected, like those reported by Bothwell *et al.* (1994), where periphytic diatom communities gained higher biomass under UV-exposure due to the high susceptibility of the grazers.

Since UVB generally inhibits phytoplankton growth and cell division, it will most often affect the food quality of these cells. First of all, nutrient deficient phytoplankton cells are poor food for zooplankton for a multitude of reasons. To the extent that UV radiation reduces nutrient uptake, it may offset the same array of effects that follow a direct nutrient limitation (*cf.* Hessen 1992, Sterner & Hessen 1994). One major effect on nutrient limitation, notably phosphorus limitation, is increased cell size, apparently cell wall thickening and reduced assimilation by grazers (Van Donk & Hessen 1993, 1995). Ultrastructure studies of the green algae *Chlamydomonas reinhardtii* (Van Donk *et al.* 1997) revealed intracellular storage of photosynthetic products, considerable thickening of the cell wall due to glycoprotein accumulation and signs of extracellular mucous cell wall covering in cells subjected to severe nutrient limitation. A cell-wall deficient mutant strain of *Chlamydomonas* was still efficiently assimilated even when severely phosphorous starved, clearly confirming that the reduced assimilation efficiency could be attributed cell wall changes.

Similarly, a number of experiments have found decreased growth and egg production of zooplankton feeding on nutrient deficient algae owing to stoichiometric constraints on the grazer (Hessen 1992, Sterner *et al.* 1993, Urabe *et al.* 1997). If UV-stress indeed put constraints on nutrient uptake, this poses a new and interesting aspect of UV-induced, qualitative food-chain effects. Besides the possible effects related to stoichiometric constraints on consumer growth, changed stoichiometry and reduced assimilation efficiency will imply changed patterns of nutrient regeneration. The impact of these processes would be most severe in upper layers during summer, where a major part of primary production is based on nutrient recycling. So far this hypothesis remains to be tested. One should, however, keep in mind that these processes are naturally occurring. Irrespective of any changes in stratospheric ozone, present day UVA and UVB radiation already affect both quantitative and qualitative food web interactions.

UV-radiation could play a major role in the food web in shallow and clear waters by

affecting fatty acid composition, in particular by reducing PUFAs. Fatty acid profiles, and in notably a few specific PUFAs have been considered as a major determinant to herbivore success and animal nutrition in general. Ahlgren *et al.* (1990) revealed a strong dependency of growth in various freshwater cladocerans, and found that long chained PUFAs in general improved cladoceran success. This was pursued by Müller-Navarra (1995), who found that 20:5ω3 (EPA) was a major determinant of the growth of *Daphnia* in a particular lake. Since this FA is found both in marine diatoms and freshwater chlorophytes and seems highly susceptible to UVB (Wang & Chai 1994, Hessen unpubl.), it could be a likely candidate if searching for UV-sensitive key FA. The ability of chain elongation is probably present in a number of zooplankton species, but will probably occur at low rates relative to the demands. Recent studies (Brett & Müller-Navarra 1997, DeMott & Müller-Navarra 1997) suggest that EPA and DHA could be limiting factors for growth of *Daphnia.* These FA are essential for membrane functioning in most organisms, and the observation of cytoplasm leakage and apparent osmotic problems in UV-stressed phyto- and zooplankton (Hessen unpubl.) could be indicative of membrane damage. The role of EPA as a nutritional requirement for aquatic grazers is not certain, however. *Daphnia* may grow well also in absence of EPA (Chapter 5, Weers & Gulati 1997). Thompson *et al.* (1993) reported higher food quality for oyster larvae feeding on *Chaetoceros* and *Isochrysis* grown under high light compared with those grown under low light. The positive response in the oyster larvae was linked to an increase in short-chain saturated fatty acids (14:0, 16:0), while long-chain unsaturated fatty acids like EPA were negatively correlated with growth and survival of the larvae.

In conclusion, it may be stated that there is a number of UV-mediated qualitative changes in phytoplankton that may affect the planktonic grazers and the entire aquatic food web. Changes in species composition, cell size and cell wall will invariably influence ingestion and digestion. Changes in stoichiometry, cell biochemistry and macromolecular composition will strongly influence the cells nutrient quality. The potential effect of UV on fatty acids call for special attention. FA play a major role in nutrition for most animals, and as such UV-mediated lipid peroxidation or reduced biosynthesis of essential FA could be a major determinant of food quality for aquatic herbivores.

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CHAPTER 4

EFFECTS OF UVB-IRRADIATED ALGAE ON GRAZING BY ZOOPLANKTON

This chapter is based on:
De Lange H.J. & Lürling M. UVB-irradiated algae cause different effects on ingestion and assimilation by *Daphnia, Bosmina* and *Brachionus*. In preparation.

Ik ga lopen tot de zon komt
Tot de zon me achterhaalt
Lopen tot de zon komt
Tot ie straalt

(Acda & de Munnik - Lopen tot de zon komt)

4.1 INTRODUCTION

The phytoplankton-zooplankton interactions have received considerable attention in aquatic ecology, because of their importance in the pelagic foodchain. Since phytoplankton requires light for photosynthesis, it may be exposed to harmful UVB radiation. Zooplankton on the other hand can swim actively through the water column. This ability to migrate and to detect UVB radiation (*e.g.* Hessen 1994, Siebeck *et al.* 1994, Smith & Macagno 1990), may allow zooplankters to avoid exposure to UVB. Therefore, indirect effects of UVB radiation on zooplankton through changes in its food may play a more important role than direct effects.

UVB radiation may have both direct and indirect effects on interspecific interactions between phytoplankton and zooplankton, but also on those between bacteria and their grazers, *e.g.* heterotrophic nanoflagellates and other protists (Karentz *et al.* 1994). A direct effect of UV radiation can be inhibition of grazing, as found for heterotrophic nanoflagellates (Ochs 1997, Ochs & Eddy 1998, Sommaruga *et al.* 1996). Preliminary studies show that this is not likely for *Daphnia* (Van Donk *et al.* subm., De Lange unpubl.). An indirect UVB effect is a reduced apparent clearance rate, as found for *Daphnia magna* feeding on UVB stressed *Selenastrum capricornutum* (Van Donk & Hessen 1995). In life history experiments with *Daphnia*, UVB irradiated algae reduced the growth rates (Chapter 5).

In situ studies on feeding of *Daphnia pulex* on *Chlamydomonas* in the Netherlands (52 °N) showed that UVB can reduce the clearance rate (Chapter 7). This is more likely caused by a reduced digestibility of the UVB stressed *Chlamydomonas* than by a direct reduction of clearance rate. In a similar experiment at Spitsbergen (Norway, 79 °N), the clearance rate of melanic *Daphnia middendorffiana* feeding on *Chlamydomonas* was stimulated by UVA radiation, but not by UVB radiation (Chapter 7).

Bothwell *et al.* (1994) found that in shallow (1 cm depth) outdoor experimental streams total algal biomass doubled in the UV-exposed community as UV radiation selectively suppressed chironomid grazers. Indoor ecosystem studies with a range of UVB intensities showed that individual phytoplankton-zooplankton interactions might have been negatively affected by the UVB stress, but at community and ecosystem levels no effects of UVB stress were found (Chapter 6).

The aim of this study was to assess if UVB-irradiated algae are grazed differently by zooplankton compared with algae cultured under normal light conditions. 4 Species of phytoplankton were tested (*Chlamydomonas reinhardtii, Cryptomonas* A, *Scenedesmus acutus,* and *Microcystis aeruginosa*), and 3 species of zooplankton (*Daphnia galeata, Bosmina longirostris,* and *Brachionus calyciflorus*), representing different taxonomic groups.

The tested zooplankton species are known to differ in their feeding behaviours: large cladocerans like *Daphnia galeata* do not exhibit taste discrimination (Kerfoot & Kirk 1991). Prey size and hardness are important in determining daphnid feeding selectivity. 'Soft' algae (naked and gelatinous flagellates) are more readily ingested than 'hard' algae (diatoms and dinoflagellates) (DeMott 1995). Daphnids can ingest some species of gelatinous green algae, but these algae pass the gut intact and in viable condition (Porter 1975). *Bosmina* shows some

degree of taste discrimination, and prefers large algae to small cells (Bogdan & Gilbert 1987, DeMott 1986, Kerfoot & Kirk 1991). For rotifers, *e.g. Brachionus*, particle size is an important feature, although this taxon is regarded as a generalist suspension feeder (Rothhaupt 1990a).

Altered cell wall morphology, *e.g.* in nutrient-deficient phytoplankton, can reduce the clearance rate of *Daphnia*, compared with non-limited cells, causing reduced somatic growth and population rate of increase for *Daphnia* (Van Donk *et al.* 1997). For *Brachionus* ingestion rate on non-limited and nutrient-limited *Scenedesmus* did not differ but the growth rate was reduced with nutrient-limited *Scenedesmus* (Rothhaupt 1995). Probable explanation for this is a lower nutritional value of nutrient-limited algae (Lürling & Van Donk 1997a).

The hypothesis in this study is that UVB-stressed algae are ingested similarly as non-stressed algae by *Daphnia*, but UVB-stressed algae may be assimilated differently. Both *Bosmina* and *Brachionus* may have a different ingestion rate and assimilation rate for UVB-stressed algae. It is expected that each phytoplankton species will invoke the same response on each species of zooplankton. The experiments were focused on the effects of UVB stressed algae on grazing by zooplankton. UVB effects on the algae are described, but only briefly.

4.2 METHODS

4.2.1 Plankton species

The phytoplankton species used are: *Microcystis aeruginosa* Kützing (Norwegian Institute for Water Research, strain NIVA-CYA 43), *Chlamydomonas reinhardtii* Dangeard (strain NIVA-CHL 13), and *Cryptomonas* A Geitler and *Scenedesmus acutus* Meyen (strains from culture collection Max Planck Institut für Limnologie, Plön, Germany). The grazing experiments were performed with *Daphnia galeata* Sars, *Bosmina longirostris* Müller, and *Brachionus calyciflorus* Pallas. All three taxa were obtained from the Max Planck Institut für Limnologie, Plön, Germany. *Daphnia* and *Bosmina* were cultured in 1 l batch cultures with *Scenedesmus acutus* as food. *Brachionus* was cultured in a 350 ml chemostat system on WC-medium (Guillard & Lorenzen 1972) with *S. acutus* as food, with a dilution rate of 0.35 d^{-1}.

4.2.2 Phytoplankton culture conditions

The algae were batch cultured, and frequently diluted in fresh WC-medium (Guillard & Lorenzen 1972) to obtain an exponentially growing culture. The UVB radiation experiment for each phytoplankton species started with an algal biovolume concentration of *ca.* $5*10^6$ μm^3 ml^{-1}, and lasted 3 days. *Chlamydomonas* and *Scenedesmus* were cultured under continuous photosynthetically active radiation (PAR, 95 $\mu mol\ m^{-2}\ s^{-1}$) with 6 hours UVB radiation per day. PAR was provided by 5 Osram L18W/19 tubes. *Cryptomonas* and

Microcystis were cultured with a L:D cycle of PAR of 16:8 h, at 80 and 50 µmol m^{-2} s^{-1} respectively, with 6 hours UVB radiation centred around the middle of the light period. PAR was provided by 4 Osram TL36W-1/29 tubes.

UVB radiation was provided by 1 Philips TL12/20W tube, UVC was filtered by cellulose acetate foil that was preburned for 5 hours. Three UVB radiation levels were applied in duplicate: no UVB, controls; low UVB, 0.3 W m^{-2}; and high UVB, 0.7 W m^{-2}. Radiation levels were measured at the surface level with a Macam SR9910 spectroradiometer. Daily biologically effective UVB dose, weighted with Setlow's DNA action spectrum (Setlow 1974) was 6.6 kJ m^{-2} d^{-1} for the low and 15.3 kJ m^{-2} d^{-1} for the high UVB treatment. The control cultures were grown in 300 ml glass Erlenmeyers, the UVB exposed cultures in 300 ml quartz Erlenmeyers.

At temperate latitude in June, noon levels of UVB radiation during clear sky can vary from 1.5 to 2.0 W m^{-2}, with PAR levels around 1500 µmol m^{-2} s^{-1}(De Lange unpubl.). We used UVB levels of 0.3 and 0.7 W m^{-2}, and PAR levels between 50 and 95 µmol m^{-2} s^{-1}. The absolute unweighted UVB doses were lower, but relative to PAR much higher than encountered in nature. The UVB levels were intended to cause stress on the algae, in order to investigate the effects of UVB stressed algae on zooplankton grazing rates.

After 2 days, one culture replicate of each treatment was labelled with 3.7 MBq ^{14}C as $NaH^{14}CO_3$ for the radiotracer feeding experiment. The other culture replicate was used to measure cell dimensions and appearance measurements. Initial and final algal densities were measured in the size range 0-30 µm equivalent spherical diameter (ESD, 100 µm capillary) using a CASY (Schärffe system) particle analyzer. Cell appearance was investigated with a Nikon light microscope.

4.2.3 Ingestion and incorporation experiments

Ingestion and incorporation rates of *Brachionus* and *Daphnia* were determined feeding on each of the 4 phytoplankton species. Ingestion and incorporation rates of *Bosmina* were determined feeding on *Chlamydomonas* and *Microcystis*. The experimental animals were fed at least for 24 hours with the respective phytoplankton species prior to the grazing experiment. The labelled algae cultured as described above were centrifuged and resuspended in ADAM medium (Klüttgen *et al.* 1994). The food concentrations in carbon equivalents (mg C l^{-1}) were determined using a calibration curve of extinction at 800 nm *vs.* carbon content. Aliquots of 50 µl labelled algae were pipetted into scintillation vials. Two food concentrations were used in the experiments, 0.1 mg C l^{-1} (low food) and 1.0 mg C l^{-1} (high food).

For the ingestion experiment the experimental animals were transferred from the cultures into 10 ml (*Brachionus* and *Bosmina*) or 25 ml (*Daphnia*) unlabeled food in 50 ml bottles and allowed to acclimate to the desired food concentration. After at least 1 h of acclimation 25 ml labelled food (at the same concentration) was added, and 2 × 1 ml food

suspension was pipetted from the bottles into scintillation vials. After 5 minutes (*Daphnia*) or 10 minutes (*Brachionus* and *Bosmina*) of feeding the experimental animals were collected on a 52 µm sieve, rinsed with deionized water, narcotized in carbonated water and killed in a Petri-dish with a few drops of formaldehyde (37%). Groups of 6 (*Bosmina*) or 12 animals (*Brachionus*) were pipetted into scintillation vials. Length of individual daphnids was measured, and the animals were pipetted into scintillation vials, to obtain a length-ingestion rate relation (Vanni and Lampert 1992). In the *Brachionus* experiments, an additional vial was filled with a similar aliquot of fluid from the Petri-dish, but without animals, to check for background radioactivity.

For the incorporation experiment the experimental animals were transferred from the cultures into 10 ml (*Brachionus*) or 25 ml (*Bosmina* and *Daphnia*) unlabeled food and allowed to acclimate to the desired food concentration. After at least 1 h of acclimation 25 ml (*Brachionus* and *Bosmina*) or 250 ml (*Daphnia*) labelled food (at the same concentration) was added, and 2 × 1 ml food suspension was pipetted from the bottles into scintillation vials. After 3 hours of grazing the experimental animals were collected on a 52 µm sieve, rinsed with deionized water, narcotized in carbonated water and killed in a Petri-dish with a few drops of formaldehyde (37%). Processing of the animals was identical to the ingestion experiments. Because the guts of the animals in this experiment contained unassimilated food when the animals were killed, the rates represent "incorporation rates", although the term "assimilation rate" is more common in the literature (*cf.* DeMott 1998, Vanni and Lampert 1992).

The animals were dissolved overnight at 60 °C in 300 µl of tissue solubilizer (Soluene 350, Packard). Animals and the 50 µl samples of labelled food were counted with 5 ml water-absorbing scintillation cocktail (Instant scint-gel II plus + 5% Carbo-sorb, Packard), the 1 ml labelled food samples were counted with 3.5 ml scintillation cocktail. The activity (DPM) of the samples was measured in a Tri-Carb 1900CA liquid scintillation analyzer (Packard). Ingestion and incorporation rates were calculated according to Peters (1984). Incorporation efficiencies were calculated as the incorporation rate as percentage of ingestion rate (*cf.* DeMott 1998).

For *Daphnia*, length-grazing rate relations were obtained, and the slope and intercept of these regression lines were tested with two-way Anova (UVB x food concentration). For *Bosmina* and *Brachionus* the ingestion and incorporation rates were tested with two-way Anova (UVB x food concentration). Differences for all statistical tests were considered significant when $p<0.05$.

4.3 RESULTS

4.3.1 Phytoplankton characteristics

UVB radiation affected the growth rate of the phytoplankton. *Cryptomonas* growth rate was reduced by almost 50% in the high UVB treatment. *Chlamydomonas* growth rate was reduced by 12% in the high UVB treatment, *Scenedesmus* and *Microcystis* growth rates were not or only slightly reduced (Table 4.1). These differences could not be statistically tested, since there were no replicate measurements.

Table 4.1: Phytoplankton characteristics, mean particle volume (MPV, measured with Casy) and estimated growth rate as biovolume increase

		MPV (μm^3)	growth rate (d^{-1})
Chlamydomonas	control	181	1.12
	low UVB	158	1.05
	high UVB	183	0.99
Cryptomonas	control	246	0.68
	low UVB	332	0.55
	high UVB	169	0.37
Scenedesmus	control	100	0.92
	low UVB	95	0.84
	high UVB	104	0.88
Microcystis	control	47	2.42
	low UVB	58	2.11
	high UVB	56	2.38

Cell volume of *Scenedesmus* and *Microcystis* was increased slightly under influence of UVB radiation. *Chlamydomonas* cell volume varied slightly between treatments. There was a huge increase in bacteria numbers in the high UVB treatment of *Cryptomonas*. This was also measured in the electronic particle analysis and resulted in a decrease in average cell volume. Microscope measurements on Lugol fixed samples however showed a significant increase in length and width of the cells under high UVB exposure. Average length and width of *Cryptomonas* in the control were 18.4 µm and 12.5 µm, in the low UVB treatment 19.4 µm and 12.6 µm, and in the high UVB treatment 20.6 µm and 14.0 µm, respectively.

Changes in appearance were visible under the light microscope. *Chlamydomonas* had some cells with larger pyrenoids in the high UVB treatment. *Scenedesmus* cells showed more variation in cell size and shape in the high UVB treatment. Cells of *Cryptomonas* had a more granular appearance in the UVB treatments.

4.3.2 *Daphnia galeata*

Ingestion and incorporation rates of *Daphnia galeata* at high food concentration were higher compared with those at low food concentrations for all four species (Figs. 4.1 and 4.2). There was no difference between ingestion or assimilation rates on control, low UVB and high UVB stressed food. *Daphnia* had the highest ingestion and incorporation rate on *Chlamydomonas* and the lowest ingestion and incorporation rate on *Cryptomonas*. Ingestion rate of a mature female on *Chlamydomonas* was around 1 µgC ind^{-1} h^{-1}, on *Cryptomonas* around 0.4 µgC ind^{-1} h^{-1}. Considerable variation between replicate individuals caused most regressions to be not significant, so further statistical analysis and calculation of incorporation efficiencies was not performed.

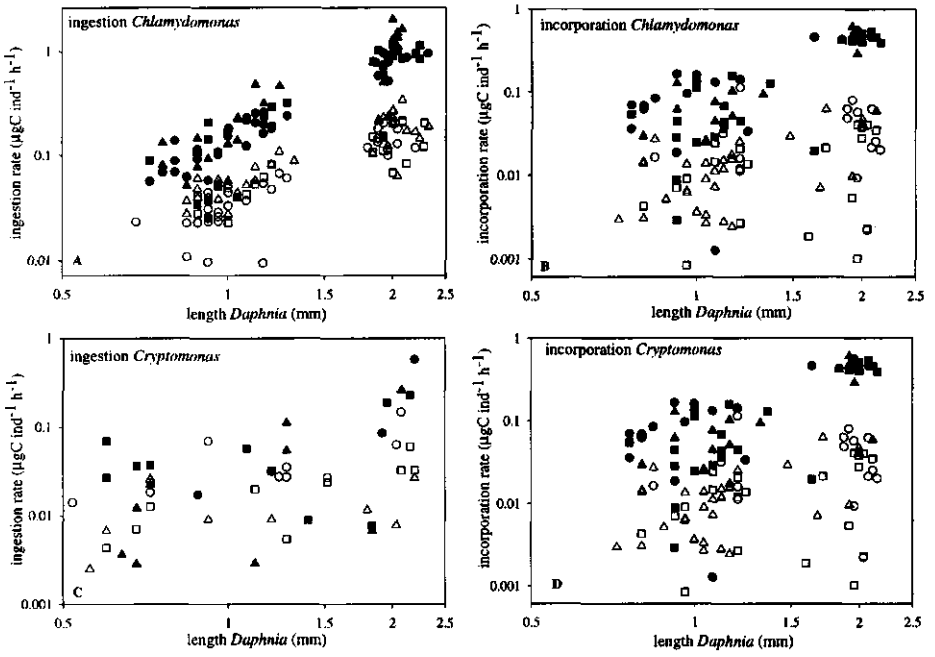

Figure 4.1: *Daphnia galeata* ingestion and incorporation rate on *Chlamydomonas* (panel A and B) and *Cryptomonas* (panel C and D); symbols are: triangles = no UVB, squares = low UVB, circles = high UVB; open symbols is low food, closed symbols is high food concentration.

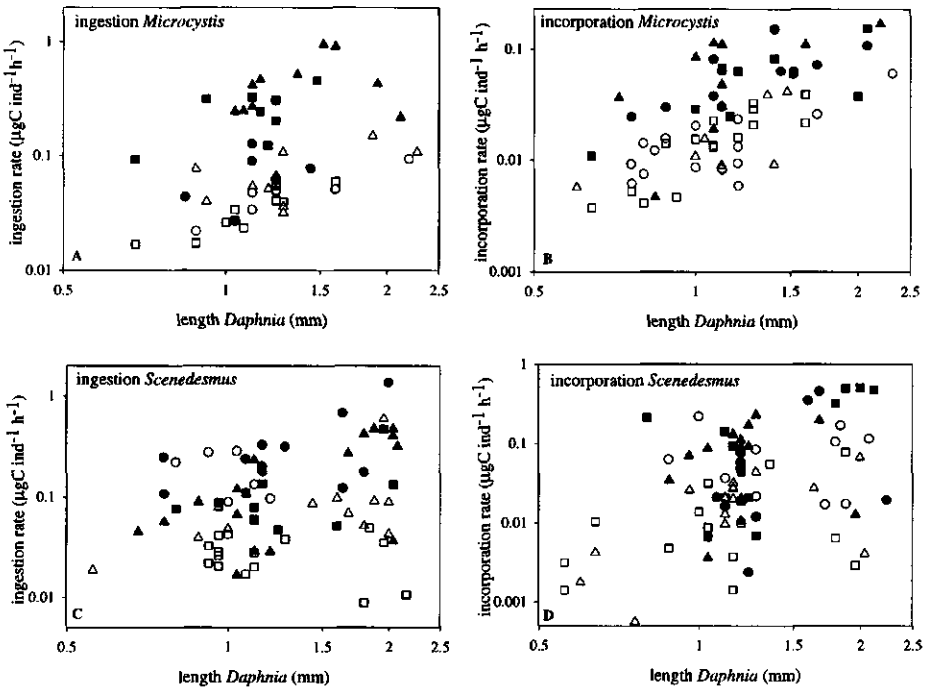

Figure 4.2: *Daphnia galeata* ingestion and incorporation rate on *Microcystis* (panel A and B) and *Scenedesmus* (panel C and D); symbols as in Fig. 4.1.

4.3.3 *Brachionus calyciflorus*

The higher ingestion and incorporation rates for the high food concentration compared with the low food concentration was significant for all 4 phytoplankton species (Fig. 4.3, Anova $p<0.05$). *Brachionus* had the highest ingestion and incorporation rate on *Cryptomonas*, and the lowest ingestion and incorporation rate on *Microcystis*.

The *Chlamydomonas* ingestion rate was not affected by the UVB treatment. The *Scenedesmus* ingestion rate increased significantly in the high UVB treatment. The increase was stronger at low food than at high food concentration. *Cryptomonas* ingestion rate increased significantly in both UVB treatments, the increase was stronger at high food concentration. The *Microcystis* ingestion rate was significantly lower in both UVB treatments compared with the control.

The *Chlamydomonas* incorporation rate at low food concentration increased significantly in the high UVB treatment. *Scenedesmus* incorporation rate at low food increased significantly for the low UVB treatment. *Cryptomonas* incorporation rate at high food

concentration increased significantly for the low UVB treatment. The *Microcystis* incorporation rate at low food concentration decreased significantly in both UVB treatments.

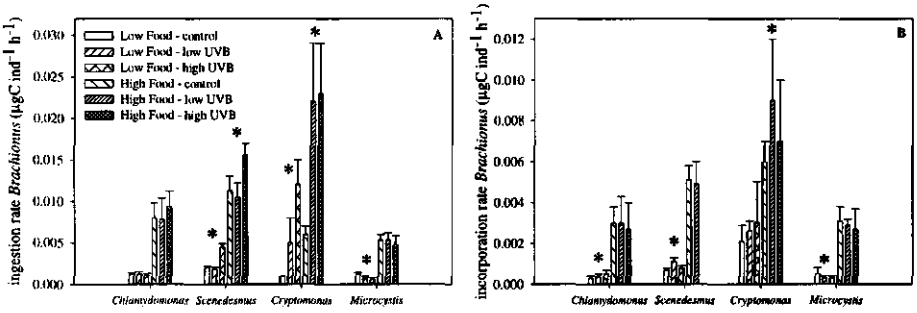

Figure 4.3: *Brachionus calyciflorus* ingestion rate (panel A) and incorporation rate (panel B). Asterisk indicates a significant difference between control, low UVB and high UVB for each food concentration.

Incorporation efficiencies of *Brachionus* feeding on control or UVB stressed algae varied without apparent UVB effect (Table 4.2). UVB stressed *Chlamydomonas* was incorporated more efficient at low food concentration, and less efficient at high food concentration. UVB stressed *Cryptomonas* was incorporated less efficient, at both food concentrations. However, efficiencies at high food concentrations were unrealistic high. UVB stressed *Chlamydomonas* was incorporated with varying efficiencies at low food concentration, and with similar efficiencies at high food concentration. UVB stressed *Microcystis* was incorporated at similar efficiencies as the control, at both food concentrations.

4.3.4 *Bosmina longirostris*

The higher ingestion and incorporation rates for the high food concentration compared with the low food concentration was significant for both phytoplankton species (Fig. 4.4, Anova $p<0.05$). *Bosmina* had higher ingestion and incorporation rates on *Microcystis* compared with *Chlamydomonas*. The *Chlamydomonas* ingestion rate at low food concentration was not affected by the UVB treatment, the *Chlamydomonas* ingestion rate at high food concentration decreased significantly. The *Microcystis* ingestion rate at low food was not affected by the UVB treatment. The ingestion rate at high food was affected significantly, it decreased in the low UVB treatment, and increased in the high UVB treatment.

Table 4.2: Incorporation as percentage of ingestion ± 1 SE of *Brachionus* and *Bosmina* for the different treatments. n.d. indicates not determined.

Food type			*Brachionus*	*Bosmina*
Chlamydomonas	low food	control	30 ± 3	79 ± 13
	low food	low UVB	33 ± 3	49 ± 9
	low food	high UVB	50 ± 7	102 ± 16
	high food	control	41 ± 6	87 ± 9
	high food	low UVB	42 ± 7	73 ± 7
	high food	high UVB	28 ± 4	123 ± 16
Cryptomonas	low food	control	37 ± 5	n.d.
	low food	low UVB	13 ± 2	n.d.
	low food	high UVB	14 ± 3	n.d.
	high food	control	641 ± 103	n.d.
	high food	low UVB	257 ± 98	n.d.
	high food	high UVB	62 ± 10	n.d.
Scenedesmus	low food	control	34 ± 2	n.d.
	low food	low UVB	60 ± 4	n.d.
	low food	high UVB	18 ± 1	n.d.
	high food	control	45 ± 2	n.d.
	high food	low UVB	47 ± 3	n.d.
	high food	high UVB	n.d.	n.d.
Microcystis	low food	control	43 ± 7	57 ± 7
	low food	low UVB	47 ± 3	106 ± 16
	low food	high UVB	52 ± 5	82 ± 11
	high food	control	59 ± 2	86 ± 9
	high food	low UVB	55 ± 1	304 ± 28
	high food	high UVB	63 ± 15	92 ± 11

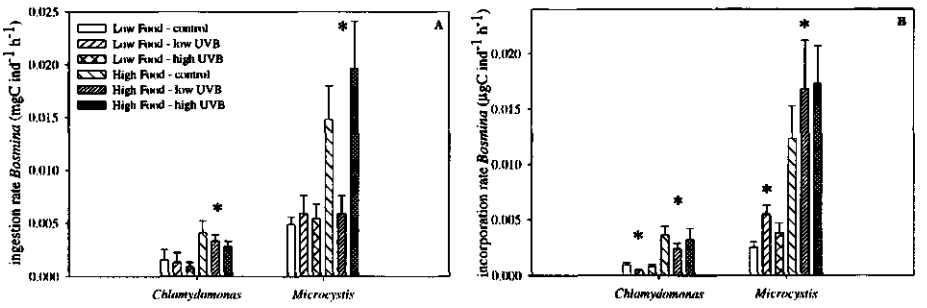

Figure 4.4: *Bosmina longirostris* ingestion rate (panel A) and incorporation rate (panel B). Asterisk indicates a significant difference between control, low UVB and high UVB for each food concentration.

The *Chlamydomonas* incorporation rate at both food concentrations decreased significantly in the UVB treatments. The *Microcystis* incorporation rate at both food

concentrations increased significantly in the UVB treatments. Incorporation efficiencies of *Bosmina* feeding on control or UVB stressed algae varied (Table 4.2). Low UVB stressed *Chlamydomonas* was incorporated less efficient, whereas high UVB stressed *Chlamydomonas* was incorporated more efficient than the control. The efficiencies of high UVB stressed *Chlamydomonas* were unrealistic high. UVB stressed *Microcystis* was incorporated more efficient than the control. The efficiencies for *Microcystis* in the low UVB treatment were unrealistic high.

4.4 DISCUSSION

Effects of UVB treatment on ingestion and incorporation rates are species-specific (Table 4.3). *Daphnia* showed no changes in ingestion and incorporation rates when offered UVB stressed algae. The magnitude of grazing rates on *Scenedesmus* is in agreement with the rates found by Vanni & Lampert (1992). The rates for feeding on *Chlamydomonas* resemble those found by Urabe & Watanabe (1991). It has been proposed that UVB exposed algae may experience reduced grazing losses because of changed cell wall properties that could hamper digestion by *Daphnia* (Van Donk 1997, Van Donk & Hessen 1995). However, our study did not confirm the earlier findings, despite indications that the algae showed similar effects. In the study by Van Donk & Hessen (1995), and the study described in Chapter 5, as well as in this study, algae appeared more granular under UVB stress. This granular appearance of UVB-irradiated algae suggests an altered biochemical composition. In general, carbohydrates may increase and total fatty acids and ω3 fatty acids may decrease under UVB stress (*e.g.* Goes *et al.* 1994, Chapter 3). Moreover, growth rates may decrease and cell volume increase due to arrested cell division (Behrenfeld *et al.* 1992, Buma *et al.* 1995, Karentz *et al.* 1991, Van Donk & Hessen 1995). As UVB radiation can inhibit uptake of nutrients, such as N (Braune & Döhler 1996, Döhler 1996) or P (Van Donk & Hessen 1995), on the long-term UVB stressed algae may become nutrient limited. Nutrient limited algae may reduce the feeding in *Daphnia* (Butler *et al.* 1989, Lürling & Van Donk 1997a, Sterner & Smith 1993, Van Donk & Hessen 1993). Since in this study UVB stressed algae were ingested and incorporated by *Daphnia* equal to non-stressed cells, they were probably not nutrient-limited to such an extent that feeding was affected. The UVB exposure of the algae lasted 3 days, this relatively short period was probably too short to cause UVB exposed cells to become nutrient limited. However, even at equal ingestion, growth in zooplankton may be affected because of lower nutritional quality of the cells as was demonstrated for the rotifer *Brachionus* feeding on nutrient limited algae (Rothhaupt 1995). The granular appearance reflects more intracellular carbohydrates (*e.g.* Van Donk *et al.* 1997) and presumably a reduced nutritional quality. In fact, growth and reproduction in *Daphnia* were reduced when feeding on UVB stressed algae (Chapter 5). Thus, even without a hampered digestion of UVB stressed algae, growth and reproduction in *Daphnia* may be affected.

Table 4.3: Effects of UVB stressed *Chlamydomonas reinhardtii* (*C.r.*), *Cryptomonas pyrenoidifera* (*C.p.*), *Scenedesmus acutus* (*S.a.*) and *Microcystis aeruginosa* (*M.a.*) on ingestion and incorporation rates under low food and high food concentrations; 0 indicates no significant effect, - indicates a significant negative effect, + indicates a significant positive effect, x indicates a significant interaction between food concentration and UVB stress, n.d. indicates not determined.

		Daphnia		*Brachionus*		*Bosmina*	
		ingestion	incorpo-ration	ingestion	incorpo-ration	ingestion	incorpo-ration
C.r.	low food	0	0	0	+	0	-
	high food	0	0	0	0	-	-
	interaction	0	0	0	0	x	0
C.p.	low food	0	0	+	0	n.d.	n.d.
	high food	0	0	+	+	n.d.	n.d.
	interaction	0	0	x	x	n.d.	n.d.
S.a.	low food	0	0	+	+	n.d.	n.d.
	high food	0	0	+	0	n.d.	n.d.
	interaction	0	0	x	x	n.d.	n.d.
M.a.	low food	0	0	-	-	0	+
	high food	0	0	0	0	+/-	+
	interaction	0	0	0	0	x	x

Bosmina showed a decreased ingestion and incorporation rate when fed with UVB stressed *Chlamydomonas*. In contrast, the rates tended to be higher when animals were fed with UVB stressed *Microcystis*. The UVB effects on ingestion and incorporation rates were stronger at high food concentration. Incorporation efficiencies for *Microcystis* were not affected by the UVB treatment. Incorporation efficiencies for *Chlamydomonas* were decreased by the low UVB treatment, and increased at the high UVB treatment. *Bosmina* may be capable of taste discrimination and prefers large algae above small cells (Bogdan & Gilbert 1987, DeMott 1986, Kerfoot & Kirk 1991). Therefore, *Bosmina* was expected to exhibit lower ingestion rates on *Microcystis* than on *Chlamydomonas* as the former is both smaller and of a lower nutritional quality (bad taste?) than the latter food species, but the opposite was observed.

Brachionus had the lowest rates on *Microcystis*. *Brachionus* exhibits a particle size-dependent generalistic feeding mode (Rothhaupt 1990a), but shows no taste discrimination (DeMott 1986). The algal size determines the relative clearance efficiency being the highest around an equivalent spherical diameter (ESD) of *ca.* 10 µm (Rothhaupt 1990a,b). *Brachionus* had higher ingestion and incorporation rates for UVB stressed *Scenedesmus* and *Cryptomonas* compared with the controls. Microscopic analysis revealed significantly enlarged *Cryptomonas* cells in the high UVB treatment, meaning that the observed ingestion pattern is consistent with the particle size-dependent feeding mode. *Brachionus* grazing rates on UVB stressed and control *Chlamydomonas* were similar. This alga may be, however, of a lower nutritional value to *Brachionus*, as at equal ingestion rates, growth rates appeared lower than those with other algae (Rothhaupt 1990c). The values of *ca.* 3 and 12 ngC $ind^{-1}h^{-1}$ for low and

high food, respectively, are in good agreement with values obtained by Rothhaupt (1990a,b,c;1995). The UVB effects on ingestion and incorporation rates of *Microcystis* and *Scenedesmus* were stronger at low food concentration. However, the UVB effects on ingestion and incorporation rates of *Cryptomonas* were stronger at high food concentration. Incorporation efficiencies were affected species-specific, and did not show a consistent UVB effect.

Only the UVB effects on phytoplankton were tested, and how it may affect zooplankton ingestion and assimilation rates. Under this short-term laboratory exposure the phytoplankton was not affected dramatically by the UVB treatment. Growth rates were reduced, and appearance was different, but the cells were still alive and growing. *Cryptomonas* showed the strongest response to UVB stress, both in growth rate reduction and in cell size increase. Except *Brachionus* feeding on *Cryptomonas*, the effects of UVB radiated algae on ingestion and incorporation rates of the three zooplankters were marginal.

Summarizing the effects as positive, negative and none (see Table 4.3) reveals nine positive, five negative and 25 cases without any effect of UVB. These experiments did not confirm the reduced digestibility hypothesis (Van Donk 1997, Van Donk & Hessen 1995). Explanation for this may be the higher UVB intensity used in the experiments by Van Donk and co-workers, and their UVB source contained a larger proportion of wavelengths below 300 nm. Effects of enhanced UVB radiation on phytoplankton-zooplankton interactions are not straightforward predictable from these grazing experiments. Moreover, extrapolation of these data to predict ecosystem response to UVB stress seems unjustified. But, since spring communities, with diatoms and cryptophytes, are the most sensitive to UVB (Gala & Giesy 1991) and rotifers may be abundant in spring (*e.g.* Lürling & Van Donk 1997b), an effect of UVB on phytoplankton-zooplankton interactions may be expected. Of course, in the real world of the pelagic, phytoplankton is exposed to a highly dynamic and heterogeneous environment. Fluctuating nutrient concentrations, temperature, turbulence, light field and grazing pressure may all interact with fluctuating UVB stress on the algae, the question however is in what direction, and with what consequences? The ecological community structure is considered as the result of the continuous struggle for life in which competitive interactions are a major driving force (MacArthur 1960, Sommer *et al.* 1986). The most dominant genera in early spring are the nanoplanktonic algae including diatoms and cryptophytes (Sommer *et al.* 1986) that experience besides high growth rates also high mortality rates due to sinking and grazing (Reynolds *et al.* 1982). Moreover, these taxa may be more sensitive to UVB than green algae and cyanobacteria (Gala & Giesy 1991). The susceptibility to UVB radiation differs among algae (Karentz *et al.* 1991), which could easily result in shifts in phytoplankton species composition that in turn could affect the energy transfer to herbivorous zooplankton to a great extent. How these effects will act on the ecosystem level is difficult to predict, but available laboratory data on biochemical changes in algae and reduced growth in zooplankton, call for *in situ* experimentation.

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CHAPTER 5

EFFECTS OF UVB-IRRADIATED ALGAE ON LIFE HISTORY TRAITS OF *DAPHNIA*

This chapter is based on:
De Lange H.J. & Van Donk E. (1997) Effects of UVB-irradiated algae on life history traits of *Daphnia pulex*. *Freshwater Biology*, **38**, 711-720.
and:
Van Reeuwijk P.L. & De Lange H.J. Effects of UVB-irradiated algae on life history traits of *Daphnia magna*. In preparation.

Als ik de aarde ga verwarmen,
laat ik haar leven in mijn armen.
Van sterren weefde
ik het verre aah, het Noorderlicht.
Maar soms ben ik als kolkend lood,
ik ben het leven en de dood,
in vuur, in liefde, in alle tijd.

(Lennaert Nijgh - Pastorale)

5.1 INTRODUCTION

As discussed in Chapter 1, UVB radiation can cause several negative effects on phytoplankton and zooplankton organisms. Zooplankters are not directly dependent on light energy. This may allow them a greater range of responses to minimize exposure to UVB in surface waters than phytoplankton. Further, the potential for directly sensing UVB, and the ability of vertical migration may allow zooplankters to regulate their exposures to UVB (Siebeck *et al.* 1994, Williamson 1995). Therefore it is likely that indirect effects of UVB, namely a change in phytoplankton community structure or food quantity and quality, will have the greatest effect on zooplankton.

There are several effects of UVB radiation on phytoplankton cells that may influence herbivorous zooplankton (Chapter 3). There has been increased interest into assessing the importance of the fatty acid composition of algae as a factor affecting the food quality of phytoplankton for zooplankton. Several studies suggest that unsaturated fatty acids, especially ω3- and ω6-PUFAs like eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3), may improve the food quality of phytoplankton for zooplankton (*e.g.* Ahlgren *et al.* 1990, Müller-Navarra 1995a, 1995b). In some studies UVB radiation caused a decrease in total lipid content of the algal cells, and a reduction of unsaturated fatty acids (Goes *et al.* 1994, Wang & Chai 1994, Chapter 3). The reduction in total fatty acid content is possibly caused by reduced biosynthesis (linked with reduced photosynthesis). Reduction of unsaturated fatty acid can also be explained by UVB induced oxidation of double bonds.

The mechanisms by which UVB could influence phytoplankton-zooplankton interactions are shown in Fig. 5.1. There are four pathways: 1) A decrease in food quantity can be expected as a result of decreased algal growth rate and primary production. 2) A change in food quality can be expected as a result of i) a change in phytoplankton species composition and ii) changes in the biochemical composition of individual cells which may affect palatability. It is not yet known whether the net result of these changes will be positive or negative for the zooplankton. 3) A change in food uptake (ingestion) might be expected as a result of i) changed cell volume of affected phytoplankton. This should affect smaller zooplankters (rotifers) more than larger zooplankters (cladocerans). ii) A change in filtering rate might occur as a direct effect of UVB radiation on zooplankton. 4) A decrease in food digestion can be expected as a result of a change in algal morphology (thicker cell wall) which may inhibit digestion (Van Donk & Hessen 1995). These four factors and their interactions could influence energy transfer between the first and second trophic level and, hence, have an effect on the life history traits of herbivorous zooplankters.

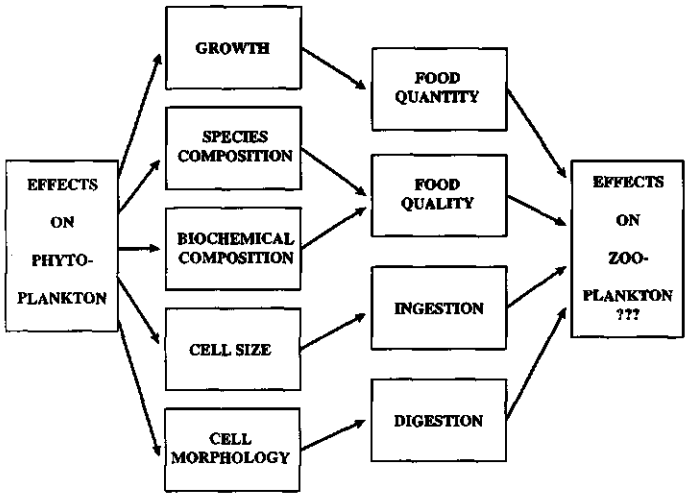

Figure 5.1: Schematic presentation of the main aspects in the phytoplankton-zooplankton interaction that may be affected by UVB radiation.

This chapter describes the results of life history experiments with *Daphnia pulex* and *Daphnia magna* fed with different species of phytoplankton that were cultured with or without exposure to UVB radiation. Four phytoplankton species cultured in batch were used in the *D. pulex* experiment. The fatty acid composition was analysed for each phytoplankton species. Emphasis was placed on the fatty acid composition because little is known about the effect of UVB radiation on the fatty acids of phytoplankton. Further, fatty acids are by some investigators regarded as an important factor in determining food quality. The results of the fatty acid analysis are therefore related to the results of the life history experiments. Two phytoplankton species were used in the experiments with *D. magna*, that were cultured in chemostat with a range of UV intensities. Morphological and biochemical characteristics of the phytoplankton species were determined and related to the results of the life history experiments.

5.2 MATERIALS AND METHODS

5.2.1 Plankton species

The following phytoplankton species were used in the life history experiments: three Chlorophyceae, *Chlamydomonas reinhardtii* Dangeard (strain from Norwegian Institute for Water Research NIVA-CHL 13); *Scenedesmus acutus* Meyen (strain from the Max Planck Institut für Limnologie, Plön, Germany); *Scenedesmus subspicatus* Chodat (strain NIVA-CHL 55); and one Cryptophyceae, *Cryptomonas pyrenoidifera* Geitler (strain NIVA-2/81).

Daphnia pulex was isolated from Lake Zwemlust. *Daphnia magna* was obtained from NIOO-Centre for Limnology. *D. pulex* was cultured at 20 °C in 1 litre jars containing a suspension of *S. acutus* in 0.45 µm membrane filtered (Schleicher & Schuell, Germany) lake water. *D. magna* was cultured at 20 °C in 1 litre jars containing a suspension of *S. acutus* in RT medium (Tollrian 1993).

5.2.2 Phytoplankton culture conditions in the *D. pulex* experiment

D. pulex life history experiments were done with *C. reinhardtii*, *C. pyrenoidifera*, *S. acutus*, and *S. subspicatus*. The three Chlorophyceae species were cultured in 1.0 litre chemostats on 20% Z8 medium (Skulberg & Skulberg 1990). Bacteria were present in low abundance (<0.5% of biovolume). The chemostats were continuously illuminated by circular fluorescent tubes (Philips TLEM 40W/33RS) at an irradiance of 125 µmol m^{-2} s^{-1} (measured at culture surface) in a temperature controlled chamber at 20 °C and with a dilution rate of 1.2 d^{-1}. The cryptophyte was cultured in semi-continuous culture, in 500 ml Erlenmeyer flasks, under similar growth conditions. The algae were inoculated daily from the (semi) continuous culture into 150 ml batch culture flasks (20% Z8 medium), giving initial concentrations of *ca.* $5*10^4$ cells ml^{-1}. The batches were grown for 3 d on a shaking table under 90 µmol m^{-2} s^{-1} PAR (14:10 L:D), and 3 h UVB radiation centred around the middle of the light period, with a daily biological effective dose of 3 kJ m^{-2}.

The biologically effective dose was calculated using Setlow's DNA action spectrum, normalized to 1 at 300 nm (Setlow 1974), based on the assumption that the primary impact of UVB irradiation is damage to DNA molecules. The unweighted UVB dose rate was 0.90 W m^{-2}, which corresponded with a biologically effective dose rate of 0.28 W m^{-2}_{DNA} and thus a daily biologically effective dose of 3 kJ m^{-2} d^{-1}_{DNA}. This daily dose was chosen based on previous experiments, and was higher than natural sunlight. On an energy basis, the unweighted UVB dose rate was 4% of PAR, and the weighted UVB dose rate was 1% of PAR. On the basis of photons, UVB was 2% of PAR. For natural sunlight these percentages are 0.6%, 0.02% and 0.4% respectively.

PAR was provided by 8 fluorescent tubes (Osram L36W/21-840 Lumilux Cool White). Two Philips TL12/40W tubes were used as UV light sources. The UVB treatment batches were grown in quartz Erlenmeyer flasks, covered with cut-off filters (3 mm WG305, Schott, Germany) to remove UVC radiation (<280 nm). The control batches were grown in glass Erlenmeyer flasks covered with perspex to remove all UVB and UVC and part of UVA radiation. Irradiation was measured at the culture surface with a Macam Spectroradiometer SR9910 (Fig. 5.2).

Every day the Erlenmeyer flasks were placed in different positions on the shaking table, to minimize variation in radiation. The batches were cultured for three days, collected shortly after the third UV period, and used in the life table experiment. Algal densities and particle size distributions were determined with an electronic particle counter (Coulter

Multisizer II). The cells were examined regularly under a light microscope to observe any changes in appearance and flagella as result of UVB stress.

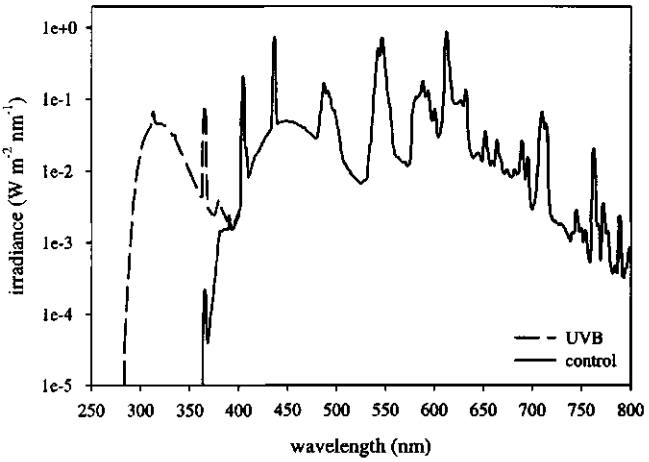

Figure 5.2: Spectral irradiance of control and UVB treatment as measured on culture surface.

5.2.3 Phytoplankton culture conditions in the *D. magna* experiment

D. magna life history experiments were done with *C. reinhardtii* and *C. pyrenoidifera* grown in a chemostat. Five different radiation treatments were applied: control, UVA, and 3 different intensities of UVB (Table 5.1). The control was grown in a 1 litre glass flask, the UVA treatment was grown in a 1 litre polycarbonate flask, wrapped in Mylar foil to remove wavelengths below 320 nm, the 3 different UVB treatments were grown in 1 litre polycarbonate flasks. All treatments were cultured on WC medium (Guillard & Lorenzen 1972). All flasks were bubbled with air, to provide sufficient carbon, and to prevent sedimentation of the algae. *C. reinhardtii* was grown at a dilution rate of 1.0 d^{-1}, *C. pyrenoidifera* was grown at a dilution rate of 0.33 d^{-1}. The chemostats were placed in a temperature controlled room at 20 °C, PAR radiation was provided by Philips TLD 30W/33 tubes at a 14:10 L:D cycle. UVA and UVB radiation was provided by Philips TL12/20W tubes at a 4:20 L:D cycle. The UV radiation was centered in the middle of the PAR radiation. The daily UVB dose was calculated with Setlow's DNA weighting function. The overflows of the chemostats were emptied every day and used in the life history experiment. Algal densities and particle size distributions were determined daily with an electronic particle counter (Coulter Multisizer II).

Table 5.1: Radiation treatments of *C. reinhardtii* and *C. pyrenoidifera* in the *D. magna* experiments. PAR intensity ± 1 SD. The UVB high treatment for *C. reinhardtii* (*C.r.*) was 1.5, for *C. pyrenoidifera* (*C.p.*) 2.5 kJ $m^{-2} d^{-1}_{DNA}$.

Treatment	Light spectrum	PAR µmol $m^{-2} s^{-1}$	UVA kJ $m^{-2} d^{-1}$	UVB kJ $m^{-2} d^{-1}_{DNA}$
control	PAR	95 ± 5	-	-
UVA	PAR+UVA	95 ± 5	8.6	-
UVB low	PAR+UVA+UVB	95 ± 5	9.0	0.5
UVB medium	PAR+UVA+UVB	95 ± 5	9.5	1.0
UVB high	PAR+UVA+UVB	95 ± 5	10	1.5 (*C.r.*), 2.5 (*C.p.*)

5.2.4 Biochemical analysis

Phytoplankton samples were concentrated by centrifugation, freeze-dried and stored at -20 °C. The fatty acid composition was analysed for the algal species used in the *D. pulex* experiments. Samples of *ca.* 1 mg were transferred to 10 ml tubes. Heneicosaenoic acid (C21:0) was added as an internal standard. Lipids were extracted with 2 ml of analytical grade chloroform:methanol (2:1 v/v). The chloroform contained 0.01% butylated hydroxytoluene as an antioxidant. The extract was then vortexed and centrifuged (5 min, 2500 rpm). This procedure was repeated twice, the supernatants were combined and washed with demineralized water with 0.88% NaCl (Folch *et al.* 1957). Lipid esters were transmethylated at 80 °C for 4 h with 1 ml of 3% H_2SO_4 in dry methanol, and extracted with hexane. The fatty acids were analyzed on a Hewlett Packard 5890 Gas Chromatograph, with a very polar 50 m silica column (SGE, BPX70, 509C3).

The algal species used in the *D. magna* experiments were analyzed for protein and carbohydrate content. Total protein content was determined according to the method described by Lowry *et al.* (1951), using a Sigma diagnostics total protein kit (catalog no. 690-A). Carbohydrates were measured using the anthrone method (Hassid & Abraham 1957). Samples were taken 3 times during the life history experiment, at the start, half-way, and at the end.

5.2.5 Life history experiments

Life history experiments were conducted with newborn *Daphnia* from mothers grown on the same phytoplankton species as used in the experiment. Newborns, collected within 20 h of birth were placed individually in 100 ml glass tubes containing 50 ml (*D. pulex*) or 100 ml (*D. magna*) food suspension. In the *D. pulex* experiments, the control and UVB treatment consisted of 15 replicates. In the *D. magna* experiments, the control and UV treatments consisted of 12 (for *C. reinhardtii*) or 10 (for *C. pyrenoidifera*) replicates. All animals were incubated at 20 °C in the dark.

The algal cells, cultured as described in § 5.2.2 and § 5.2.3, were diluted in 50 ml 0.45 µm filtered (Schleicher & Schuell, Germany) water from Lake Maarsseveen (*D. pulex* experiment) or 100 ml RT medium (*D. magna* experiment) to a standard food concentration of $6*10^6$ µm^3 ml^{-1}. This biovolume corresponded with a carbon concentration of *ca.* 1.5 mg l^{-1} and is well above the Incipient Limiting Level for *Daphnia* (Lampert 1987). Every day the individuals were transferred to clean tubes with fresh food suspension.

Length and moulting frequency were examined daily. Time to maturity, survival and the number of newborns were also recorded. Newborns were removed from the tubes. The length of newborns of the first clutch of *D. pulex* was also measured in the experiments with *C. reinhardtii* and *C. pyrenoidifera*. In the *D. magna* experiments the length of newborns of the first three clutches was measured. Growth and reproduction were measured until the animals reached the fourth adult instar and consequently had released their third clutch. This procedure was adopted because population growth rate is mainly determined by the first three clutches (Porter *et al.* 1983, Vanni & Lampert 1992).

The intrinsic rate of population increase (r) was estimated using the Euler-Lotka equation:

$$1 = \sum_{x=0}^{n} e^{-rx} l_x m_x$$

where r = rate of population increase (d^{-1}), x = age class (0...n), l_x = probability of surviving to age x, m_x = fecundity at age x. The standard errors of the population parameter r were computed with a Jackknife procedure (Meyer *et al.* 1986).

For each experiment the mean value (r) and the standard error (se) of the Jackknife pseudo-values of the intrinsic rate of population increase for the control (r_{Jc}) and UVB (r_{Juvb}) treatment were tested as follows:

$$z = \frac{(r_{Jc} - r_{Juvb})}{\sqrt{(se_{Jc}^2 + se_{Juvb}^2)}}$$

where z is assumed to be normally distributed with variance=1.

To test for a general effect of UVB irradiated phytoplankton on r, a t-test for matched pairs was used. The difference between r_{Jc} and r_{Juvb} for the four experiments was tested to be different from zero (with df=3). Differences in growth rates and cell volumes of the phytoplankton, and other life history parameters were also tested with this t-test for matched pairs.

5.3 RESULTS

5.3.1 Effects of UVB radiation on phytoplankton growth and morphology

In the batch cultures used in the *D. pulex* experiments, UVB radiation reduced the algal growth rates by *ca.* 10 to 20% compared with the controls (Table 5.2; t-test: p=0.02, df=3). The cell volume increased under UVB radiation almost significantly (Table 5.2; t-test: p=0.08, df=3). The appearance of the cells of all four species was more granular in the UVB irradiated cultures, compared with the control cultures. In the control cultures of *C. reinhardtii* 95% of the cells had two flagella. In the UVB treatment only 40% of the cells had two flagella, 60% of the cells had lost one or both flagella. Unfortunately, no data on flagella are available for *C. pyrenoidifera*.

Table 5.2: Results from the batch cultures used in the *D. pulex* experiments: relative growth rates (μ in day^{-1}) and biovolumes (μm^3 $cell^{-1}$) of algae in the UVB and control treatments (± 1 SD).

	$\mu_{UVB}/\mu_{control}$	Cell volume control (μm^3 $cell^{-1}$)	Cell volume UVB (μm^3 $cell^{-1}$)
C. pyrenoidifera	0.77 ± 0.17	620 ± 100	670 ± 135
C. reinhardtii	0.86 ± 0.15	140 ± 20	155 ± 25
S. acutus	0.93 ± 0.15	90 ± 10	103 ± 10
S. subspicatus	0.86 ± 0.14	95 ± 20	110 ± 25

In the chemostat cultures used in the *D. magna* experiments, UVB radiation did not affect the cell density of *C. reinhardtii* or *C. pyrenoidifera*. Cell densities were *ca.* $9 * 10^5$ cells ml^{-1} and *ca.* $4 * 10^5$ cells ml^{-1} for *C. reinhardtii* and *C. pyrenoidifera* respectively. The cell volume of *C. pyrenoidifera* increased significantly in the UVB treatments (Anova, $p<0.05$). The differences in *C. reinhardtii* cell volume were not significant (Table 5.3).

Table 5.3: Results from the chemostat cultures used in the *D. magna* experiments: cell volume ± 1 SD, differences were tested with Anova, homogeneous groups (Tukey test) are indicated by letters.

Treatment	*C. reinhardtii*	*C. pyrenoidifera*
control	121 ± 6 a	573 ± 29 a
UVA	111 ± 6 a	592 ± 20 a
UVB low	129 ± 8 a	752 ± 27 b
UVB medium	106 ± 5 a	836 ± 31 bc
UVB high	109 ± 6 a	874 ± 30 c

5.3.2 Biochemical composition

The results of the fatty acid analyses of the batch cultures used in the *D. pulex* experiments are given in Table 5.4. Because measurements were made without replicates, differences in fatty acid composition could not be statistically tested. Total fatty acid content for *C. reinhardtii*, as percentage of freeze dried weight, decreased from 25% to 14%. The ratio of saturated to unsaturated fatty acids did not change and was 0.41 in both control and UVB treatment. For *S. acutus* total fatty acid content decreased from 14% to 12%. The ratio saturated to unsaturated decreased from 0.30 in the control to 0.26 in the UVB treatment, this was caused by a small decrease in saturated fatty acids. For *S. subspicatus* total fatty acid content decreased from 8% to 6%. The ratio saturated to unsaturated increased from 1.3 to 2.2, indicating a relative increase in saturated fatty acids under UVB stress. This was mainly caused by a reduction in PUFA. For *C. pyrenoidifera* total fatty acid content decreased from 26% to 17%. The ratio saturated to unsaturated decreased from 0.46 in the control to 0.39 under UVB exposure, indicating a shift to more unsaturated fatty acids as result of UVB stress. The content of EPA (20:5ω3) doubled under UVB radiation, whereas the content of DHA (22:6ω3) decreased under UVB. The total PUFA content was similar under UVB compared with control. The smaller ratio was mainly caused by a reduction in saturated fatty acids. The relative change in total fatty acid content was tested with a t-test for matched pairs, the decrease in total fatty acid content was significant (df=3, p=0.02).

Results of the biochemical analysis of the chemostat cultures of *C. reinhardtii* and *C. pyrenoidifera* are given in Table 5.5. Carbohydrate content of *C. reinhardtii* fluctuates, but without apparent time or UV effect. Protein content of *C. reinhardtii* decreases in time, especially in the UVB low and UVB medium treatment. Carbohydrate content of *C. pyrenoidifera* increases in the UV treatments.

Table 5.4: Fatty acid composition as % of freeze dried weight for control and UVB irradiated *Chlamydomonas reinhardtii (C.r.), Cryptomonas pyrenoidifera (C.p.), Scenedesmus acutus (S.a.)* and *Scenedesmus subspicatus (S.s.)*, nd = not detected.

	C.p. control	*C.p.* UVB	*C.r.* control	*C.r.* UVB	*S.a.* control	*S.a.* UVB	*S.s.* control	*S.s.* UVB
14:0	0.75	0.48	0.25	0.20	0.10	0.34	0.29	0.20
16:0	3.53	4.15	7.19	3.92	2.57	2.16	3.45	1.99
16:1ω7	0.43	0.45	1.09	0.56	0.27	0.73	0.04	0.46
16:2ω4	nd	nd	0.20	0.12	nd	nd	nd	nd
16:3ω?	nd	nd	0.80	0.45	0.33	0.30	0.05	nd
16:4ω3	0.38	0.06	5.44	3.11	2.66	2.70	0.26	0.08
18:0	3.85	0.18	nd	nd	nd	nd	nd	nd
18:1ω9	5.35	0.44	0.30	0.20	1.02	0.73	1.49	0.69
18:1ω7	0.51	0.30	0.79	0.54	nd	nd	nd	nd
18:2ω6	0.26	0.51	0.78	0.35	0.79	0.45	0.44	0.16
18:3ω6	nd	nd	1.22	0.65	0.04	0.04	0.03	0.03
18:3ω3	1.58	3.31	7.37	4.17	5.04	4.41	0.93	0.37
18:4ω3	2.13	4.13	nd	nd	0.53	0.39	0.10	nd
20:1ω9	0.14	0.06	nd	nd	nd	nd	nd	nd
20:5ω3	0.83	1.64	nd	nd	nd	nd	nd	nd
22:6ω3	6.14	1.60	nd	nd	nd	nd	nd	nd
24:0	nd	nd	nd	nd	0.53	nd	0.70	1.72
total	25.87	17.31	25.43	14.30	13.87	12.23	7.78	5.68
SAFA	8.13	4.81	7.44	4.17	3.20	2.50	4.44	3.90
MUFA	6.43	1.24	2.18	1.29	1.29	1.46	1.52	1.14
PUFA	11.32	11.25	15.81	8.84	9.38	8.28	1.81	0.64
sat/unsat	0.46	0.39	0.41	0.41	0.30	0.26	1.33	2.19
ω3	11.06	10.74	12.81	7.28	8.22	7.49	1.29	0.45
ω6	0.26	0.51	1.99	1.00	0.82	0.49	0.47	0.19
ω3/ω6	43.03	21.07	6.42	7.31	9.99	15.29	2.77	2.35

Table 5.5: Carbohydrate (carbo.) and protein content as percentage of freeze dried weight. Measurements were made at the start (A), halfway (B), and at the end (C) of the *D. magna* life history experiment; - = not determined.

		C. reinhardtii		*C. pyrenoidifera*	
treatment	time	carbo.	protein	carbo.	protein
control	A	12	53	42	26
	B	14	47	28	34
	C	11	47	34	28
UVA	A	-	-	31	31
	B	16	48	20	45
	C	12	42	39	24
UVB low	A	-	-	39	30
	B	14	50	35	40
	C	17	14	58	10
UVB medium	A	12	40	43	28
	B	12	42	29	34
	C	9	24	47	11
UVB high	A	-	-	34	31
	B	12	51	30	38
	C	14	47	44	18

5.3.3 Life history traits *D. pulex*

The intrinsic population growth rate r was slightly lower for the UVB treatments, but this difference was only significant for *S. subspicatus* (Table 5.6; z-test: $p<0.01$). Combining the four experiments in a paired t-test resulted in an almost significant decrease in intrinsic population growth rate in the UVB treatment ($p=0.08$, $df=3$). Age at maturity showed no consistent trend, whereas length at maturity was depressed by the UVB treatment in all four experiments. This difference was only significant for *C. pyrenoidifera* (t-test: $p<0.01$; Table 5.6, Fig. 5.3). Survival to day 16 was not significantly different between treatments (Table 5.6).

Table 5.6: Population growth rate (± 1 SD), age (± 1 SD) and length at maturity (± 1 SD), interclutch duration (± 1 SD) and survival to day 16 of *Daphnia pulex* fed on control and UVB irradiated *Cryptomonas pyrenoidifera* (*C.p.*), *Chlamydomonas reinhardtii* (*C.r.*), *Scenedesmus acutus* (*S.a.*) and *Scenedesmus subspicatus* (*S.s.*); * indicates a significance level of $p<0.01$ (z-test)

	growth rate (d^{-1})	Age at maturity (d)	Length at maturity (mm)	Interclutch duration (d)	Survival to day 16 (%)
C.p. con	0.41 ± 0.02	5.7 ± 0.5	2.19 ± 0.10*	2.82 ± 0.39	75
C.p. UVB	0.41 ± 0.03	5.4 ± 0.5	2.02 ± 0.11	2.83 ± 0.48	80
C.r. con	0.46 ± 0.06	5.3 ± 0.4	2.15 ± 0.12	2.86 ± 0.35	75
C.r. UVB	0.42 ± 0.09	5.7 ± 0.5	2.14 ± 0.10	3.08 ± 0.36	69
S.a. con	0.51 ± 0.06	4.4 ± 0.5	2.04 ± 0.13	2.54 ± 0.50	65
S.a. UVB	0.49 ± 0.03	4.4 ± 0.5	2.03 ± 0.11	2.58 ± 0.49	80
S.s. con	0.39 ± 0.05*	5.2 ± 0.4	1.95 ± 0.14	2.81 ± 0.53	53
S.s. UVB	0.32 ± 0.04	5.3 ± 0.4	1.90 ± 0.08	2.75 ± 0.64	67

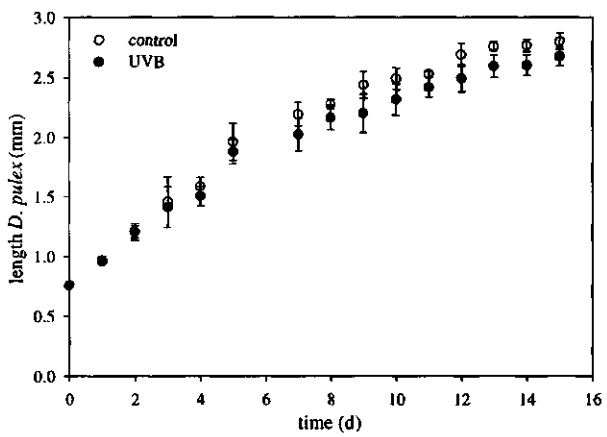

Figure 5.3: Body length of *Daphnia pulex* fed UVB irradiated and control *Cryptomonas pyrenoidifera*, error bars indicate 1 SD.

The number of newborns was smaller in the UVB treatment for all four experiments (Table 5.7). This difference was most pronounced for *S. subspicatus*. The net reproductive rate is smaller for all species except *C. reinhardtii*, this is mainly determined by the clutch sizes, because the survival is similar between control and UVB treatment. T-test for matched pairs showed that the difference was not significant ($p=0.15$, $df=3$). The first clutch and third clutch were significantly smaller in the UV-B treatment (t-test matched pairs, $p<0.05$)). Other life history parameters were not significantly different. For *C. reinhardtii* and *C. pyrenoidifera* the length of the newborns in the first clutch was significantly smaller in the UVB treatment compared to the control (t-test: $p<0.01$; Table 5.7).

Table 5.7: Clutch sizes (± 1 SD), net reproductive rate (R_0) and length of newborns of first clutch (± 1 SD) of *Daphnia pulex* fed on control and UVB irradiated *Cryptomonas pyrenoidifera (C.p.), Chlamydomonas reinhardtii (C.r.), Scenedesmus acutus (S.a.)* and *Scenedesmus subspicatus (S.s.)*, - = not determined; * indicates a significance level of $p<0.01$ (t-test)

	Clutch 1 (n)	Clutch 2 (n)	Clutch 3 (n)	R_0	Length clutch 1 (mm)
C.p. con	6.1 ± 1.7	14.7 ± 3.0	18.0 ± 1.6	37.3	0.76 ± 0.02*
C.p. UVB	5.4 ± 0.9	12.6 ± 2.3	16.7 ± 2.8	34.4	0.71 ± 0.03
C.r. con	9.3 ± 1.7	14.5 ± 3.6	32.5 ± 7.4	46.3	0.74 ± 0.04*
C.r. UVB	8.8 ± 3.3	14.4 ± 2.7	30.0 ± 4.9	46.3	0.69 ± 0.06
S.a. con	9.9 ± 2.7	16.5 ± 1.9	18.8 ± 1.9	40.5	-
S.a. UVB	8.5 ± 2.0	14.7 ± 3.5	17.1 ± 2.4	38.0	-
S.s. con	8.3 ± 1.8	11.1 ± 2.4	7.8 ± 1.6	22.4	-
S.s. UVB	6.6 ± 1.6	5.6 ± 2.6	3.3 ± 1.5	13.7	-

5.3.4 Life history traits *D. magna*

The intrinsic population growth rate r was not affected for *C. reinhardtii*, and slightly lower in the UVB treatments for *C. pyrenoidifera*. Differences were not significant (Table 5.8). Age at maturity was not affected, whereas length at maturity was depressed by the UVB treatments in both experiments. This difference was only significant for *C. pyrenoidifera* (Anova, $p<0.05$; Table 5.8). Interclutch duration and survival to day 16 was not significantly different between treatments (Table 5.8).

Clutch sizes are given in Table 5.9. There were no significant differences between the treatments in the *C. reinhardtii* experiment. The differences found between the treatments in the *C. pyrenoidifera* experiment were significant. Clutch size was largest in the control.

The average length of the juveniles born in each treatment is given in Table 5.10. There are no significant differences in the average length of juveniles born in the first clutch. Juveniles born in the second and third clutch have significantly different body lengths (Anova, $p<0.05$). Differences are however small, and there is no apparent relation with the UV treatments.

Table 5.8: Population growth rate (r, ± 1 SD), age at maturity (± 1 SD) and length at maturity (± 1 SD), interclutch duration (± 1 SD) and survival to day 16 of *Daphnia magna* feeding on *Chlamydomonas reinhardtii* (*C.r.*) or *Cryptomonas pyrenoidifera* (*C.p.*) from the different radiation treatments. Differences were tested with Anova and Tukey test, homogeneous groups are indicated by letters.

Treatment	Growth rate (d^{-1})	Age at maturity (d)	Length at maturity (mm)	Interclutch duration (d)	Survival to day 16 (%)
C.r. control	0.52 ± 0.00	5.9 ± 0.1	3.03 ± 0.06	3.0 ± 0.1	83
C.r. UVA	0.50 ± 0.00	6.1 ± 0.1	2.95 ± 0.03	3.0 ± 0.0	92
C.r. UVB low	0.51 ± 0.00	6.0 ± 0.0	2.92 ± 0.03	3.0 ± 0.0	100
C.r. UVB medium	0.58 ± 0.03	6.3 ± 0.4	2.80 ± 0.11	3.0 ± 0.1	92
C.r. UVB high	0.51 ± 0.00	6.0 ± 0.0	2.91 ± 0.03	3.0 ± 0.0	92
C.p. control	0.48 ± 0.00	6.0 ± 0.0	2.84 ± 0.02 a	4.0 ± 0.1	100
C.p. UVA	0.46 ± 0.00	6.0 ± 0.0	2.73 ± 0.02 b	4.0 ± 0.1	100
C.p. UVB low	0.47 ± 0.00	6.0 ± 0.0	2.71 ± 0.03 b	4.0 ± 0.0	100
C.p. UVB medium	0.46 ± 0.00	6.0 ± 0.0	2.66 ± 0.02 c	4.0 ± 0.0	100
C.p. UVB high	0.45 ± 0.01	6.0 ± 0.0	2.68 ± 0.03 c	4.0 ± 0.1	100

Table 5.9: Clutch sizes (± 1 SD) and net reproductive rate (R_0) of *Daphnia magna* feeding on *Chlamydomonas reinhardtii* (*C.r.*) or *Cryptomonas pyrenoidifera* (*C.p.*) from the different radiation treatments. Differences were tested with Anova, homogeneous groups (Tukey test) are indicated by letters.

	Clutch 1 (n)	Clutch 2 (n)	Clutch 3 (n)	R_0
C.r. control	22.6 ± 0.7	38.7 ± 1.5	45.3 ± 0.6	89.0
C.r. UVA	20.0 ± 0.8	35.8 ± 0.8	44.3 ± 1.2	91.2
C.r. UVB low	19.8 ± 1.1	34.8 ± 1.5	48.3 ± 1.2	102.1
C.r. UVB medium	17.8 ± 0.0	32.6 ± 3.0	46.8 ± 2.4	97.2
C.r. UVB high	19.6 ± 0.6	31.5 ± 3.2	44.7 ± 1.0	88.9
C.p. control	17.2 ± 1.7 a	29.5 ± 5.1 a	35.1 ± 7.7 a	81.8 a
C.p. UVA	15.7 ± 0.4 ab	23.0 ± 0.5 bc	27.6 ± 6.3 bc	66.3 b
C.p. UVB low	14.2 ± 1.5 b	25.2 ± 3.4 b	29.2 ± 2.2 b	68.5 c
C.p. UVB medium	13.6 ± 0.6 b	20.7 ± 1.6 c	25.7 ± 2.0 bc	59.9 d
C.p. UVB high	14.3 ± 1.1 b	23.6 ± 5.6 bc	21.3 ± 7.2 c	59.1 d

Table 5.10: Average length of juveniles (± 1 SD) of *Daphnia magna* feeding on *Chlamydomonas reinhardtii* (*C.r.*) or *Cryptomonas pyrenoidifera* (*C.p.*) from the different radiation treatments. - = not determined. Differences were tested with Anova, homogeneous groups (Tukey test) are indicated by letters.

	Clutch 1 (mm)	Clutch 2 (mm)	Clutch 3 (mm)
C.r. control	0.73 ± 0.03	0.81 ± 0.04 a	0.87 ± 0.02
C.r. UVA	0.74 ± 0.02	0.81 ± 0.04 a	0.86 ± 0.04
C.r. UVB low	0.74 ± 0.02	-	0.85 ± 0.03
C.r. UVB medium	0.72 ± 0.05	0.77 ± 0.04 b	0.86 ± 0.03
C.r. UVB high	-	0.83 ± 0.04 a	0.88 ± 0.03
C.p. control	0.69 ± 0.04	0.78 ± 0.04 ab	0.80 ± 0.04 a
C.p. UVA	0.69 ± 0.04	0.81 ± 0.03 ab	0.80 ± 0.04 ab
C.p. UVB low	0.68 ± 0.05	0.78 ± 0.02 a	0.81 ± 0.04 ab
C.p. UVB medium	0.70 ± 0.05	0.81 ± 0.04 ab	0.83 ± 0.03 b
C.p. UVB high	0.72 ± 0.04	0.82 ± 0.04 b	0.79 ± 0.03 ac

The length at maturity of *D. magna* in the *C. pyrenoidifera* experiment was smaller in the UV treatments, compared with the control. Clutch sizes were also smaller in the UV treatments. This might be caused by the smaller body length of the mother. However, Fig. 5.4 shows that the daphnids in the UV treatments have smaller juveniles than in the control at similar body length. The slopes and intercepts of the regression lines are tested, and the control regression line is significantly different from the UV treatment regression lines (t-test, $p<0.05$).

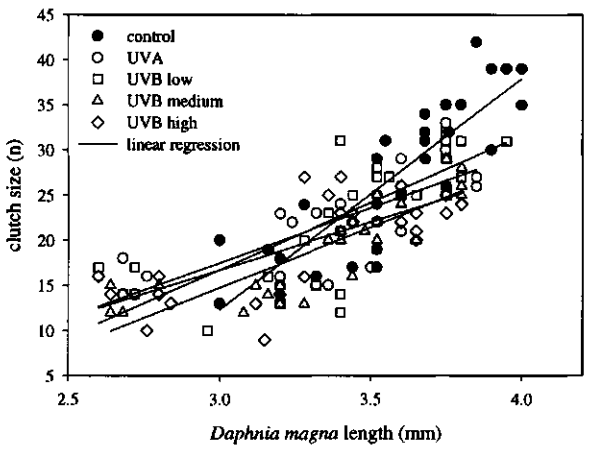

Figure 5.4: Relation between length of *D. magna* feeding on *C. pyrenoidifera* from the different radiation treatments and clutch size. Linear regression lines are fitted, slope of control = 25.6, UVA = 11.9, UVB low = 15.2, UVB medium = 14.7, UVB high = 10.0.

5.4 DISCUSSION

The intrinsic population growth rate (r) of *D. pulex* showed a modest decrease in the UVB treatment compared with the control. Because only 4 phytoplankton species were tested, generalization is difficult. However, the almost significant p-value of 0.08 for the difference of r between control and UVB treatment suggests that UVB irradiated phytoplankton can negatively affect the growth and reproduction of *D. pulex*. The difference of r between control and UVB treatment was mainly determined by the smaller number of offspring. The first clutch and the third clutch were significantly smaller in the UVB treatment. Generation time and survival were not significantly different in the UVB treatment compared with the control. The values of r found in these experiments resembled the value of *ca.* 0.40 d^{-1} at similar high carbon food level as reported by Lynch (1989) and Lürling & Van Donk (1996).

D. magna had similar responses to UVB irradiated phytoplankton as *D. pulex*. No significant effects on life history traits were found in the *C. reinhardtii* experiment. In the *C.*

pyrenoidifera experiment, the intrinsic population growth rate was not affected. However, number of offspring was decreased in the UV treatments, as was R_0. Since survival was 100% in all treatments, the difference in R_0 was fully determined by the smaller clutch sizes. Length at maturity was also smaller in the UV treatments, but clutch sizes were even smaller than could be expected form the smaller body length of the mother (Fig. 5.4). Two additional experiments showed that juveniles born in the UVB medium and UVB high treatment were more vulnerable to UVB radiation, and to starvation (Van Reeuwijk & De Lange, in prep.).

C. reinhardtii had a dilution rate of 1.0 d^{-1} in the chemostat, and was therefore on average exposed to one UVB radiation period before being used in the life history experiment. The total UVB dose received might have been too low to cause effects on the cells, let alone cause effects on *D. magna*. The lack of UVB effects on *C. reinhardtii* may also indicate that this species is relatively resistant to UVB radiation. This is supported by the lack of effects on the life history traits of *D. pulex* feeding on UVB-irradiated *C. reinhardtii* cultured in batch.

C. pyrenoidifera had a dilution rate of 0.33 d^{-1}, and was therefore exposed to three UVB radiation periods before being used in the life history experiment. The cells were affected by the UVB treatment, cell volume had increased, protein content had decreased and carbohydrate content increased. These changes in biochemical composition might explain the observed differences in life history traits of *D. magna*.

Food quantity was sufficient during all experiments. The biovolume concentration was $6*10^6$ μm^3 ml^{-1} in all treatments, so the differences in growth rate and cell volume of the phytoplankton would not have affected the growth of *Daphnia*. The maximum clearance rate of a mature female of *D. pulex* is *ca.* 1 ml ind^{-1} h^{-1}; of *D. magna ca.* 2 ml ind^{-1} h^{-1}; with juveniles having a slightly lower rate (De Lange unpubl.). Because food was refreshed every 24 hours, it was unlikely that *D. pulex* or *D. magna* had cleared the whole volume of food during the incubation. Therefore it is assumed that the measured differences in growth of *Daphnia* feeding on control and UV irradiated algae were not caused by food limitation.

The life history results from the *D. pulex* and *D. magna* experiments are comparable. For both *Daphnia* species, the effect of UVB irradiated phytoplankton on population growth rate r was minor. Clutch sizes and net reproductive effort were smaller in the UVB treatments. The juveniles born in the UV treatments were smaller. Additional experiments showed that juveniles born in the UV treatments had a lower resistance against starvation or UVB radiation. These quality aspects are not taken into account in the calculation of r, but are of ecological importance.

UVB radiation may change the morphology (cell wall) of the algal cells, this may result in a reduced digestibility. However, UVB-irradiated cells can be digested by the daphnids in the time course between refreshing the food suspension (24 h) after repeated egestions and ingestions, *i.e.* after several gut passages (Van Donk & Hessen 1995). Repeated gut passages however cost extra energy, and will result in a decreased assimilation rate. This will also have a negative effect on the growth of the daphnid.

A second explanation for the observed difference in growth rate in the life history experiments can be the change in biochemical composition, especially the fatty acid

composition. It is suggested by several studies that unsaturated fatty acids, especially ω3-PUFA's, improve the food quality for zooplankton (*e.g.* Ahlgren *et al.* 1990, Müller-Navarra 1995a, 1995b, Chapter 3). The fatty acid composition is a highly dynamic component of cellular physiology, which responds to variation in irradiation. This response seems to be species specific (Thompson *et al.* 1990).

The fatty acid measurements in this study were made on the batch cultures used in the *D. pulex* experiment. Assuming that the growth stage was similar in batch and chemostat, UVB effects on the fatty acid composition measured in batch cultures may be extrapolated to the chemostat cultures. The results shown here demonstrate that the fatty acid composition was influenced by the UVB treatment. Total fatty acid content decreased significantly in the UVB treatment. For *C. pyrenoidifera* there was a shift to unsaturated fatty acids under UVB stress. The observed increase in the content of EPA for *C. pyrenoidifera* was in contradiction with the expectation of a decrease in unsaturated fatty acids, based on results known from literature (Goes *et al.* 1994, Wang & Chai 1994). This EPA increase may be associated with the decline in DHA content, assuming that DHA is converted into EPA. For *C. reinhardtii* and *S. acutus* the ratio between saturated and unsaturated fatty acids remained constant under UVB. For *S. subspicatus* the ratio indicated a shift to relatively more saturated fatty acids. The observed shifts in fatty acid composition under UVB stress seem to be species specific, whereas the observed decrease in total fatty acid content under UVB stress seems to be more general. This is confirmed by fatty acid analyses of *Rhodomonas lacustris*, *Stephanodiscus hantzschii* and *Synedra tenuis* (Chapter 3). Total fatty acid content decreased under UVB radiation in these species and the PUFA content, especially the ω3 fatty acid content, decreased. Analysis of marine diatoms showed that the levels of EPA and DHA are reduced under UVB, but the degree of reduction varies between species (Wang & Chai 1994). Total lipid content is also reduced as result of UVB. Total lipid content in the marine prasinophyte *Tetraselmis* sp. decreased under UVB exposure, with a shift to relatively more saturated fatty acids (Goes *et al.* 1994, Wang & Chai 1994).

In these experiments, *D. pulex* had the highest growth rate when fed *S. acutus*, *D. magna* had the highest growth rate on *C. reinhardtii*. The unsaturated fatty acids EPA and DHA were not detected in *S. acutus* or in *C. reinhardtii*. This suggests that in these experiments EPA and DHA were not the determining factor for the population growth of *Daphnia*, contradicting with Müller-Navarra (1995a).

There are two possible explanations for the lower growth rate of *D. pulex* feeding on *S. subspicatus* compared with the other phytoplankton species. First, the fatty acid composition has relatively more saturated fatty acids. Second, the spines of *S. subspicatus* can possibly reduce the ingestion rate. This is not further tested in this study. As result of UVB radiation, the fatty acid composition of *S. subspicatus* changes to even more saturated fatty acids. This may explain the significantly reduced population growth rate of *D. pulex* feeding on UVB irradiated *S. subspicatus*.

The life history experiments were conducted with non-limiting food concentration in control and UVB treatment. Differences in life history traits are therefore only caused by

differences in food quality. However, in nature food may become limiting as result of decreased primary production (*e.g.* Gala & Giesy 1991). A reduction in food quantity was not accounted for in the set-up of our experiments. It might enhance the effects shown here, and might result in even smaller population growth rate, smaller adults and smaller offspring. In the absence of predation, *Daphnia* tend to reach levels where food is limiting and growth is low. Thus productivity is more likely to affect the carrying capacity than the growth rate or to affect both.

5.5 CONCLUSIONS

Daphnia life history traits were negatively affected by UVB irradiated phytoplankton. The magnitude of this effect was small and species specific. Effects on the population growth rate were only minor. Effects on clutch sizes and quality of offspring were more pronounced. In general, less juveniles of poorer quality were produced in the UVB treatments. This has implications for the food web functioning. The cause of these UVB effects may be a change in food quality combined with reduced digestibility of UVB irradiated phytoplankton.

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CHAPTER 6

EFFECTS OF UVB RADIATION ON EXPERIMENTAL AQUATIC MICROCOSMS

This chapter is based on:
De Lange H.J., Verschoor A.M., Gylstra R., Cuppen J.G.M. & Van Donk E. The effects of artificial UVB radiation on experimental aquatic microcosms. Submitted.

Zes dagen bijver zijn als nijen,
Op Vrondag vredig liggen zijen,
Dat maakt een woede geek.

(Rudy Kousbroek - Larkensviedje)

6.1 INTRODUCTION

Initially, most research on the effects of enhanced levels of UVB radiation was focused on marine ecosystems in the Southern Ocean (Smith *et al.* 1992, Weiler & Penhale 1994). Reports of increases in UVB radiation for both north and south temperate latitudes increased interest in freshwater ecosystem response (Williamson 1995, Williamson & Zagarese 1994). This far, most emphasis has been put on the effects of UVB radiation on single species of phytoplankton, and include DNA damage, inhibition of photosynthesis and growth rate reduction (Chapter 1).

When studying trophic interactions, the phytoplankton-zooplankton interaction receives great interest because it is an important step in the pelagic foodchain. There are different mechanisms by which UVB irradiated phytoplankton may affect herbivorous zooplankton (see Chapters 3, 4 & 5). Few studies have examined aquatic community response to UVB radiation (Bothwell *et al.* 1994, Cabrera *et al.* 1997, Halac *et al.* 1997, Keller *et al.* 1997, Worrest *et al.* 1981). These studies on trophic interactions show that indirect effects of UV radiation on foodweb interactions may play an important role in ecosystem response to UV radiation.

The long-term effects of UVB-radiation on ecosystem response was tested in indoor freshwater microcosms. The experiment was designed to imitate a clear, mesotrophic, macrophyte-dominated system, specifically a shallow drainage ditch. Such systems are very common in the Netherlands and can be compared with other shallow, macrophyte-dominated systems. The hypothesis in the experiment was that phytoplankton communities would be inhibited by exposure to low and realistic doses of UVB radiation. Further, the zooplankton community would not be affected directly by UVB radiation, but indirectly, as a result of a change in phytoplankton quantity and quality. This hypothesis was tested in an additional laboratory bio-assay, where *Daphnia pulex* was used as a standard grazer, feeding under standard conditions on seston from the microcosms.

Microcosms were chosen as a tool to study the effects of UVB stress on a multitrophic system. Microcosms, and model ecosystems in general, are widely used in stress-ecology (Brock *et al.* 1995). The advantage of microcosms versus single species tests is that interactions are incorporated between various species, between trophic levels, and between the biotic and abiotic components. The disadvantage is that results integrate individual direct and indirect effects, and are therefore harder to interpret. The advantages of microcosms *versus* field tests are that they allow for experimental control and replication (Brock *et al.* 1995).

6.2 MATERIALS AND METHODS

6.2.1 Experimental design

Twelve microcosms were situated in a climatized room (temperature 20 ± 2 °C). Each microcosm consisted of a glass aquarium (length 1.1 m, width 1.1 m, height 0.7 m, water volume 600 litres), filled with a 5 cm layer of sandy clay lake sediment and a 50 cm water column (groundwater). High pressure metal halide lamps (Philips HPI-T, 400 W) were used to provide artificial daylight, with an average light intensity of 120 µmol m^{-2} s^{-1} at the water surface, and a daily photoperiod of 16 h. Details of the construction can be found in Brock *et al.* (1992a).

The twelve microcosms were positioned in two rows of six. Six duplicate UVB treatments were divided in a randomized block design. The microcosms were exposed to UVB for 8 weeks. Samples were taken before the UV exposure period (week 0) and at 1 week intervals thereafter (week 1 to 9). The experiment started by switching on the UVB lamps.

Prior to the experiment, macro-invertebrates, planktonic organisms and macrophytes (*Elodea nuttallii*) were obtained from an undisturbed ditch and introduced into the microcosms. The experimental biocoenosis developed over an acclimation period of 10 weeks. Meanwhile, all microcosms were interconnected and water circulated at a flow rate of 3.8 l min^{-1} to achieve similarity between the planktonic communities in the systems. The flow rate was sufficient to mix the volume of one microcosm in 1.5 hours, and to circulate the twelve microcosms within 55 hours (Hartgers *et al.* 1998).

Nutrients were added as NH_4NO_3 and as KH_2PO_4 weekly or twice a week to maintain low concentrations of N and P (concentrations in the microcosm when added were 90 µg N l^{-1} and 15 µg P l^{-1}). Bicarbonate (as $NaHCO_3$, concentration in the microcosm when added was 2.4 mg C l^{-1}) was added twice a week in the first 4 weeks of the experiment. Nitrogen to phosphorus ratios were chosen to prevent blooms of nitrogen-fixing cyanobacteria.

6.2.2 UVB treatment

The UVB radiation above each microcosm was provided by 2 Philips TL12/20W tubes. Absolute radiation figures will be discussed first, in the last paragraph the biologically effective doses will be discussed. The UVB exposure period was 3 hours per day, centred in the middle of the PAR light period. UVC radiation from the tubes was removed with cellulose acetate foil. The foil was preburned for 5 hours and changed every 5 days to ensure constant UVB intensity. UVB intensities were measured at the water surface. The UVB intensities were calculated with a radiation model described by Veen (1995), based on a model by Gregg & Carder (1990). Surface UVB and PAR intensities for 52 °N in May (Julian day 140) at noon for cloudless conditions were calculated for several ozone concentrations, and the ratio of UVB to PAR was calculated (Table 6.1). These ratios were used as guideline to obtain the

desired UVB intensity relative to the PAR intensity of 120 μE m^{-2} s^{-1} available for the microcosms (Table 6.2, Fig. 6.1). The ratio of UVB to PAR is chosen as the determining factor in comparing the artificial UVB and PAR radiation with natural solar radiation. The UVB:PAR ratio is important in the mechanistic processes determining the balance between UVB damage and repair processes (Mitchell & Karentz 1993). It also compromises desired absolute PAR and UVB intensities and practical limitations of the light sources. The UVB radiation was kept at the noon ratio intensity for 3 hours. With this square-wave design we hoped to achieve daily UVB dose levels comparable with the natural situation.

Table 6.1: Calculated UVB and PAR irradiance at noon, 52 °N, day 140, cloudless sky, for several ozone concentrations (Veen 1995).

Ozone concentration (DU)	PAR (W m^{-2})	UVB (W m^{-2})	ratio UVB:PAR (%)
350	360	2.50	0.69
320	363	2.65	0.73
280	371	2.87	0.77
210	372	3.36	0.90
140	374	4.05	1.08
70	376	5.21	1.39
0	377	9.25	2.45

Solar radiation levels of PAR, UVA and UVB measured at Wageningen (51°58' N; 5°40' E) in May at noon under clear sky are given for comparison (Table 6.2, Fig. 6.1). The unweighted level of UVB radiation in the highest treatment is *ca.* 50% of the solar UVB radiation. The PAR and UVA intensities are *ca.* 10% of the solar levels.

When regarding the UVB:PAR ratio, treatment 2 is comparable with the ratio measured at Wageningen, the ratio in treatment 5 is higher than can be expected without ozone layer. In the experiment PAR and UVB intensities are constant during the light period. Under natural conditions, due to changing solar zenith angle, more UVB radiation is absorbed by stratospheric ozone early and later in the day compared with solar noon (Bothwell *et al.* 1993). Changes in cloud cover can reduce solar noon UVB levels below 0.1 W m^{-2} and PAR levels below 250 μE m^{-2} s^{-1} (De Lange unpubl.). Our constant light intensities did not take into account these natural fluctuations in intensity, but are an approximation of the average UVB and PAR intensities.

Table 6.2: UVB irradiance and daily cumulative UVB dose for each treatment in (W m^{-2}), unweighted (unw.) and weighted with Plant action spectrum (Plant; Caldwell 1971), DNA action spectrum (DNA; Setlow 1974), and photosynthesis inhibition (PI; Cullen *et al.* 1992). Between brackets is the UVB irradiance as percentage of PAR irradiance (120 µE m^{-2} s^{-1} ≡ 25.7 W m^{-2}). Solar irradiance data are from measurements at noon and clear sky, at Wageningen (the Netherlands, 52°N, 5°E), 11 May 1998. Between brackets the solar UVB irradiance as percentage of PAR (345 W m^{-2}).

	UVA (W m^{-2})	UVB (W m^{-2})			
treatment	unw.	unw.	Plant	DNA	PI
control	3.72	0.00 (0%)	0.00 (0%)	0.00 (0%)	0.00 (0%)
1	3.95	0.08 (0.31%)	0.02 (0.08%)	0.02 (0.08%)	0.03 (0.12%)
2	3.93	0.13 (0.51%)	0.04 (0.16%)	0.04 (0.16%)	0.05 (0.19%)
3	4.03	0.32 (1.25%)	0.11 (0.43%)	0.09 (0.35%)	0.14 (0.54%)
4	4.16	0.50 (1.95%)	0.18 (0.70%)	0.15 (0.58%)	0.22 (0.86%)
5	4.42	0.89 (3.46%)	0.31 (1.21%)	0.26 (1.01%)	0.38 (1.48%)
Solar	37.9	1.82 (0.53%)	0.16 (0.05%)	0.06 (0.02%)	0.38 (0.11%)
		Daily UVB dose (kJ m^{-2} d^{-1})			
treatment		unw.	Plant	DNA	PI
control		0.00	0.00	0.00	0.00
1		0.83	0.23	0.19	0.32
2		1.37	0.45	0.38	0.57
3		3.44	1.17	0.97	1.47
4		5.41	1.89	1.63	2.38
5		9.61	3.33	2.79	4.15

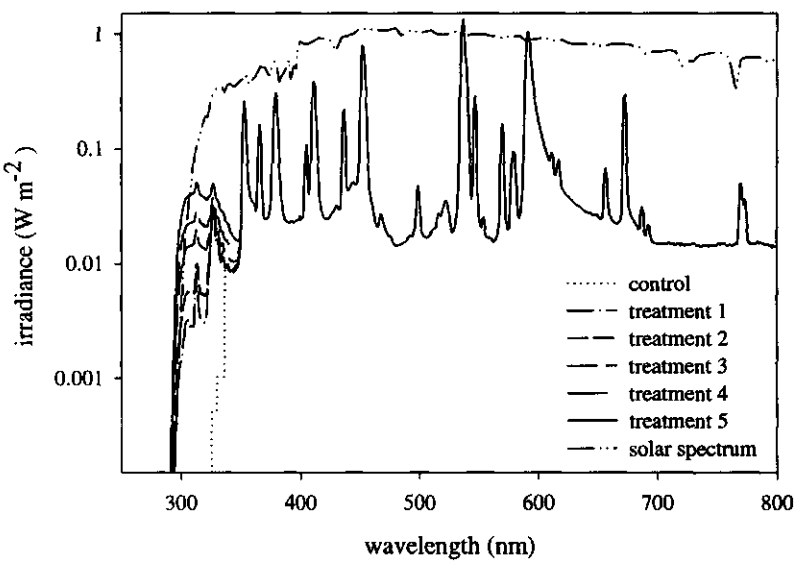

Figure 6.1: Irradiance spectra for each treatment at the watersurface (see Table 6.2).

Biological effects of absorbed UV radiation are generally a function of wavelength, with shorter wavelengths having a larger deleterious effect. The artificial UVB radiation in the experiments contained relative to solar radiation shorter wavelengths at higher intensities. Therefore, the biological effective irradiance was calculated with 3 different action spectra: the DNA action spectrum (Setlow 1974), the general plant action spectrum (Caldwell 1971), and the photosynthesis inhibition action spectrum (Cullen *et al.* 1992). All action spectra were normalized to 1 at 300 nm (Table 6.2).

Scattering and attenuation processes give a heterogeneous irradiance field in the microcosm, which makes it more difficult to precisely define the UVB irradiance in the system. This heterogeneity is comparable with natural systems. Therefore the UVB intensities were measured at the water surface.

6.2.3 Measurements

Irradiation spectra (range 280 to 800 nm, interval of 1 nm) were measured several times during the experiment using a Macam SR9910 spectroradiometer with a flat cosine head. Measurements were made just above and below the watersurface, and at depth intervals of 10 cm. Care was observed that the measurements were not influenced by the macrophytes. Attenuation coefficients K_d for PAR (400-700), UVA (320-400 nm) and UVB (280-320) were calculated according to Kirk (1994).

Temperature (T, °C), dissolved oxygen (DO, mg l^{-1}), pH, electric conductivity (µS cm^{-1}) and alkalinity (meq l^{-1}) were measured weekly following UVB exposure. Measurements were made at a water depth of 10 cm using a WTW Oximeter (OXO 196), a WTW pH meter (pH 196) and a WTW Conductometer (LF 191). Alkalinity estimation was done by titration of 100.0 ml of water sample with 0.05 N HCl to pH 4.4.

Weekly water samples were analysed for nutrients (NO_3^-, NO_2^-, NH_4^+ and PO_4^{3-}) using a Skalar SA40 autoanalyser. Organic and inorganic carbon were analysed in three size fractions using a TOC analyser model 700, O.I. Analytical; dissolved (<0.2 µm), filtered over 3µm, and total (unfiltered) carbon. Humic acids were estimated using a qualitative approach by measuring the extinction at 250 nm (E_{250}) and 365 nm (E_{365}), using a Beckman DU-64 spectrophotometer. The ratio E_{250}/E_{365} is a qualitative indication for humic acids with a ratio of around 5 indicating the presence of mostly large humic acid molecules, and a ratio of around 9 indicating the presence of mostly small humic acid molecules (De Haan & De Boer 1987).

Phytoplankton was sampled weekly, taking depth-integrated water samples with a perspex tube (length 40 cm, volume 1 l). Five samples were taken (total *ca.* 5 l) and used for the study of the species composition (1 l) and chlorophyll-*a* analysis (1 l). Samples were preserved with 3 ml of Lugol's solution and stored in the dark in bottles for sedimentation.

After 4 days, 90% of the water of each sample was siphoned and algae were thus concentrated 10-fold. Phytoplankton identification and enumeration was done at bi-weekly intervals (weeks 0, 3, 5, 7, 9) using an inverted microscope (Reichert, at 240x magnification). Counts were made in duplicate, to a total of 400 organisms. Seston biovolume concentration was measured weekly with an electronic particle counter (Coulter Multisizer II) for the size range of 2 to 3 µm equivalent spherical diameter (ESD; 'bacterioplankton'), and 3 to 30 µm ESD ('edible fraction'). For the chlorophyll-*a* analysis 1 litre of water was filtered through a GF/C filter (Schleicher & Schuell, pore size 1.2 µm). Chlorophyll-*a*, extracted with ethanol at 70 °C, was analysed spectrophotometrically at 750 and 665 nm, with correction for phaeopigments (Moed & Hallegraeff 1978).

Zooplankton was sampled weekly by taking depth-integrated water samples as described above. A sample volume of exactly 5 litres was concentrated using a nylon zooplankton net (mesh size 30 µm) and preserved in 80% ethanol. Zooplankton identification and enumeration was done at bi-weekly intervals (weeks 0, 2, 4, 6, 8, 9) at 40x magnification using an inverted microscope. An approximate conversion to biomass was made by multiplying individual numbers with literature values of dry weight per individual (rotifers: Stemberger & Gilbert 1985; cladocerans: Guisande & Gliwicz 1992, Pont *et al.* 1991, Sterner 1993; copepods: Adrian & Frost 1992, Hart 1990).

The macro-invertebrates were studied bi-weekly (weeks 0, 2, 4, 6, 8) using 2 multiplates and 2 pebble-baskets placed at the bottom of the microcosms as artificial substrates (Brock *et al.* 1992a). Animals were counted and identified alive, then released back into the corresponding microcosm. The artificial substrate protected the animals from UVB radiation.

Periphyton was sampled bi-weekly using glass slides (7.6 by 2.6 cm) as artificial substrate. The glass slides were positioned vertically in a transparent perspex frame, at 2 cm depth, and had been exposed for 4 weeks before sampling. Direction of orientation was vertical, so surface exposed to UVB radiation was not maximal. Species composition and total biomass as dry weight and chlorophyll-*a* concentration were measured bi-weekly (weeks 0, 2, 4, 6, 8). Macrophyte biomass as dry weight was measured at the end of the experiment.

6.2.4 Bio-assay with *Daphnia pulex*

With a laboratory clone of *Daphnia pulex* the indirect effects of UVB on zooplankton growth and fecundity, caused by possible changes in seston quantity and/or quality, were tested. Twenty juvenile *D. pulex* (within 12 hours of birth) were placed in an aquarium, filled with 1 litre water from one of the 12 microcosms. The water was filtered over a mesh size of 30 µm, to remove the microcosm zooplankton. This way, *D. pulex* was fed with the seston fraction smaller than 30 µm in exactly the same concentration and composition as present in that microcosm. In total, 12 aquaria (from 12 microcosms) with 6 food qualities in duplicate, ranging from control to treatment 5, were placed in a temperature regulated room at 20 ± 1 °C

and 120 µmol m^{-2} s^{-1} PAR at 16:8 L:D. Every second or third day, water and food was refreshed, survival was recorded, length of every female was measured, development of eggs per female, and total number of neonates born per aquarium during the experiment (N) were recorded. The bioassay lasted 19 days and was conducted in weeks 3 to 5 of the microcosm experiment.

With the data on survival (l_x) and reproduction per female (m_x) for each time interval, a life table was constructed (Stearns 1992). The net reproductive rate R_0 ($=\Sigma l_x m_x$) could then be calculated. The somatic growth rate g_s (d^{-1}) for the juvenile instars was calculated with the length data, as:

$$g_s = (\ln(l_6/l_0))/6$$

where l_6 is length after 6 days, and l_0 is length at the start.

6.2.5 Data analysis

The effects of the UVB treatment on biological, physical and chemical variables were tested with Anova. Biological data were ln-transformed (ln(ax+1)), as described in Van den Brink *et al.* (1995). In addition, univariate analysis was also carried out using the Williams test (Williams 1971) to test for the effects of UVB radiation on each of the individual taxa of phytoplankton, zooplankton, periphyton and macro-invertebrates, and on physical and chemical variables. The Williams test was performed for each species at each sampling date. Treatment effects were considered statistically significant from the control at p-values <0.05, and when significance occurred at 2 consecutive sampling dates. The Williams test assumes an increasing effect with increasing dose.

Multivariate analysis of the phytoplankton, zooplankton, periphyton and macro-invertebrate community was done by using Principal Response Curves (PRC) based on Redundancy Analysis (RDA) using the CANOCO computer program, version 3.14 (Ter Braak 1988, 1990). The PRC method is introduced and explained in Van den Brink & Ter Braak (1999). The RDA method places more emphasis on abundant species than on rare species, and assumes a linear response. The PRC method then distils the complexity of time-dependent, community-level effects of a stress factor to a graphic form that is easy to comprehend. The control treatment is taken as reference to which the other treatments are contrasted, and time is defined as the horizontal axis of the diagram. The significance of the PRC diagram was tested by performing a Monte Carlo permutation of the microcosms, by permuting whole time series, using an F-type test statistic based on the eigenvalue of the component.

Results from the bio-assay were tested with the Williams test, and with Anova. Seston variables during the bio-assay were compared with somatic growth rate and net reproductive rate using linear regression (Genstat 1993). Influence of the UVB treatment on R_0 and g_s was tested using Spearman Rank Correlation (Fowler & Cohen 1990).

6.3 RESULTS

6.3.1 PAR and UV radiation measurements

The light spectrum at the watersurface for each treatment, and for comparison the solar spectrum measured in Wageningen, is given in Fig. 6.1. The wavelengths below 305 nm are higher in the treatments than in the solar spectrum. The UVA and PAR part of the solar spectrum have higher intensities than the treatments.

Vertical attenuation coefficients for PAR (400-700 nm), UVA (320-400 nm) and UVB (280-320 nm) were calculated for each microcosm (Table 6.3). There was some variation between replicate microcosms, but not significant (Anova, $p>0.05$). The UVB radiation at bottom depth was very low in all treatments. From the K_d value for UVB follows a 1% UVB depth of 30 cm. The actual exposure levels for organisms in the microcosms are more difficult to assess since it also depends on their position in the water column.

Table 6.3: Vertical attenuation coefficients (K_d) ± 1 SD for PAR, UVA and UVB radiation for each treatment, and the absolute UVB irradiance at 10 cm depth and at bottom depth (45 cm).

Treatment	K_dPAR (m^{-1})	K_dUVA (m^{-1})	K_dUVB (m^{-1})	UVB -10 cm ($W\ m^{-2}$)	UVB -45 cm ($W\ m^{-2}$)
control	2.4 ± 0.8	8.0 ± 0.4	-	0	0
1	1.8 ± 0.1	8.3 ± 2.9	19.6 ± 5.1	$2.98\ 10^{-3}$	$1.65\ 10^{-6}$
2	1.9 ± 1.8	7.4 ± 3.1	15.4 ± 1.9	$4.03\ 10^{-3}$	$9.49\ 10^{-6}$
3	2.1 ± 0.5	7.5 ± 0.4	15.5 ± 1.9	$1.88\ 10^{-2}$	$4.24\ 10^{-5}$
4	2.8 ± 1.4	7.2 ± 0.2	15.3 ± 0.8	$4.53\ 10^{-2}$	$1.45\ 10^{-4}$
5	1.6 ± 0.1	6.7 ± 1.3	13.7 ± 3.3	$9.21\ 10^{-2}$	$4.40\ 10^{-4}$

6.3.2 Physical and chemical variables

Average values of the physical and chemical variables are given in Table 6.4. Variation in time was larger than the variation between treatments. Variables T, DO, electric conductivity, pH and alkalinity did not vary significantly during the experimental period or among treatments (Anova, $p>0.05$). Temperature decreased slightly during the experiment. Nutrients varied according to the weekly supplementation.

The inorganic carbon concentration showed a strong relationship with the added bicarbonate, but not with the UVB radiation treatment. Similarly, organic carbon concentrations increased slightly during the experiment, but no effects of UVB radiation could be detected. In all treatments, humic acid absorption ratios decreased slightly during the experimental period, from around 6.5 to around 5.5. The decrease in ratio occurred in all microcosms, with no apparent influence of effects of UVB treatment (Anova, $p>0.05$). The high value of chlorophyll-*a* concentration in treatment one is caused by the bloom of *Volvox aureus* occurring in one microcosm.

Table 6.4: Physical and chemical variables during the experimental period for each treatment ± 1 SD.

Variable	control	treatment 1	treatment 2	treatment 3	treatment 4	treatment 5
temperature (°C)	20.4 ± 1.4	20.4 ± 1.3	20.3 ± 1.3	20.4 ± 1.2	20.0 ± 1.5	20.3 ± 1.2
DO (mg l^{-1})	10.9 ± 1.3	10.5 ± 0.7	10.4 ± 1.1	10.6 ± 0.9	10.5 ± 1.0	10.8 ± 1.3
pH (-)	9.4 ± 0.7	9.1 ± 0.4	9.1 ± 0.7	9.2 ± 0.5	9.1 ± 0.5	9.2 ± 0.7
conductivity (µS cm^{-1})	244 ± 24	254 ± 31	251 ± 29	252 ± 32	255 ± 29	245 ± 22
alkalinity (meq l^{-1})	1.15 ± 0.22	1.27 ± 0.31	1.21 ± 0.29	1.25 ± 0.34	1.29 ± 0.31	1.18 ± 0.21
humic ratio (-)	6.2 ± 0.9	6.0 ± 1.1	6.1 ± 0.9	5.6 ± 1.3	6.1 ± 1.0	6.2 ± 0.9
DOC <0.2 µm (mg l^{-1})	4.6 ± 1.2	5.3 ± 1.8	4.3 ± 0.9	4.9 ± 0.7	4.5 ± 1.5	4.1 ± 0.9
DOC <3 µm (mg l^{-1})	4.9 ± 1.5	5.8 ± 1.5	4.7 ± 1.1	5.5 ± 1.7	4.4 ± 0.8	4.7 ± 1.2
TOC (mg l^{-1})	5.4 ± 1.4	6.1 ± 1.4	4.8 ± 1.0	5.7 ± 1.5	5.0 ± 1.2	4.8 ± 1.2
DIC <0.2 µm (mg l^{-1})	12.7 ± 2.5	14.6 ± 3.8	13.9 ± 3.7	14.3 ± 4.4	14.7 ± 4.0	13.6 ± 2.4
DIC <3 µm (mg l^{-1})	12.9 ± 2.6	14.6 ± 3.9	14.0 ± 3.7	14.3 ± 4.5	14.8 ± 4.1	13.7 ± 2.4
TIC (mg l^{-1})	13.2 ± 2.5	14.9 ± 3.8	14.1 ± 3.7	14.6 ± 4.5	15.1 ± 3.8	13.8 ± 2.3
Chlorophyll-*a* (µg l^{-1})	5.8 ± 8.8	11.1 ± 12.4	5.8 ± 6.5	6.9 ± 5.3	3.3 ± 1.6	4.7 ± 4.5
$NO_3^- + NO_2^-$ (mg N l^{-1})	0.02 ± 0.03	0.02 ± 0.03	0.04 ± 0.07	0.02 ± 0.04	0.03 ± 0.04	0.03 ± 0.06
NH_4^+ (mg N l^{-1})	0.07 ± 0.08	0.07 ± 0.04	0.08 ± 0.06	0.05 ± 0.03	0.06 ± 0.03	0.06 ± 0.06
PO_4^{3-} (µg P l^{-1})	1.5 ± 4.9	2.5 ± 7.8	0.4 ± 1.3	1.1 ± 2.8	0.7 ± 2.5	0.8 ± 2.1

6.3.3 Biological variables

Phytoplankton numbers were low and variable throughout the experiment. Biovolume estimates for edible algae (3 to 30 µm ESD) showed variation during the experiment without influence of the UVB treatment (Fig. 6.2; Anova, $p>0.05$).

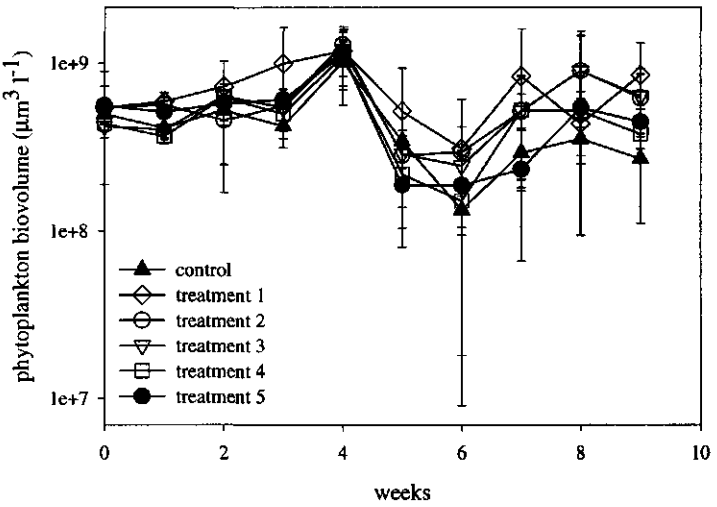

Figure 6.2: Total phytoplankton biovolume concentration ($\mu m^3 l^{-1}$) per treatment in time, error bar indicates 1 SD.

The phytoplankton community was dominated by the chlorophytes *Chlamydomonas* sp. and *Volvox aureus* (Fig. 6.3B). A bloom of *V. aureus* occurred in four of the microcosms (Fig. 6.3E). At the start of the experiment, cryptophytes *Cryptomonas* sp. and *Rhodomonas* sp. were abundant, but these decreased during the course of the experiment (Fig. 6.3C). Diatoms and cyanobacteria were low in abundance throughout the experiment (Figs. 6.3A & 6.3D). Univariate analysis (Williams test) did not show significant effects of the UV treatment on any individual species abundance.

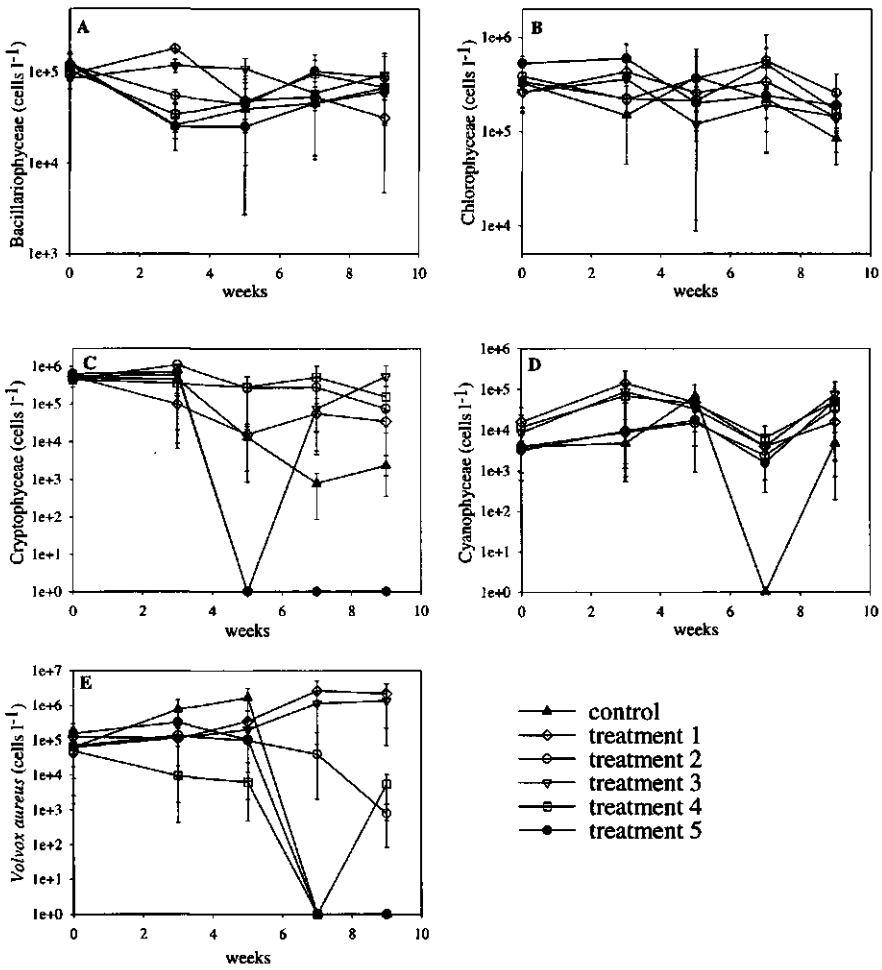

Figure 6.3: Phytoplankton abundance (cells ml^{-1}) per treatment in time of Bacillariophyceae (panel A), Chlorophyceae (panel B), Cryptophyceae (panel C), Cyanophyceae (panel D), and *Volvox aureus* (panel E), error bar indicates 1 SD.

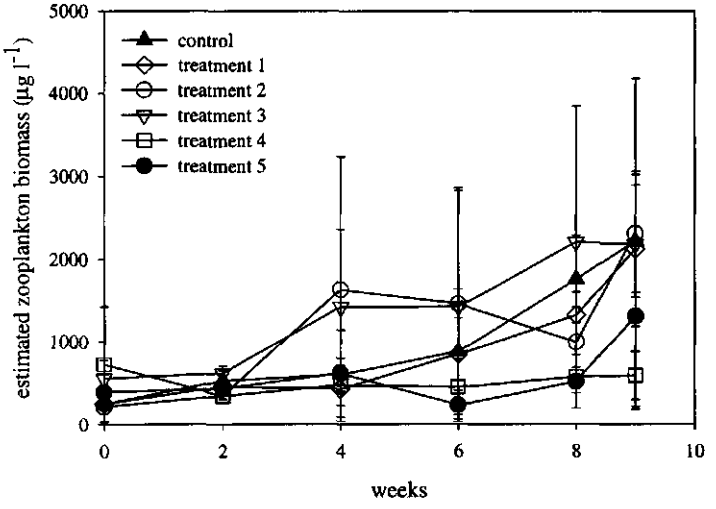

Figure 6.4: Estimated zooplankton biomass ($\mu g\ l^{-1}$) per treatment in time, error bar indicates 1 SD.

Zooplankton biomass fluctuated between treatments and during the experiment. The differences were not significant (Fig. 6.4; Anova, $p>0.05$). The cladocerans showed an increase in abundance during the experiment (Fig. 6.5A). Cladoceran abundance in week 2 was negatively affected by the UVB treatment (Anova, $p<0.05$). This, however, could not be demonstrated for individual taxa, including the dominant species *Daphnia galeata* var. *longispina* and *Chydorus sphaericus*. Rotifers were constantly present in abundances of *ca.* 150 l^{-1} (Fig. 6.5B). Dominant species were *Brachionus* sp., *Polyarthra* sp. and *Lecane* sp., total rotifer abundance was unaffected by UVB-radiation (Anova, $p>0.05$). Copepods were dominated by cyclopoids. The total number of copepods decreased slightly during the experiment, without significant effects of the UVB treatment (Anova, $p>0.05$; Fig. 6.5C). Ostracods increased in abundance during the experiment (Fig. 6.5D). This increase was largest in the control microcosms, although differences were not significant (Anova, $p=0.07$). Univariate analysis (Williams test) did not show significant effects of the UVB treatment on any individual species abundance.

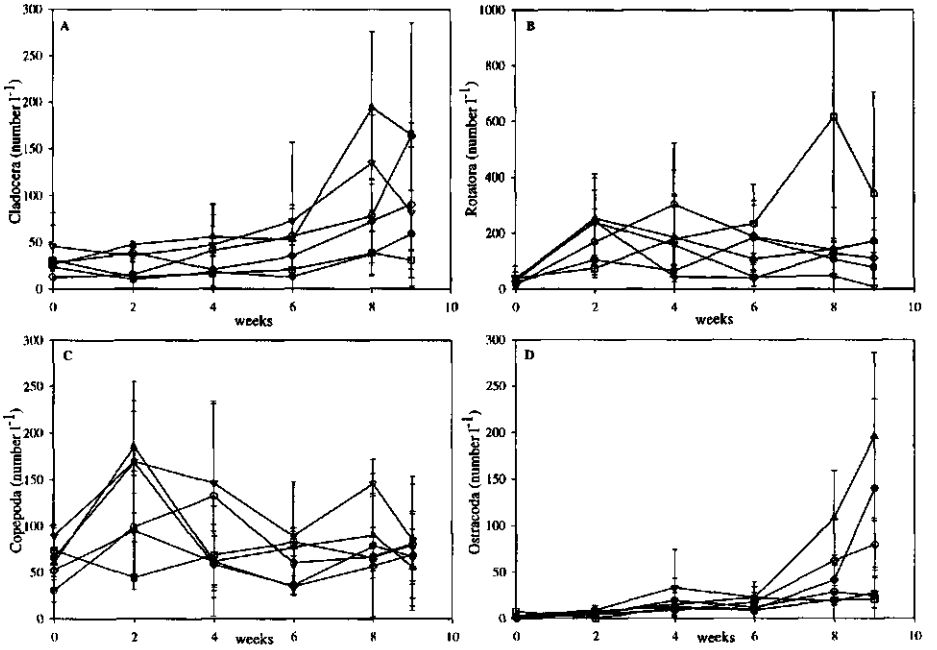

Figure 6.5: Zooplankton abundance (individuals l^{-1}) per treatment in time of Cladocera (panel A), Rotatora (panel B), Copepoda (panel C) and Ostracoda (panel D), error bar indicates 1 SD, symbols as in Fig. 6.4.

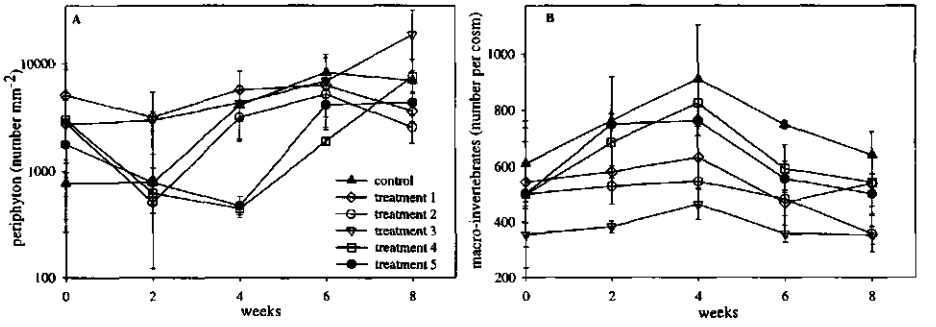

Figure 6.6: Periphyton abundance (number mm^{-2}) per treatment in time (panel A), and macro-invertebrate abundance (number $cosm^{-1}$) per treatment in time (panel B), error bar indicates 1 SD.

Periphyton abundance and biomass fluctuated throughout the experiment, and changes were not related to the UVB treatment (Fig. 6.6A, Anova, $p>0.05$). Periphyton was dominated by chlorophytes and diatoms. Univariate analysis (Williams test) did not show significant effects of the UVB treatment on any individual species abundance.

Macro-invertebrate abundance fluctuated between treatments and during the experiment (Fig. 6.6B). Differences were small and not significant (Anova, $p>0.05$). Detritivores as *Asellus aquaticus* and *Gammarus pulex* were dominant. *Proasellus coxalis* was negatively affected by the UVB treatment in weeks 6 and 8 (Williams test, lowest observed effect level at treatment 1). *Dugesia lugubris* was negatively affected by the UVB treatment in weeks 2 and 4 (Williams test, lowest observed effect level at treatment 1).

The macrophytes were dominated by *Elodea nuttallii*. Mean total biomass per treatment level at the end of the experiment varied between 82 and 136 g dry weight per microcosm. The UVB treatment had no significant effect on the macrophyte biomass (Anova, $p>0.05$).

All data were analysed with Anova, no block effect in the randomized block design was observed for any of the variables. Multivariate analysis showed no significant effect of the UVB treatment on the phytoplankton community, the zooplankton community, the periphyton community, nor the macro-invertebrate community (Table 6.5). Monte Carlo permutation tests showed that none of the PRC plots was significant.

Table 6.5: Results CANOCO analyses using PRC, percentage of variance explained by time, treatment and replicate, and significance of PRC.

	time	treatment	replicate	significance
phytoplankton	34%	28%	38%	$p>0.05$
zooplankton	39%	26%	35%	$p>0.05$
periphyton	23%	39%	38%	$p>0.05$
macro-invertebrates	32%	32%	34%	$p>0.05$

6.3.4 Bio-assay with *Daphnia pulex*

Daphnia pulex feeding on phytoplankton from the control microcosms had the best survival (Fig. 6.7, Table 6.6). Survival to the end of the experiment was significantly lower in treatments 2 to 5, compared with the control (Williams test, $p<0.05$; lowest observed effect level at treatment 2).

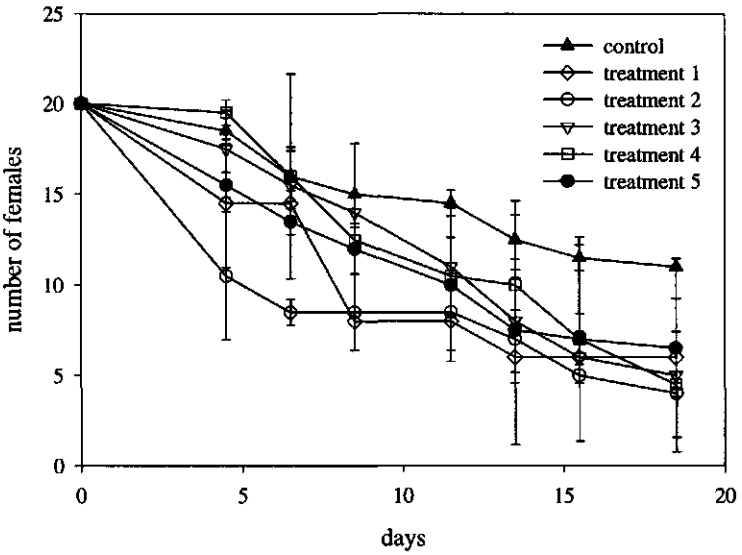

Figure 6.7: Survival of *Daphnia pulex* in the bio-assay per treatment in time, error bar indicates 1 SD.

Table 6.6: Results of the bio-assay, where L1 is length ± 1 SD at day 6, L2 is length ± 1 SD at end of experiment, S1 is survival to day 6, S2 is survival to end of experiment, N is total number of neonates, g_s is the somatic growth in the first 6 days, R_0 is the net reproductive rate; a or b indicates replicate.

treatment	L1 (mm)	L2 (mm)	S1 (%)	S2 (%)	N (n)	g_s (d^{-1})	R_0 (-)
controla	1.65 ± 0.21	1.99 ± 0.11	85	55	87	0.15	4.35
controlb	1.62 ± 0.11	1.87 ± 0.14	75	55	91	0.14	4.55
1a	1.58 ± 0.23	2.04 ± 0.04	70	25	67	0.14	3.35
1b	1.37 ± 0.16	1.82 ± 0.09	60	35	11	0.11	0.55
2a	1.38 ± 0.27	1.99 ± 0.10	40	20	52	0.12	2.60
2b	1.40 ± 0.16	2.08 ± 0.07	50	20	64	0.12	3.20
3a	1.75 ± 0.15	2.07 ± 0.10	70	40	56	0.16	2.94
3b	1.43 ± 0.14	1.84 ± 0.06	75	10	29	0.12	2.00
4a	1.60 ± 0.09	2.00 ± 0.04	60	25	65	0.14	3.25
4b	1.37 ± 0.14	1.81 ± 0.13	100	20	35	0.11	1.75
5a	1.22 ± 0.22	2.13 ± 0.05	65	15	61	0.09	3.05
5b	1.43 ± 0.17	1.88 ± 0.13	70	50	47	0.12	2.35

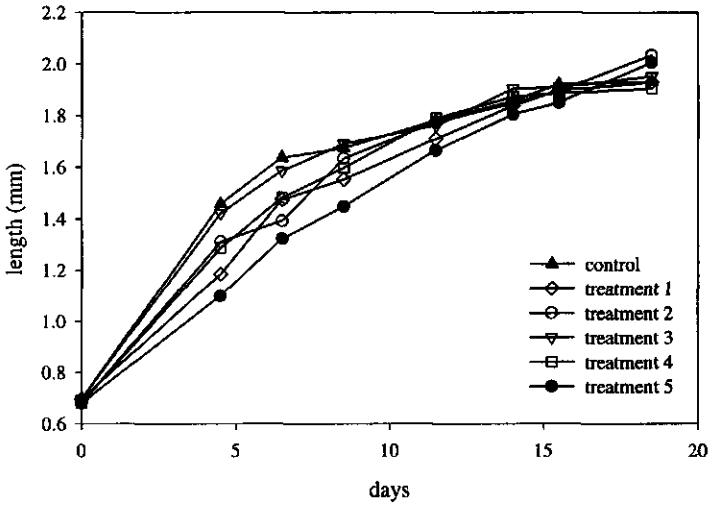

Figure 6.8: Carapace length of *Daphnia pulex* in the bio-assay per treatment.

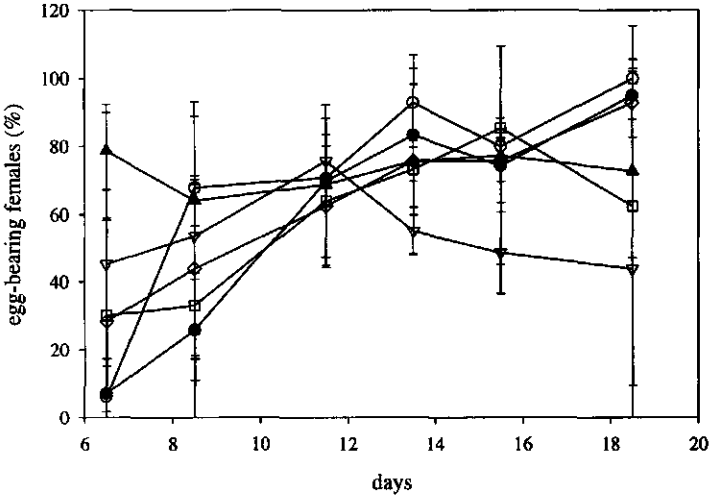

Figure 6.9: Egg-bearing females as percentage of surviving females, error bar indicates 1 SD, symbols as in Fig. 6.8.

Carapace length (Fig. 6.8, Table 6.6) was different in the first 9 days. Females in the control were larger than females in treatment 5. At t = 4 and t = 6 days females in treatment 5 were significantly smaller than in the other treatments (Williams test, $p<0.05$). After 12 days the females were of approximately equal size. Females in the control had the highest somatic growth rate for the juvenile instars (Table 6.6), but differences were not significant (Williams test, $p>0.05$). The Spearman Rank Correlation Coefficient between treatment and g_s was -0.39, which means that there was a significant weak negative relation between UVB treatment and somatic growth rate (Fowler & Cohen 1990).

Females in the control developed eggs from day 6 onwards (Fig. 6.9). Females in treatment 5 were retarded in reaching maturity. The number of eggs per female varied, but without apparent food quality or quantity effect. From day 11 onwards there was no significant difference in percentage egg-bearing females between control and treatments (Williams test & Anova, $p>0.05$).

Total number of neonates per aquarium during the experiment (N) was highest in the control, and significantly different from the treatments (Table 6.6, Williams test, $p<0.05$, lowest observed effect level at treatment 1). This is related with the higher number of survivors in the control.

The net reproductive rate (R_0) was highest in the control, and significantly different from the UVB treatments (Table 6.6, Williams test, $p<0.05$). This is mainly caused by a higher survival (l_x) of the females. The reproduction per female (m_x), for those surviving, is similar in all treatments. The Spearman Rank Correlation Coefficient between treatment and R_0 is -0.44, which indicates that there is a significant modest negative relation between UVB treatment and R_0 (Fowler & Cohen 1990).

The linear regression analyses showed that the seston variables had no significant relation with the somatic growth of *Daphnia*. Diatom abundance, biovolume concentration of the bacterioplankton, and the edible fraction had a significant negative effect on R_0 (Table 6.7). *Volvox* abundance in the microcosms, on the other hand, had a slightly positive effect on R_0. Both chlorophyll-*a* concentration and POC concentration had no effect on somatic growth or reproductive rate.

Table 6.7: Results of the significant linear regressions (p-values, R^2_{adj} and fitted equation) with seston variables as explanatory variables, and R_0 as response variable.

Explanatory variable	p-value	R^2_{adj} (%)	fitted equation: R_0 =
ln edible fraction ($\mu m^3\ l^{-1}$)	< 0.001	68.0	56.9 - 2.683 * ln edible fraction
ln bacterioplankton ($\mu m^3\ l^{-1}$)	< 0.001	67.9	48.0 - 2.53 * ln bacterioplankton
bacterioplankton ($\mu m^3\ l^{-1}$)	< 0.01	61.8	4.74 - 3.1 10^{-8} * bacterioplankton
edible fraction ($\mu m^3\ l^{-1}$)	< 0.01	58.4	4.74 - 3.1 10^{-9} * edible fraction
Bacillariophyceae (cells l^{-1})	< 0.01	49.3	3.56 - 9.8 10^{-4} * Bacillariophyceae
ln Bacillariophyceae (cells l^{-1})	< 0.01	45.7	8.62 - 0.93 * ln Bacillariophyceae
Cyanobacteria (cells l^{-1})	0.02	37.6	3.35 - 9.8 10^{-4} * Cyanobacteria
ln *Volvox* (cells l^{-1})	0.03	34.0	2.264 + 0.12 * ln *Volvox aureus*
ln POC (mg l^{-1})	0.03	31.7	2.465 - 0.45 * ln POC
ln Cyanobacteria (cells l^{-1})	0.04	31.1	3.61 - 0.18 *ln Cyanobacteria

6.4 DISCUSSION

No significant effect of UVB radiation was found on the phytoplankton community, zooplankton community, periphyton community or macro-invertebrate community. A few species showed a significant response at some of the sampling dates, but there was no negative UVB effect at community level. The few significant differences observed even may be explained as the result of the large number of tests carried out and the level of significance of 5%, resulting in a few significant differences by chance (Type I error).

The phytoplankton community was not affected in total biovolume estimates or in composition. This result can be compared with results from an enclosure experiment in a high-altitude mountain lake (Central Alps, 47 °N) that lasted for 16 days, in which no effect of UVB on the phytoplankton growth and species composition was found (Halac *et al.* 1997). However, in an outdoor mesocosm experiment in a high-altitude Andean lake (33 °S) that lasted 48 days, some effects on the phytoplankton community were found. The chlorophyte *Ankyra judayi* was more abundant in the UVB treatment, and the diatoms *Fragilaria construens* and *F. crotonensis* were more abundant in the UVB-excluded treatments (Cabrera *et al.* 1997).

The effect of UVB radiation on a phytoplankton community seems to depend on the species composition. Chlorophytes dominated the systems in this study, and these are generally less vulnerable to UVB than diatoms or cryptophytes (Gala & Giesy 1991). When diatoms dominate, negative responses to UVB radiation may be found (Worrest *et al.* 1981, Cabrera *et al.* 1997).

The highest dose in this experiment (2.79 kJ m^{-2} d^{-1} BED_{DNA}) can cause damage at single species level in laboratory experiments (Buma *et al.* 1995, Chapter 5). In field experiments, Gala and Giesy (1991) estimated that a cumulative UVB dose of 6.0 kJ m^{-2} d^{-1} (unweighted, comparable with our treatment 4) could reduce the primary production of spring phytoplankton assemblage by 25%. The exposure level in single-species experiments can be controlled better than in deeper systems. The actual exposure of organisms in the systems in this study is more difficult to assess. That no effect was found on the phytoplankton community could be a result of low actual UVB exposure, but, alternatively, it might be more resistant or adapted to UVB stress. Adaptation can happen on molecular level, *e.g.* by synthesizing protective pigments as mycosporine-like amino acids (Carreto *et al.* 1990, Karentz *et al.* 1991), carotenoids (Karentz *et al.* 1994, Paerl *et al.* 1985) or scytonemin (Garcia-Pichel & Castenholz 1991). Defence against toxic end products of UVB induced photochemical reactions is also possible by synthesizing scavenging molecules as carotenoids and enzymes as superoxide dismutase (Karentz *et al.* 1994). Phytoplankton organisms are unicellular and can reproduce asexually. As a result, responses to environmental stress, such as UVB radiation, will likely occur more rapidly than in larger zooplankton organisms, with generation times in the order of weeks (Karentz 1994).

The zooplankton community in this experiment was not significantly affected by the UVB treatment. Cladoceran abundance was negatively affected by UVB radiation only in

week two of the experiment, so any UVB effect is questionable. UVB radiation can be damaging to freshwater zooplankton in single-species assays, *e.g.* copepods (*Acanthodiaptomus denticornis*: Ringelberg *et al.* 1984), and cladocerans (*Daphnia*: Siebeck 1978, Zagarese *et al.* 1994). In a mesocosm study, Cabrera and co-workers found that UVB radiation strongly reduced the abundance of the cladoceran *Chydorus sphaericus* and the rotifer *Lepadella ovalis* (Cabrera *et al.* 1997). Direct effects of UVB radiation may be avoided by migrating zooplankton (*e.g.* Hessen 1994, Williamson 1995), this could be responsible for the lack of zooplankton community effects in our experiment.

The bio-assay showed some potential negative effects of UVB-treated seston on the growth, survival, and, to a lesser extent, reproduction of *Daphnia pulex*. The effects were most pronounced in the first 9 days of the experiment, especially on body length and percentage egg-bearing females (Figs. 6.8 and 6.9). This implies that the juvenile instars of *D. pulex* were more susceptible to differences in food than the surviving mature instars. The individuals that survived the first 9 days and reached maturity had similar fecundity and body length. However, since the total number of survivors was smaller in the treatments, total number of neonates (N) and the net reproductive rate (R_0) were smaller in the treatments. R_0 showed a stronger response than somatic growth (g_s) to differences in seston and UVB treatment, this might suggest a trade-off between somatic growth and future reproductive success. The results of the bio-assay are similar to results of life history experiments with *Daphnia pulex* feeding on UVB irradiated phytoplankton, where the intrinsic population growth rate showed a modest decrease in the UVB treatment (Chapter 5).

The higher somatic growth rate and net reproductive rate in the controls could not be explained by any of the seston variables. The explanation may be in the quality of the phytoplankton cells, in either biochemical composition or morphology, which may affect their ingestion, digestion or palatability. With the inverted microscope analysis of the phytoplankton we were not able to show differences in morphology. Unfortunately, the biochemical composition and stoichiometry of the seston was not analysed. The physical and chemical variables of the water in the microcosms were analysed, and proved not to be different for the UVB treatments. It therefore remains speculative which variable(s) was responsible for the differences in *D. pulex* growth. There is a difference in the results from the bioassay (indirect negative UVB effect on *Daphnia*) and from the microcosm (no UVB effect on zooplankton). The explanation for this discrepancy may be the multitude of zooplankton species in the microcosm ecosystems, compared with only one species in the bio-assay.

The question remains why no effect of the UVB treatment was found at the ecosystem level, in contradiction to the original hypothesis based on results from single-species experiments. Several differences between experimental and solar irradiance may have contributed to the lack of UVB response in our experiments. The surface irradiance level in the experiment was lower than solar surface irradiance, UVB levels (in the highest treatment) were 50% of solar irradiance, UVA and PAR *ca.* 10% of solar irradiance. The UVB:PAR ratio was higher than solar irradiance. The experimental design had a spectrally fixed light field, at constant intensities. In nature the intensity and spectral balance of sunlight may vary several-

fold with the solar zenith angle, cloud cover, ozone thickness, atmospheric conditions, etc. (Santas *et al.* 1997). The biologically effective UVB irradiance and daily UVB dose in the experiment, weighted with any of the three used action spectra, was higher than in nature. The UVB intensities were not extremely high, but was aimed to be comparable with current UVB intensities at temperate latitude. A point of discussion regarding the experimental design may be the spectral differences between the artificial spectrum and the solar spectrum. With this in mind, the results might not be an accurate prediction of natural community responses to increased solar UVB. This caution does not change the result of our experiment that the UVB treatments did not affect the ecosystems in the microcosms.

The UVB attenuation processes in the microcosms were similar to natural Dutch systems. DOC levels in the microcosms were low compared with Dutch waters, where values range between 5 and 40 mg DOC l^{-1} (see Chapter 2). According to the model described by Scully & Lean (1994), DOC levels are the most important factor in the attenuation of UV radiation in lakes. This is confirmed by other studies for North American lakes (Laurion *et al.* 1997, Morris *et al.* 1995, Williamson *et al.* 1996). For eutrophic systems in Japan, the UVB attenuation coefficient was only weakly correlated with DOC concentration (Hodoki & Watanabe 1998). The DOC concentration in our systems was *ca.* 5 mg l^{-1}, in which case the predicted UVB attenuation coefficient from Scully & Lean (1994) would be *ca.* 9 m^{-1}, but predicted from Hodoki & Watanabe (1998) it would be *ca.* 25 m^{-1}. In a survey of Dutch systems, UVB attenuation coefficients are rarely below 10 m^{-1}, DOC levels are generally well above 5 mg l^{-1}, and do not have a strong correlation with K_dUVB (Chapter 2). The UVB attenuation coefficient in the microcosms ranged from 15 to 19 m^{-1}, which means that the 1% UVB depth was 30 cm. It is likely that the systems were to some extent protected from UVB radiation. UVB irradiance was still detectable throughout the water column, though intensities were very low. The presence of the macrophytes in the microcosms will have influenced the scattering processes, and thus the attenuation coefficient. These scattering, shading and attenuation processes give a heterogeneous irradiance field in the microcosm, which makes it more difficult to precisely define the UVB irradiance in the system. This heterogeneity is comparable with natural systems, it does imply that refugia from UVB radiation were present, either in the deeper parts of the water column, or under macrophytes. Actual exposure of organisms to UVB was difficult to assess.

It is possible that no effect was found because the statistical power was insufficient by using only two replicates per treatment. However, the same set of model ecosystems has been used earlier to investigate the ecosystem effects of pesticides and in these cases differences between treatments were demonstrated easily and convincingly (*e.g.* Brock *et al.* 1992b, 1993, Van den Brink *et al.* 1995). But even if an effect in this experiment would have been significant, the data show that it would have been small.

In addition, it is possible that all species present in our systems were either not vulnerable to UVB radiation, or able to repair the UVB damage, or that the zooplankton species were able to hide from the UVB radiation by means of vertical migration. Damaging effects of UVB radiation have been shown for several individual species (*e.g.* Karentz *et al.*

1994, Siebeck *et al.* 1994). UVB damage can be repaired. However, UVA fluxes were low relative to UVB, so any UVA-induced repair mechanisms would not have been operating at peak efficiency. As discussed above, the attenuation of UVB in the systems provided depth refugia for organisms with the ability of vertical migration.

Another possibility is that the ecosystems are able to compensate for disturbances, in this case enhanced levels of UVB radiation. Through functional redundancy the part played by one species may be taken over by another one. That this may be true is suggested by the phytoplankton data. The systems behaved more or less identically with regard to phytoplankton biovolume, however they showed considerable differences in cell numbers between taxonomic groups. Similar behaviour seems to be reflected by the comparison of zooplankton biomass, that behaved identically between treatments, and the numbers of individuals of various zooplankton groups, that showed some differences. Such functional redundancy has been suggested earlier to occur in model ecosystem experiments (Levine 1989, Brock *et al.* 1993). However, in those studies, although ecosystem functioning was not affected by the disturbance, ecosystem structure was clearly affected. This was less so in our experiments; the CANOCO analyses did not support a significant effect of the UVB treatment on the plankton and macro-invertebrate communities.

6.5 CONCLUSIONS

The UVB treatment had no significant effect on the plankton, periphyton or macro-invertebrate communities. The plankton communities in the microcosms resembled natural plankton communities. The levels of UVB radiation and exposure time per day were intended to simulate the natural situation in springtime at temperate latitude. UVB scattering, attenuation and shading processes were similar to natural systems. The bio-assay showed that the seston from the treatment microcosms had a negative effect on *Daphnia pulex* growth and survival, and to a lesser extent on fecundity. The seston from the control microcosms proved to be the best food, resulting in a higher somatic growth and a higher net reproductive rate than UV-treated seston. These results indicate that the transfer of energy from phytoplankton to zooplankton can be negatively influenced by UVB radiation. Overall, the ecosystems in the microcosms were not affected by the UVB stress. From these results we conclude that an ecosystem with similar DOC levels and UVB attenuation properties can be resistant to UVB radiation presently occurring at temperate latitude.

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CHAPTER 7

IN SITU EFFECTS OF SOLAR RADIATION ON SEVERAL PHYTOPLANKTON AND ZOOPLANKTON SPECIES

This chapter is based on:
De Lange H.J., Van Donk E. & Hessen D.O. *In situ* effects of UV radiation on 4 species of phytoplankton and 2 morphs of *Daphnia longispina* in an alpine lake (Finse, Norway). Submitted.
and:
Van Donk E., De Lange H.J. & Faafeng B.A. Impact of UV-radiation on grazing activity of *Daphnia middendorffiana*, and use of *Chlamydomonas reinhardtii* as UV-biodosimeter in an arctic freshwater lake (Spitsbergen, Norway). Submitted.

Ik laat mijn ogen over dit eenvoudige landschap gaan, nergens door bomen versluierd en toch zo geheimzinnig. Het is kaal en maakt geen kale indruk door de talloze kleurschakeringen van de nietige planten, de mossen, de grote keien en de lege plekken. Er is niemand in de wijde omtrek en er zal ook niemand komen opdagen en toch kun je het geen eenzaam landschap noemen.

(W.F. Hermans - Nooit meer slapen)

7.1 INTRODUCTION

The harmful effects of UVB radiation on aquatic organisms can be easily demonstrated in laboratory experiments. Extrapolation of results acquired in laboratory experiments to implications for field situation has, however, several pitfalls. First, the artificial light source used in the lab has usually a very different spectrum than the solar spectrum (see Chapters 2 & 6). In most cases the UVB light source has shorter wavelengths in stronger intensities relative to UVA and PAR. Those shorter wavelengths are more harmful. To correct for this difference between the solar spectrum and the artificial light spectrum and to estimate the total biologically effective dose, several biological weighting functions have been developed (*e.g.* Caldwell & Flint 1997, Cullen *et al.* 1992, Setlow 1974). Second, the light treatment is usually with a light and dark period, and constant intensity during the light period. This square wave design is different from the daily sinusoid light intensity curve. Third, other environmental variables, like cloud cover and aerosols in the atmosphere, result in a variable light intensity reaching the water surface. Wave actions influence the reflectance (Kirk 1994), and the water quality determines the attenuation processes (Kirk 1994, Scully & Lean 1994). Mixing processes influence the position of plankton in the water column. All these processes combined result in a fluctuating radiation regime for aquatic organisms. This fluctuating radiation regime is extremely difficult to imitate in the lab, but is important in determining the balance between damage and repair processes (*e.g.* Cullen & Lesser 1991, Neale *et al.* 1998, Santas *et al.* 1997, Smith 1989, Zagarese *et al.* 1998). In field experiments some or all of the above described difficulties can be overcome.

This chapter describes the effects of solar ultraviolet radiation on phytoplankton and zooplankton examined in three separate field experiments conducted at Zwemlust (the Netherlands), Finse (Norway), and Spitsbergen (Norway). These three locations represent a latitudinal gradient, and consequently differences in solar angle, daylength, and daily irradiance.

Experiments in Zwemlust (52°12' N, 5°00' E, sea level) were conducted in May 1996. Experiments in the low-arctic alpine area of Finse (60°35' N, 7°30' E, altitude 1200 m) were conducted in July 1996. Experiments at Spitsbergen (79°00' N, 12°00' E, sea level) were conducted in July 1997. In these experiments, incubations were done at fixed depths. The effect of vertical mixing is therefore not taken into account. The purpose of these experiments was threefold:

- Is it possible to demonstrate effects of solar UVB radiation on planktonic organisms?
- If so, is the interaction between phytoplankton and zooplankton affected by solar UVB radiation?
- If so, are UVB effects comparable between the 3 locations?

In areas with high irradiation, *e.g.* mountain ponds or arctic regions, darkly pigmented *Daphnia* are common. The pigment (melanin) is believed to serve as protection against UVB

radiation (Hebert & Emery 1990, Hessen 1996). In the Finse area in Norway, both non-pigmented (hyaline) as well as pigmented (melanic) *Daphnia longispina* occur. The pigmented morphs usually occur in ponds with little or no vegetation, the hyaline morphs in ponds with macrovegetation or slightly humic water (Wolf & Hobæk 1986). Also at Spitsbergen melanic morphs of *Daphnia* are common (Hobæk *et al.* 1993).

Typical characteristics of oligotrophic arctic lakes are their high transparency for short-wave radiation and often their shallowness offering no depth refugium for harmful radiation (Hessen 1996). In arctic regions, the continuous light during summer offers low potential for dark repair of photodamage.

When studying trophic interactions, the phytoplankton-zooplankton interaction should receive great interest because it is an important step in the pelagic foodchain. UVB stress can cause an increased cell wall thickness in phytoplankton, which might result in reduced assimilation efficiency for zooplankters (Van Donk & Hessen 1995). Other experiments showed that UVB stressed phytoplankton reduced the population growth rate of *Daphnia* (Chapter 5). UVB radiation can also alter the biochemical composition of phytoplankton in a number of ways that affects the food quality for zooplankton (Chapter 3). A loss of flagella in the green flagellate *Chlamydomonas* after exposure to UV was found in both lab and field experiments (Hessen *et al.* 1995, Van Donk & Hessen 1996).

7.2 METHODS

7.2.1 *Zwemlust*

Solar radiation and temperature measurements

Spectral measurements were made with a Macam Spectroradiometer SR9910 at the end of each experiment. Biological effective UVB doses were calculated with Setlow's DNA action spectrum, normalized to 1 at 300 nm (Setlow 1974). A datalogger (Squirrel 1202, Grant Instruments, Cambridge UK) was used during the second experiment to record temperature and PAR intensity at 2 depths, at 2 minute intervals. The PAR diffuse attenuation coefficient was calculated according to Kirk (1994).

Chlamydomonas growth and *Daphnia* grazing

Chlamydomonas reinhardtii (Chlorophyceae, strain from Norwegian Institute for Water Research NIVA-CHL 13), was cultured in semi-continuous culture before the start of the experiments. *Daphnia pulex* was isolated from Lake Zwemlust, and has been cultured in the laboratory for more than 1 year. *C. reinhardtii* was inoculated in WC medium (Guillard & Lorenzen 1972) to a starting concentration of *ca.* 10,000 cells ml^{-1}. Particle concentration and biovolume were determined in the size range 3.0-20.0 µm equivalent spherical diameter (ESD) with an electronic particle counter (Coulter Multisizer II, 100 µm capillary).

The experimental design consisted of two light treatments, PAR only and PAR + UVA + UVB, and three different incubations. The different incubations were: *C. reinhardtii*, 5 cm below surface, *C. reinhardtii*, 30 cm below surface, and *C. reinhardtii* + 10 juvenile *D. pulex*, 5 cm below surface. One litre glass bottles were used for the PAR only control, one litre polycarbonate bottles were used for the UV treatment. These bottles are UV transparant, with a cut-off point at 290 nm (Fig. 7.1).

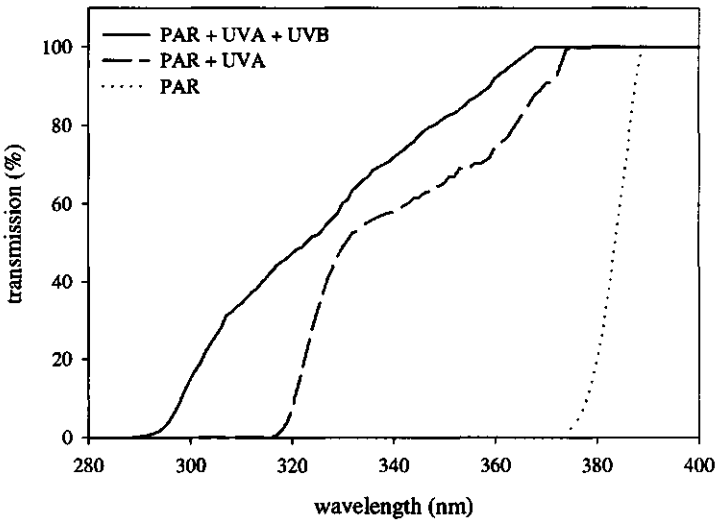

Figure 7.1: Transmission spectra of the incubation bottles. PAR = glass bottles, PAR+UVA = polycarbonate bottles, PAR+UVA+UVB = polycarbonate bottles

Each treatment was conducted in triplicate, and lasted 7 days. The whole experiment was repeated. Concentrations were measured at day 0 and at day 7, and daily growth rates μ (d^{-1}) were calculated as:

$$\mu = \ln(C_7/C_0)/7$$

where C_0 and C_7 are biovolume concentrations at day 0 and day 7 respectively.
Clearance rates (ml ind^{-1} h^{-1}) for *D. pulex* were calculated as:

$$\text{clearance rate} = (\ln(C_0/C_7)) * V/(t*N)$$

where C_0 and C_7 are biovolume concentrations at day 0 and day 7 respectively, V = volume of incubation bottle (ml), N = number of individuals, and t = grazing time (hours). A correction for algal growth was made.

Experiment 1 was from 8 May 1996 to 15 May 1996. Experiment 2 was from 22 May 1996 to 29 May 1996. In the first experiment the juvenile *D. pulex* were within 24 hours after birth. In the second experiment the juveniles were 2 days old. The following additional

measurements were made at the end of the second experiment: samples of *Chlamydomonas* were examined with a Nikon light microscope to observe cell appearance and flagella. *D. pulex* individuals were measured using a dissecting microscope, and the combined weight of the surviving individuals per incubation bottle was measured with a Sartorius balance (R160P, accuracy 10 µg).

7.2.2 *Finse*

Solar radiation and temperature

Radiation levels and water temperature were recorded 3 times per day, at 9.00 h, 13.00 h and 18.00 h. Integrated PAR was measured above the water surface and at 2 depths with a LiCor quantummeter. The PAR diffuse attenuation coefficient was calculated according to Kirk (1994). Integrated UVB intensity (integrated in the range 295-330 nm) above the water surface was measured with a Vilber Lourmat meter (VLX-3W).

Phytoplankton growth

Phytoplankton growth experiments were performed with 4 species: *Rhodomonas lacustris* (Cryptophyceae, strain NIVA-8/82), *Chlamydomonas reinhardtii* (Chlorophyceae, strain NIVA-CHL 13), *Cyclotella* sp. (Bacillariophyceae, strain NIVA-BAC 8), and *Selenastrum capricornutum* (Chlorophyceae, strain NIVA-CHL 1). Three treatments were used, each with three replicates: PAR (1 litre glass bottles), PAR+UVA (1.5 litre polycarbonate bottles, 1.5 mm thick, hereafter referred to as UVA treatment), and PAR+UVA+UVB (1 litre polycarbonate bottles, 1.0 mm thick, hereafter referred to as UVB treatment). Transmission spectra for each bottle are given in Fig. 7.1. The bottles were incubated in an oligotrophic rocky pond close to the field station (cabin pond), 5 cm below the water surface.

R. lacustris and *C. reinhardtii* were inoculated (50 ml culture) in 800 ml filtered (150 µm) pond water, 35 ml Z8 medium (Skulberg & Skulberg 1990), and 1 ml vitamin solution. *Cyclotella* sp. was inoculated (100 ml culture) in 900 ml filtered (150 µm) pond water, 35 ml Z8 medium, and 1 ml silica solution. *S. capricornutum* was inoculated with 40 ml culture in 950 ml filtered (150 µm) pond water, and 35 ml Z8 medium.

Samples were taken at time intervals of 4 days and fixed with Lugol's solution. Particle concentration and biovolume were determined in the size range 3.0-20.0 µm equivalent spherical diameter (ESD) with an electronic particle counter (Coulter Multisizer II, 100 µm capillary). Growth rates were calculated as particle increase and as biovolume increase. Cell morphology was determined with a light microscope. The growth experiments lasted for 18 days.

Daphnia survival

Survival experiments were done with two morphotypes of *Daphnia longispina*, a hyaline and a melanic morph. The hyaline morph was isolated form a small, shallow (depth 0.5 m) pond, the melanic morph was isolated from the cabin pond (depth 3 m). The incubations of *D. longispina* were performed with different types of food: pond water with the natural seston composition, *C. reinhardtii* (biovolume concentration at t=0 was $7*10^6$ μm^3 ml^{-1}), daily refreshed *C. reinhardtii* (biovolume concentration each day $7*10^6$ μm^3 ml^{-1}), daily refreshed UVB irradiated *C. reinhardtii* (irradiated for 6 hours with 0.15 mW cm^{-2} UVB, biovolume concentration each day $7*10^6$ μm^3 ml^{-1}). The *C. reinhardtii* cells came from a laboratory culture. The UVB radiation for the fourth food type was provided by one Vilber Lourmat 15W lamp, with preburned cellulose acetate foil to filter out UVC wavelengths, resulting in a dose rate of 0.15 mW cm^{-2} UVB. Each treatment was performed in triplicate, with 5 *D. longispina* placed in 200 ml glass bottles (control) and in 200 ml quartz bottles (UV treatment). The bottles were incubated in the cabin pond, at 5 cm depth for 5 days (hyaline morph) or 7 days (melanic morph).

Daphnia grazing

10 Melanic *Daphnia longispina* were incubated with *Chlamydomonas reinhardtii*. Particle concentration and biovolume were determined in the size range 3.0-20.0 µm ESD with an electronic particle counter (Coulter Multisizer II, 100 µm capillary). Treatments were PAR, PAR+UVA, and PAR+UVA+UVB, using the same type of bottles as in the phytoplankton experiment. For each light treatment triplicate bottles with and without *D. longispina* were incubated in the cabin pond, at 5 cm depth. The experiment lasted 11 days. Growth and disappearance rates were calculated according to Van Donk & Hessen (1995).

7.2.3 *Spitsbergen*

Solar radiation

UVB (280-320 nm), UVA (320-400 nm) and PAR (400-700 nm) surface irradiance was measured with a GUV541 multichannel filter instrument (Biospherical Instruments). The attenuation coefficients in the enclosures were calculated according to Kirk (1994) from measuring intensity of UVA and UVB at 5 depths with a PUV500 system (Biospherical Instruments).

Chlamydomonas as UV- biodosimeter

Enclosures (polyethylene, ca. 2 m^3, depth 2 m) filled with lake water were placed in a shallow freshwater lake Brandallaguna (12 ha; 4-6 µg total-P l^{-1}; chlorophyll-*a* 0.8-1.4 µg l^{-1}; max. depth 2 m; TOC *ca.* 0.5 mg l^{-1}) near Ny-Ålesund, Spitsbergen (79^0N). An artificial UV-gradient was set up by adding to the enclosures different amounts of freeze-dried humus (TOC = total organic carbon), isolated by reverse osmosis filtration of water from a Norwegian lake

(Encl.1 = control, no addition; Encl. 2, 1 mg TOC l^{-1}; Encl. 3, 10 mg TOC l^{-1}). The potential use of the flagellated, planktonic green alga *Chlamydomonas reinhardtii* (NIVA-CHL 13) as a biodosimeter for UV stress was tested in these enclosures. Three types of flasks were used for incubation of *Chlamydomonas* in the enclosures: 150 ml quartz flasks, the same bottles wrapped with Mylar (single layer) and 150 ml glass bottles. While quartz flasks are transparent to UVB, UVA and PAR, the Mylar filter blocks radiation below 320 nm (transparent to UVA and PAR, but not to UVB), and glass bottles block all UVB and most UVA. The flasks were incubated at 20 cm depth during 55 hours starting at 11.00 h on 23 July 1997 (Julian day 204). The experiments were performed in triplicate. After 30 hours and after 55 hours of incubation, 5 ml subsamples were fixed with Lugol's solution and algae were examined for number of flagella. Results were tested with 2-Way Anova, with type of enclosure and type of incubation bottles as factors. Significant differences ($p<0.05$) were distinguished by applying the Tukey test.

Phytoplankton growth and *Daphnia* grazing

A phytoplankton growth experiment was performed with *Chlamydomonas reinhardtii* (Chlorophyceae, strain NIVA-CHL 13) and *Selenastrum capricornutum* (Chlorophyceae, strain NIVA-CHL 1). Incubation took place in Lake Brandallaguna, at 5 cm depth. Light treatments were identical to the phytoplankton growth experiments at Finse. Both phytoplankton species were inoculated in 0.45 µm filtered lake water with nutrients added to obtain 10% Z8 medium (Skulberg & Skulberg 1990). A grazing experiment was conducted at the same time. 10 Melanic *Daphnia middendorffiana*, isolated from Lake Brandallaguna, were incubated with *C. reinhardtii* using the same light treatments.

Samples were taken at time intervals of 4 days and fixed with Lugol's solution. Particle concentration and biovolume were determined in the size range 3.0-20.0 µm ESD with an electronic particle counter (Coulter Multisizer II, 100 µm capillary). Growth rates were calculated as particle increase and as biovolume increase. Cell morphology was determined with a light microscope. The experiment lasted for 11 days. In the grazing experiment, *Daphnia* clearance rates were calculated according to the Zwemlust experiment.

7.3 RESULTS

7.3.1 *Zwemlust*

Solar radiation and temperature

Weather conditions during the first experiment were mostly cloudy, and cold (water temperature *ca.* 12 °C). Weather conditions were more bright and warmer during the second experiment, mean water temperature was 16.4 °C (Fig. 7.2). The K_dPAR increased during experiment 2 from 1.6 to 3.0 m^{-1}. PAR and UV intensities measured at the end of each experiment are given in Table 7.1.

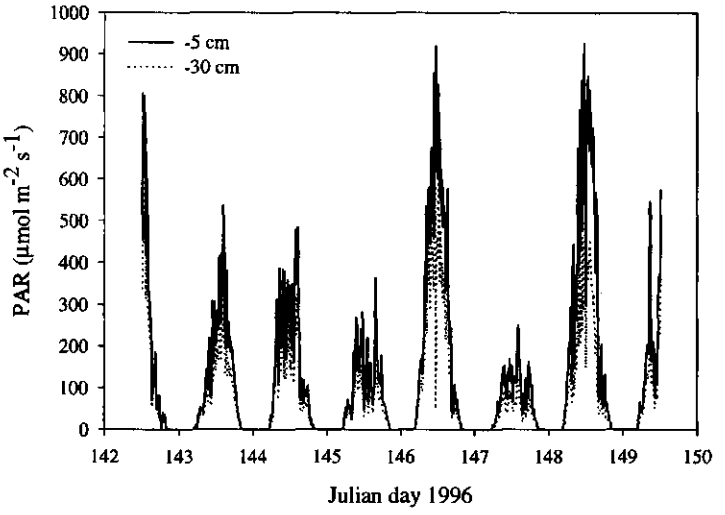

Figure 7.2: PAR intensities in Zwemlust during the second experiment, May 1996, at 5 cm and 30 cm depth.

Table 7.1: PAR, UVA and UVB intensities (W m^{-2}) measured at different depths, and attenuation coefficients K_d (m^{-1}), nd = not determined.

	+ surface	-0 cm	-30 cm	K_d (m^{-1})
Experiment 1: UVB_{DNA}	0.049	0.028	$1.2\ 10^{-5}$	29
UVB	1.73	1.0	0.0016	24
UVA	30	20	1.2	11
PAR	250	135	54	3.4
Experiment 2: UVB_{DNA}	nd	0.012	$2.0\ 10^{-6}$	23
UVB	0.76	0.29	0	21
UVA	17.2	8.6	0.5	9.0
PAR	115	73	42	1.6

Chlamydomonas growth

The growth rate of *C. reinhardtii* in both experiments was not significantly affected by the UVB treatment (Fig. 7.3). The proportion of *Chlamydomonas* cells having lost both flagella was significant in the UVB treatment at -5 cm depth, but not at -30 cm depth (Fig. 7.4).

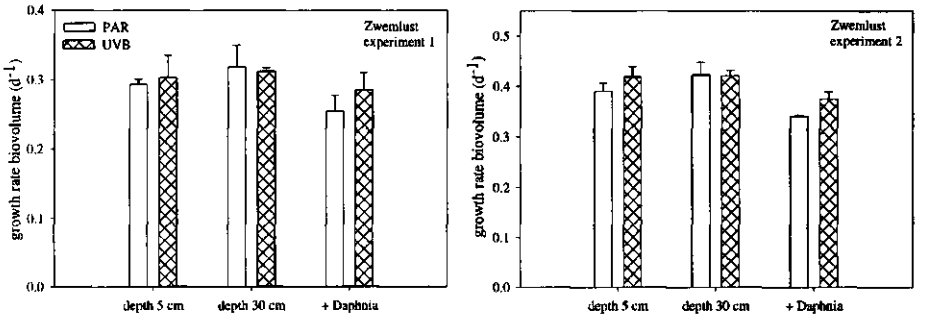

Figure 7.3: Growth rate of *Chlamydomonas*, in Zwemlust, at 5 cm and at 30 cm depth, and with *Daphnia*. Left hand panel is experiment 1, right hand panel is experiment 2; error bars indicate 1 SD.

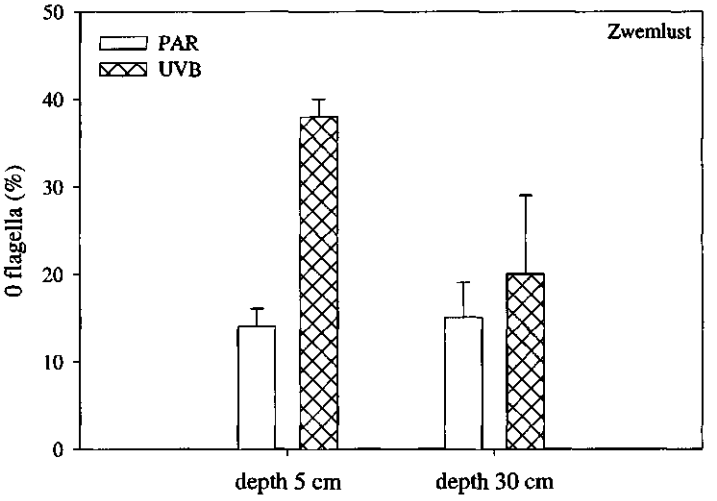

Figure 7.4: Percentage of *Chlamydomonas* cells without flagella, at the end of experiment 2 in Zwemlust; error bar indicates 1 SD.

Daphnia clearance rate

The clearance rate of *D. pulex* was reduced in both experiments, however the differences were not significant (t-test $p>0.05$; Fig. 7.5). Survival of *Daphnia* was similar in

control and UV treatment. However, the daphnids in the UVB treatment remained smaller and developed a smaller number of eggs in the broodpouch (Table 7.2).

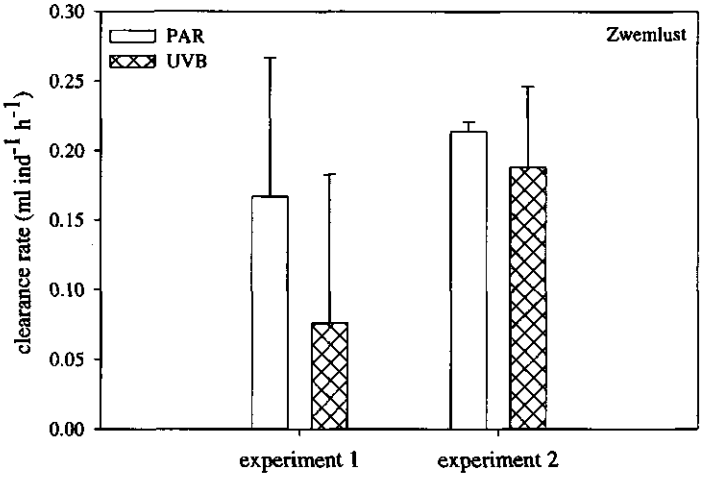

Figure 7.5: Estimated clearance rate of *Daphnia*, Zwemlust experiments; error bars indicate 1SD.

Table 7.2: Results *Daphnia pulex* ± 1 SD at end of experiment 2, ** indicates a significant UVB effect (t-test, $p<0.01$).

	PAR	UVB	
length (mm)	2.25 ± 0.12	2.09 ± 0.21	**
weight (mg ind^{-1})	0.152 ± 0.028	0.121 ± 0.019	
eggs (number ind^{-1})	5.28 ± 1.62	3.91 ± 1.41	**
survival (%)	90.0 ± 0.0	93.3 ± 11.5	

7.3.2 *Finse*

Solar radiation and temperature

Radiation levels varied strongly (Fig. 7.6). Maximum PAR levels were around 1400 µE m^{-2} s^{-1}, but at cloudy days only 200 µmol m^{-2} s^{-1}. The PAR diffuse attenuation coefficient in the pond was 0.5 m^{-1} (SD=0.1). Integrated UVB intensities varied similarly, maximum levels at noon were above 1 mW cm^{-2}, at cloudy periods below 0.1 mW cm^{-2}. Water temperature ranged from 6 to 15 °C, with an average value of 11 °C (SD=2.3).

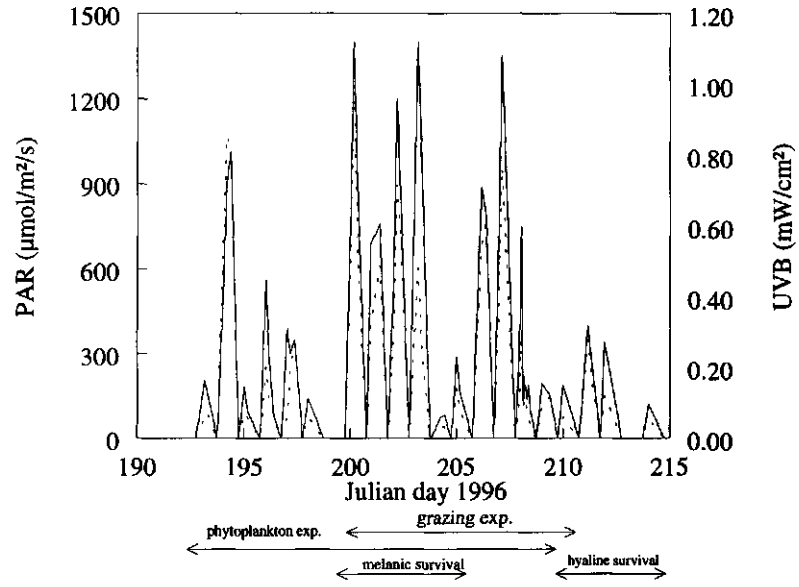

Figure 7.6: PAR and UVB light intensities in Finse in July 1996. The period of the experiments is indicated. PAR intensity is solid line, UVB intensity is dashed line.

Phytoplankton growth

A significant increased particle growth rate was found for *Rhodomonas* in both UVA and UVB treatments (Anova $p<0.05$, Fig. 7.7). This was mainly caused by an increase in the number of small round cells with one large flagellum (spores). The increase in *Rhodomonas* biovolume growth rate in the UVA and UVB treatments was not significant. The biovolume growth rate of *Cyclotella* was significantly increased in both UVA and UVB treatments (Anova, $p<0.05$).

The appearance of *Chlamydomonas* was normal in all treatments, all having two flagella. There were no marked differences between the different light treatments for *Cyclotella* and *Selenastrum* as revealed by the microscope analysis.

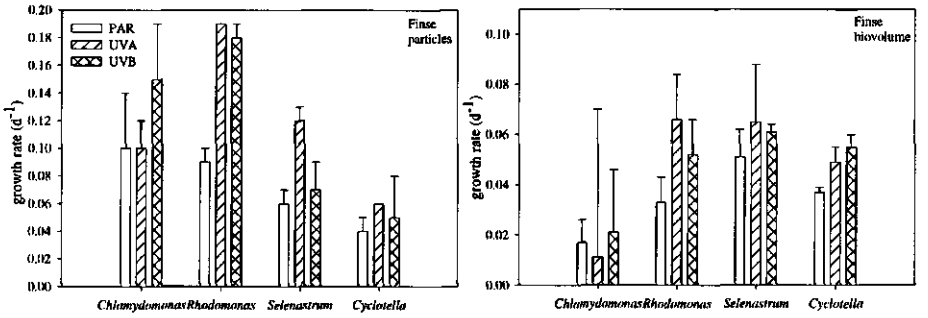

Figure 7.7: Phytoplankton growth, as particle increase (left hand panel) and biovolume increase (right hand panel); error bars indicate 1 SD.

Daphnia longispina survival

Survival was generally higher for the melanic *Daphnia* relative to the hyaline *Daphnia* (Fig. 7.8). For the hyaline *Daphnia*, the difference between control and UV treatment was significant for all 4 types of food (repeated measurements Anova, $p<0.05$). For the melanic *Daphnia*, food type A (pond water) did not have a significant UV effect ($p=0.49$). For the other food types however, the UV treatment was significant ($p<0.05$, repeated measurements Anova). Daily refreshed UVB-irradiated *Chlamydomonas*, was the best food. Daily refreshed *Chlamydomonas* and *Chlamydomonas* were intermediate, pond water was the poorest food.

Daphnia grazing

The growth rates of the algae without grazing clearly showed a stimulating effect of UVA radiation, and an inhibiting effect of UVB compared to UVA (Table 7.3). Both UV treatments had higher growth rates than PAR only. The apparent particle clearance rates were not significantly affected by the UV treatments. Due to mortality of *Daphnia* during the experiment, it was not possible to calculate a clearance rate per individual. Cell volumes were reduced in the UVB treatment, and the apparent biovolume clearance rate in the UVB treatment was much lower than in the other treatments. These differences however were not significant.

Table 7.3: *Chlamydomonas* growth rate (as particles increase, d^{-1}) ± 1 SD in the grazing experiment, apparent clearance rate (c.r., d^{-1}), calculated with particles and with biovolume.

	no *Daphnia*	with *Daphnia*	c.r. particles	c.r.biovolume
PAR	0.09 ± 0.01	0.11 ± 0.04	-0.01 ± 0.04	0.07 ± 0.05
UVA	0.18 ± 0.02	0.14 ± 0.04	0.04 ± 0.04	0.08 ± 0.02
UVB	0.14 ± 0.01	0.18 ± 0.02	-0.04 ± 0.02	0.00 ± 0.07

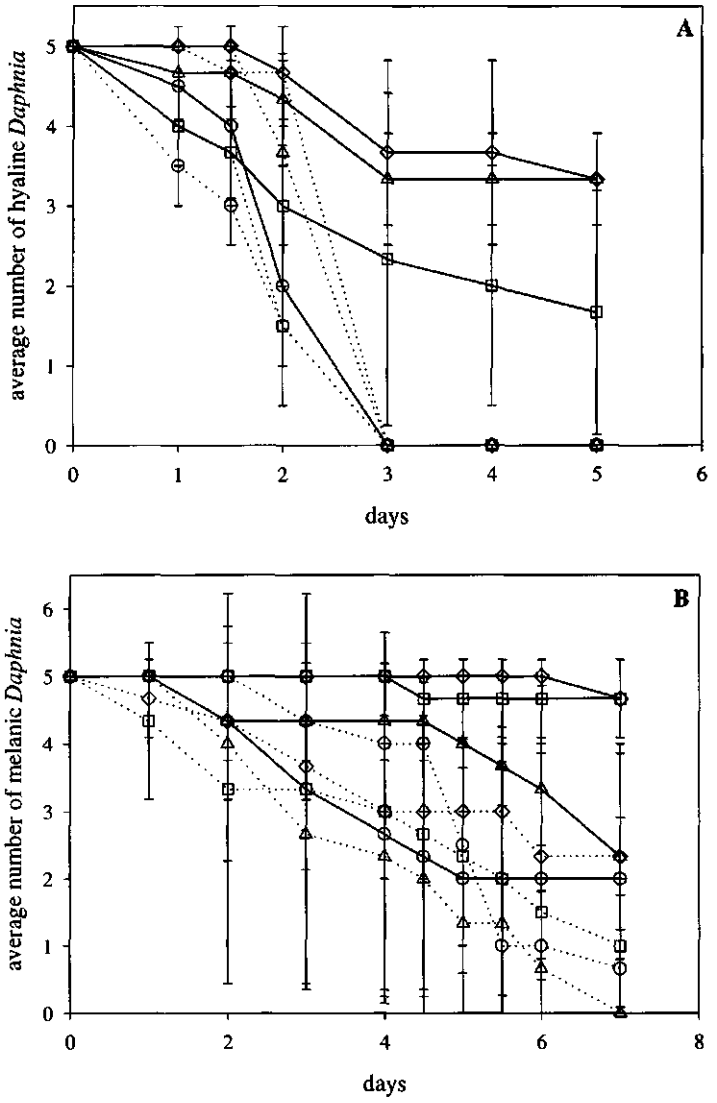

Figure 7.8: Survival of *Daphnia longispina* on different food, panel A for the hyaline clone, panel B for the melanic clone. Solid lines are control, dashed lines are UV treatment; circles = pond water, squares = *Chlamydomonas*, triangles = daily refreshed *Chlamydomonas*, diamonds = daily refreshed UVB irradiated *Chlamydomonas* (6 hours 0.15 mW cm^{-2}); error bars indicate 1 SD.

7.3.3 *Spitsbergen*

Solar radiation

The surface radiation at Brandallaguna (PAR, UVA and UVB) during the last two weeks of July 1997 is given in Fig. 7.9. Table 7.4 shows the attenuation coefficients of UVA and UVB radiation (m^{-1}) and the percentage of UVA and UVB still present at 0.2 m, measured in the three enclosures during the incubation experiments with *Chlamydomonas*. The extinction of UVA and UVB was higher in the enclosure with 10 mg TOC l^{-1} than in the enclosure with 1 mg TOC l^{-1} and that with no addition of TOC.

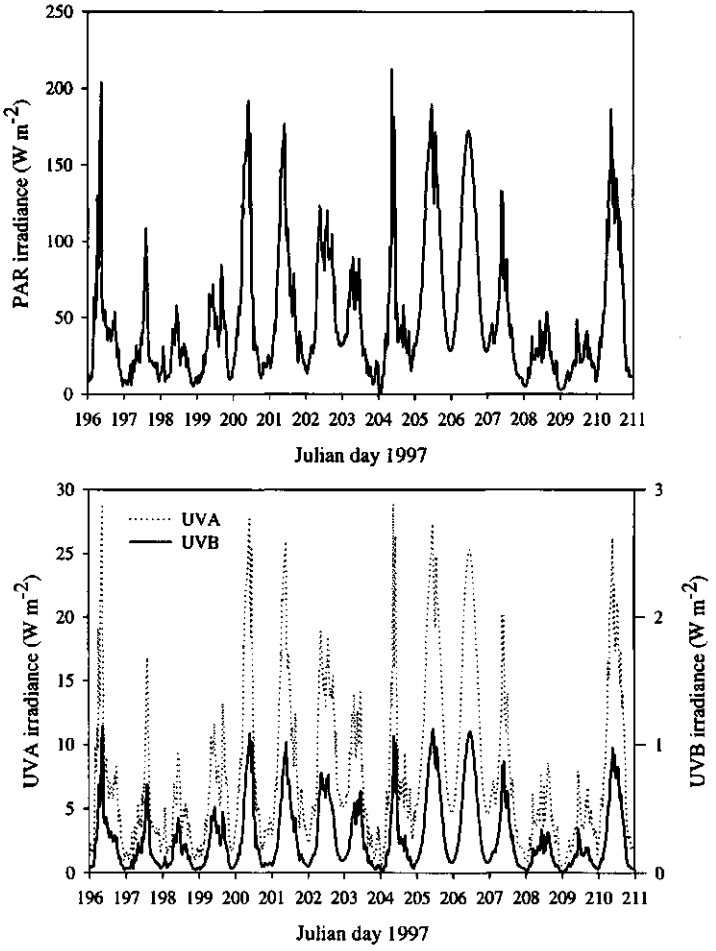

Figure 7.9: Solar radiation at Ny-Ålesund, Spitsbergen, July 1997, upper panel is PAR irradiance, lower panel is UVA and UVB irradiance.

Table 7.4: Attenuation coefficients per wavelength (m^{-1}) and percentage of UV surface irradiation still present at 0.2 m in the three enclosures.

	lake water		1 mg l^{-1} TOC		10 mg l^{-1} TOC	
Wavelength (nm)	m^{-1}	%	m^{-1}	%	m^{-1}	%
308 (UVB)	2.9	56	4.0	45	8.5	18
320 (UVA)	2.3	63	3.1	54	7.5	22
340 (UVA)	1.7	71	2.5	61	5.6	33
380 (UVA)	0.9	84	1.7	71	3.2	53

Chlamydomonas as UV-biodosimeter

Chlamydomonas incubated in the three enclosures in Lake Brandallaguna showed a significant loss of flagella with increasing UVB radiation (Fig. 7.10). At both 30 hours and 55 hours, there is a significant enclosure and light treatment effect, and a significant interaction (2-way Anova, $p<0.05$). Differences in percentage without flagella between light treatments and enclosures are larger after 30 hours incubation than after 55 hours incubation. This might indicate some adaptation to UV stress.

After 30 hours, both UVA and UVB caused an increase in percentage of cells without flagella in the enclosure with lake water and that with 1 mg TOC l^{-1}. This increase was significant for the UVB treatment. After 55 hours, the percentage of cells without flagella in the UVB treatment was significantly different from the UVA treatment in lake water. The percentage of cells without flagella in the UVA treatment was lower than in the PAR treatment. In the enclosure with mg TOC l^{-1} the differences between the light treatments after 55 hours were not significant (Anova, $p>0.05$). In the enclosure with 10 mg TOC l^{-1} no significant differences were found between the treatments at both 30 hours and at 55 hours.

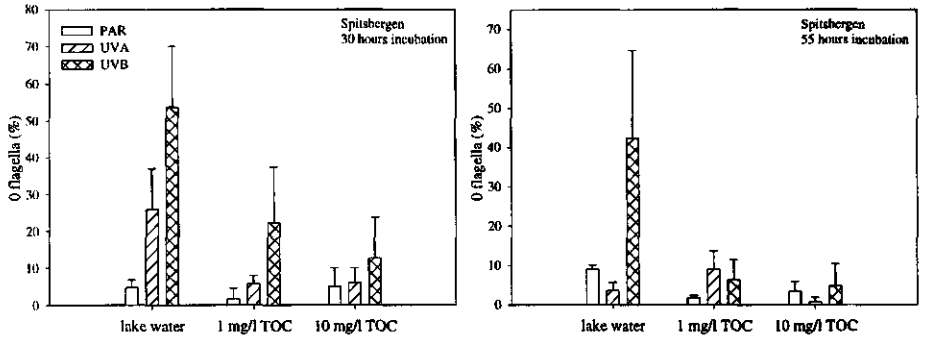

Figure 7.10: Percentage of *Chlamydomonas* cells without flagella, Spitsbergen, after 30 h (left hand panel) and 55 h incubation (right hand panel) at a depth of 20 cm in enclosures with different UV absorption characteristics (see Table 7.4); error bars indicate 1 SD.

Phytoplankton growth and *Daphnia* grazing

There were no significant differences in growth rates of *S. capricornutum, C. reinhardtii* and *C. reinhardtii + Daphnia* (Fig. 7.11). Growth rates were low, and comparable to the growth rates in Finse.

Survival of the Daphnids in the grazing experiments was 100%, therefore clearance rates per individual could be calculated. The clearance rate is higher in the UVA and UVB treatment. These differences are significant for the clearance rate calculated with biovolume concentration (Anova, $p<0.05$), but not when the clearance rate is calculated with the particle concentration (Table 7.5). The cell volume was not significantly different in the UV treatments.

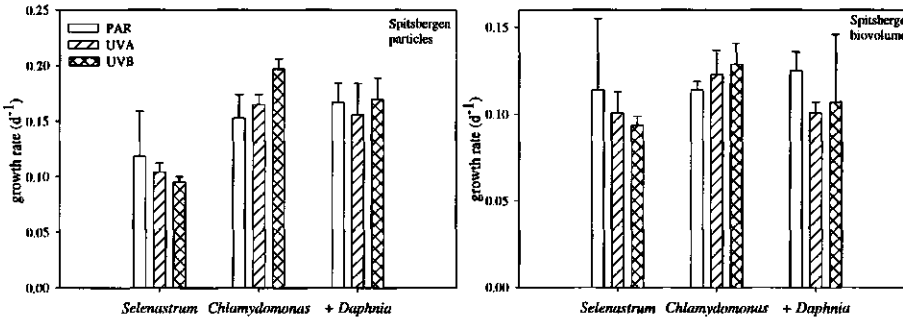

Figure 7.11: Growth rates of *Selenastrum, Chlamydomonas* and *Chlamydomonas + Daphnia*, Spitsbergen, as particle increase (left hand panel) and biovolume increase (right hand panel).

Table 7.5: *Daphnia* clearance rates (ml ind^{-1} h^{-1}), letter indicates different homogeneous groups (identified by the Tukey test).

	biovolume	particles
PAR	0.12 ± 0.04 a	-0.06 ± 0.07
UVA	0.27 ± 0.03 ab	0.04 ± 0.12
UVB	0.39 ± 0.14 b	0.12 ± 0.08

7.4 DISCUSSION

7.4.1 *Zwemlust*

Growth of *Chlamydomonas reinhardtii* was not negatively affected by solar UVB radiation. Differences in growth rate were not significant. It may seem that UVB was slightly stimulating growth at -5 cm. At -30 cm differences in growth rate between control and UV-B

treatment were negligible. Cell volume of *C. reinhardtii* was similar under control and UVB conditions. The percentage of cells having one or both flagella was significantly decreased under UVB radiation. It seems that loss of flagella is a more sensitive bio-dosimeter than growth rate or cell volume. Van Donk & Hessen (1996) also concluded that *C. reinhardtii* can be used as a sensitive bio-dosimeter for UVB stress.

Daphnia pulex survival was not affected by UVB. The clearance rate however was much lower in the UVB bottles, compared with the control bottles. Because of the large within treatment variance, this difference was not significant. *D. pulex* remained significantly smaller, and produced a significantly smaller number of eggs in the UVB treatment compared with the control. Because during the incubation both *Chlamydomonas* and *Daphnia* were exposed to UVB radiation, it is not possible to distinguish the sequence of cause and effects. The following explanations are plausible. i) UVB radiation directly inhibited the grazing activity of *Daphnia*, this resulted in a decreased food uptake by *Daphnia*, this resulted in a smaller body size and weight. ii) *Daphnia* was not directly affected by UVB, the reduced clearance rate was caused because UVB stressed *Chlamydomonas* was harder to digest, this decreased food uptake by *Daphnia* and resulted in a smaller body size. iii) *Daphnia* was not directly affected by UVB, UVB caused a different biochemical composition of *Chlamydomonas*, this reduced food quality, this resulted in a reduced growth rate of *Daphnia*, the smaller body size resulted in a lower clearance rate.

Preliminary lab experiments show that direct UVB radiation did not affect the clearance rate of *Daphnia* (De Lange unpubl., Van Donk *et al.* subm.), this weakens support for the first explanation. Both the second and the third explanation are supported by literature (Chapter 5, Van Donk & Hessen 1995).

These results show that on the short term UVB is not lethal for *Daphnia pulex*. The phytoplankton-zooplankton interaction is influenced and might influence on the long term *Daphnia* population, because of the fewer eggs and smaller size in the UVB treatment.

Zwemlust is a eutrophic lake, with K_dUVB values around 22 (m^{-1}) during the experiments. This related to a 1% UVB penetration depth of *ca.* 20 cm. The K_d values are dependent on the algal biomass, and have therefore strong seasonal variation. At 30 cm depth, no UVB effects were found on *Chlamydomonas* growth and flagella. UVB effects can be found in eutrophic systems like Zwemlust, but are limited to the upper decimetres of the water column.

7.4.2 *Finse*

Phytoplankton growth rates were generally low, due to the low temperature and the low light intensities. At the low light intensities, UV radiation did not inhibit growth, but apparently rather stimulated growth of *Rhodomonas* and *Selenastrum*. The UVA treatment yielded higher growth rate than did the PAR treatment. UVA thus apparently stimulated growth under the prevailing conditions during the experiment. The UVB treatment yielded a

higher growth rate than the PAR treatment, but a lower growth rate than the UVA treatment. The UVB and UVA had antagonistic effects, where UVA stimulated and UVB inhibited growth.

For *Rhodomonas*, the increased growth rate in both UV treatments was mainly caused by an increase in spores, small cells with one large flagellum. Growth rate calculated with biovolume increase is a better measurement. However, the Lugol fixation affects the cell volume. Assuming that the change in cell volume is similar in all samples, biovolume growth rates can be calculated. However, care should be observed in interpreting cell volume results. Cell volume showed an increase under UVB radiation for *Chlamydomonas*, and a significant decrease for *Rhodomonas* and *Cyclotella*. In contrast to Van Donk & Hessen (1996) no loss of flagella in *Chlamydomonas* was found after exposure to UVB. Maybe the UVB levels were too low to cause loss of flagella. Another possibility is that the long incubation time could facilitate adaptation to UVB stress and possible regeneration of the flagella.

In the *Daphnia* survival experiment, the different food types gave different results. Pond water was the poorest food type for survival, probably due to quantity rather than quality constraints. Daily refreshed UVB-irradiated *Chlamydomonas* was the best food type for survival. Studies of Zellmer (1996) showed that UVB tolerance of *Daphnia pulex* significantly improved with increasing food quantity. But her results could not identify the mechanism how food quantity affects UVB tolerance. Both absorption of UVB radiation by pigmentation as well as repair of radiation damage are possibly affected by the nutritional status of the animals (Zellmer 1996). The nutritional status is determined by both food quantity and food quality aspects. The melanic morph had a better survival than the hyaline morph, especially when considering that the irradiation levels were higher during the melanic experiment (Fig. 7.6). The pigment melanin is believed to serve as protection against UVB radiation (Hebert & Emery 1990, Hessen 1996). The hyaline morph was isolated from a shallow pond with vegetation. There would have been no depth refugium in this pond, but the vegetation and slightly humic water may provide protection from UVB. The melanic morph was isolated from a 3 m deep clearwater pond, individuals in this pond exhibited a vertical migration in response to increase in light levels (Hessen 1993, De Lange pers. obs.).

In the grazing experiment, the particle disappearance rate showed large variation. The biovolume disappearance rate showed that PAR- and UVA-irradiated *Chlamydomonas* were grazed at the same rate, but that UVB-irradiated *Chlamydomonas* was grazed at a lower rate. A probable explanation is a thicker cell wall for UVB-irradiated *Chlamydomonas*, this was however not examined in this study. The reduced disappearance rate is similar to results obtained in laboratory experiments (Van Donk & Hessen 1995) and the Zwemlust experiments.

The levels of PAR and UVB radiation during the experiments were variable. The phytoplankton species used in the experiments could benefit from the UVA radiation, and the UVB radiation had only a small negative effect. The different species of phytoplankton show different responses to the UVA, UVB and PAR radiation. It is therefore difficult to draw general conclusions. The effects of UV radiation on *Daphnia* were more pronounced, the UV

treatment caused significant mortality. The melanic morph of *Daphnia* had a better survival under UV radiation than the hyaline morph. This confirms that the pigment melanin serves as a protection against UV radiation.

7.4.3 *Spitsbergen*

Chlamydomonas incubated *in situ* showed a pronounced loss of flagella with increasing UVB radiation. This corresponds well with earlier studies in the laboratory (Hessen *et al.* 1995) and field experiments (Van Donk & Hessen 1996), where a strong relation was found between loss or withdrawal of flagella and UV-exposure. The algae incubated in the enclosure with lake water revealed effects at relatively low ambient UVB intensity. Due to continuous light during the 24 h diel period in the arctic summer, the plankton is exposed to UV-radiation continuously, without a dark period. These results emphasize the potential role of *C. reinhardtii* as a susceptible and biologically relevant dosimeter. The susceptibility of *Chlamydomonas* to UVB *vs.* UVA and the role of dark repair needs further evaluation. The loss of flagella was strongest in the enclosure with lake water, and the enclosure with 1 mg TOC l^{-1} added, and not in the enclosure with 10 mg TOC l^{-1} added. This confirms the role of DOC in attenuating UVB and thus protecting aquatic organisms against harmful effects of UVB radiation.

Growth rates of *Chlamydomonas reinhardtii* and *Selenastrum capricornutum* were not affected by UV radiation. The grazing of *Daphnia middendorffiana* on *C. reinhardtii* was increased under UVA radiation. The within treatment variation was quite large in this experiment, more replicates per treatment are advisable to reduce this variation. The increase in clearance rate is in contradiction with laboratory experiments (Van Donk & Hessen 1995), and with the result from the Zwemlust experiments. The different *Daphnia* species used may be the explanation, in this experiment a melanic morph of *Daphnia middendorffiana* was used. As shown in the Finse experiments, melanic morphs of *Daphnia* have a higher UVB tolerance than hyaline morphs (see Hessen 1996). Further *in situ* studies are necessary to fully quantify the impact of UV radiation on grazing zooplankton.

7.5 CONCLUSIONS

In the three experiments, growth of *Chlamydomonas reinhardtii* was not affected by present levels of UVB radiation. Growth of *Selenastrum capricornutum* was stimulated by UVA in the Finse experiment, but not affected in the Spitsbergen experiment. In Finse, UVA radiation stimulated the formation of spores of *Rhodomonas lacustris*. The different phytoplankton species show different responses to UVB, UVA and PAR radiation. This implies that increased levels of UVB radiation could lead to shifts in phytoplankton community structure. Further these results suggest that phytoplankton communities dominated by diatoms and cryptophytes (spring assemblages) are more sensitive to UVB radiation than

phytoplankton communities dominated by green algae and cyanobacteria (summer and autumn assemblages) (see Sommer *et al.* 1986, Gala & Giesy 1991).

The loss of flagella in *Chlamydomonas* seems to be a more sensitive UV-biodosimeter than inhibition of growth. Both in Zwemlust and at Spitsbergen, UVB radiation caused a significant loss of flagella while it did not effect growth. The role of UVA *vs.* UVB, and the mechanism of flagella loss needs further experimental evaluation.

Daphnia clearance rate in Zwemlust was more sensitive to UVB than survival, clearance rate was affected while survival was unaffected. The mechanism of the clearance rate reduction could not be identified in this experimental setup. Grazing rates in Finse and Spitsbergen with melanic *Daphnia* had large within treatment variations, but seemed to be stimulated by UVA radiation, and slightly inhibited by UVB radiation.

The results of these experiments showed that present levels of solar UVB radiation in the Netherlands can cause effects on both phytoplankton (loss of flagella in *Chlamydomonas*) and zooplankton (smaller body size and reduced clearance rate of *Daphnia*). Solar UVB radiation in potential can have an effect on the phytoplankton-zooplankton interaction. In all three locations the grazing rates of *Daphnia* were affected, though variance was large, and differences marginal. UVB effects are comparable between the three locations. The magnitude of the UVB effects was quite different, due to very different weather conditions.

ACKNOWLEDGEMENTS

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CHAPTER 8

DAPHNIA GROWTH RELATED TO SESTONIC VARIABLES: INFLUENCED BY UV RADIATION?

This chapter is based on:
De Lange H.J. & Arts M.T. *Daphnia* growth related to sestonic variables: influenced by UV radiation? Submitted

Make hay while the sun shines.

(traditional)

En wanneer we zonder opgave, moe en leeg maar meestal ook tevreden de laatste honderden meters naar het plankier aflegden, roeide ik in mijn verbeelding vaak op een spiegelglad meer waarvan de oevers niet te zien waren en dat achter ons zonder dat we er erg in hadden zou overgaan in het volgende, en daarna weer een. Er kwam geen eind aan, we konden zonder tijd en zonder tellen en zonder ooit moe te worden doorroeien want achter iedere einder lag weer een volgend meer met een volgende horizon. De zon, in Finland, zou ook altijd blijven schijnen.

(H.M. van den Brink - Over het water)

8.1 INTRODUCTION

The light climate and nutrient concentrations (and ratios) are two of the most important factors determining biological processes in aquatic systems (Horne & Goldman 1994). The light climate penetrating into an aquatic system is affected by physical and chemical factors, such as dissolved organic carbon (DOC), chlorophyll, particles, and humic substances (Kirk 1994a). The DOC concentration, in particular, is important in the attenuation of UVB radiation (280-320 nm) (Scully & Lean 1994, Chapter 2). However, solar UV radiation can also cause a decrease in DOC, and a shift in molecular size towards smaller molecules, indicating photodegradation of humic substances (*e.g.* Allard *et al.* 1994, De Haan 1993). These two phenomena, coupled with recent declines in stratospheric ozone concentration and the concomitant threat of increases in ultraviolet-B radiation, have stimulated renewed research into the effects of UVB radiation on aquatic organisms and ecosystems (Williamson & Zagarese 1994).

UV radiation can have negative effects on phytoplankton, typically resulting in a decrease of photosynthetic rate (*e.g.* Smith *et al.* 1992). In both laboratory and field studies, the allocation of photosynthates into lipid, protein and carbohydrates was affected, however it was not possible to identify one aspect of photosynthate allocation as consistently diagnostic of UV stress because the results were species-specific (Arts & Rai 1997, Smith *et al.* 1998). Other effects of UVB on phytoplankton include changes in the morphology and biochemical composition (Chapter 3). These types of morphological and biochemical changes might also affect herbivorous zooplankton, as shown in laboratory studies with *Daphnia* (Chapter 5).

Light and nutrients interact to affect rates of macromolecular synthesis, because light regulates many enzymatic processes. For example, the key enzyme for nitrogen assimilation is activated by light. Amino acid and protein syntheses are therefore dependent to some extent on light intensity. It has been suggested that the influence of light on lipid synthesis is likely to be far larger than variations in nutrient supply (Wainman *et al.* 1999). In this regard, Sterner and co-workers (1997) examined the balance between light and phosphorus in freshwater, pelagic ecosystems. They concluded that when light energy is high, the base of the food web is carbon rich and P-limited. When light energy is low, the base of the food web is relatively P rich.

Fluctuations in nutrients can affect the quantity of phytoplankton, the species composition, and the quality in terms of biochemical and mineral content. In laboratory experiments phytoplankton cells usually produce more lipid under nutrient stress. In general nitrogen limitation causes a lower protein content, and phosphorus limitation causes a higher carbohydrate and lipid content (*e.g.* Kilham *et al.* 1997, Reitan *et al.* 1994, Shifrin & Chisholm 1981). One of the most predictable responses of natural algal assemblages to nutrient limitation is shifts in phytoplankton community species composition (Sommer 1989). Several field studies show seasonal changes of the biochemical composition of the seston, generally related to phytoplankton succession (*e.g.* Ahlgren *et al.* 1997, Arts *et al.* 1997, Kreeger *et al.* 1997).

The phytoplankton-zooplankton interaction has been the focus of a large number of ecological studies. A considerable amount of literature is available on the topic of the nutritional quality of algal food particles for zooplankton. Studies on zooplankton have focused on mineral limitation, especially phosphorus (P) limitation (*e.g.* Hessen 1992, Sterner 1993) and the importance of biochemical limitation for zooplankton, especially the content of highly unsaturated fatty acids (HUFA) (*e.g.* Ahlgren *et al.* 1990, Müller-Navarra 1995). The fatty acid versus phosphorus limitation controversy in zooplankton ecology is still not resolved (Gulati & DeMott 1997). The importance of phosphorus content for *Daphnia* can be explained by the higher P-demand of *Daphnia* (Andersen & Hessen 1991). The importance of fatty acids in the food of *Daphnia* can be explained by the fact that fatty acid synthesis is generally low in daphnids, more than 98% of the accumulated lipid is derived from the diet, and only 1.6% of the fatty acid pool is synthesized *de novo* (Goulden & Place 1990). Ahlgren and co-workers (1997) found in a field study that the polyunsaturated fatty acid (PUFA) content of lake seston showed good correlation with the P-content. They concluded that the discussion concerning biochemical versus mineral limitation of zooplankton may not be relevant, because high-quality phytoplankton apparently contains great amounts of phospholipids which consist mainly of ω3-PUFA (Ahlgren *et al.* 1997, 1998).

This chapter describes the results of a field survey (20 ponds and 7 lakes) of seston variables in relation to the potential for *Daphnia* growth. The ponds and lakes differed in DOC content and consequently in light climate but were all mesotrophic to eutrophic. Preliminary stoichiometric analysis in 7 ponds showed that C:P ratios were below 100, and C:N ratios below 10 (Arts unpubl.). The hypothesis of this study was that differences in light climate, especially UVB penetration, would affect the phytoplankton community, and subsequently higher trophic levels. The aim of this study was to assess how the clarity of the water was related to phytoplankton species composition and biomass as well as the biochemical composition. A biotest was performed to establish the potential quality and quantity of the seston to support zooplankton growth. In the biotest the somatic growth of *Daphnia magna* fed natural seston was measured. *D. magna* is considered to be a non-selective herbivorous filter-feeder, feeding mainly on particles smaller than 40 µm (Lampert 1987), and is therefore a useful test organism. The biotest is a powerful tool to compare differences in lake seston for herbivorous zooplankton (*cf.* Müller-Navarra 1995). Correlation and regression techniques were used to examine the relationships amongst the physicochemical variables of the water, biochemical seston variables, and *Daphnia* growth.

8.2 Materials and Methods

8.2.1 Sample procedure

In a period of 8 weeks from May to July 1997, 16 ponds and 7 lakes were sampled in south-central Saskatchewan, Canada. The ponds were all located in St. Denis National Wildlife Area, a wetland area of 361 ha, located *ca.* 40 km east of Saskatoon, Saskatchewan, Canada. There are numerous ponds in this area, ranging in size, water chemistry and UV penetration (Arts unpubl., Arts *et al.* in prep.). Two ponds (pond 15 and pond 4857) were sampled 3 times during the 8 week period, the other ponds and lakes were sampled once.

10 litre water samples were collected from the surface, and taken back to the laboratory. The samples were filtered through a 40 µm mesh to remove zooplankton and to retain the edible fraction for *Daphnia*. Various biochemical seston variables were measured (see below), and *Daphnia* biotests were performed with the samples.

8.2.2 *Daphnia magna* biotest

Daphnia magna was obtained from Aquatic Research Organisms, New Hampshire (USA). This culture was originally from the Environmental Protection Agency, Duluth, Minnesota (USA). *Daphnia magna* was cultured in artificial ADAM medium (Klüttgen *et al.* 1994), in 1 litre jars at a density of 15 to 20 individuals l^{-1}. Feeding was daily with *Scenedesmus acutus* f. *alterans* (Chlorophyceae, strain T10 from University of Texas Culture Collection) at a concentration of 1 to 2 mg C l^{-1}. The daphnids were cultured at 20 °C, at a light-dark cycle of 12:12 h, and a PAR intensity of 25 µmol m^{-2} s^{-1}. Juveniles were collected every day, and cultured in cohorts under the same conditions. Every week, all animals were transferred into fresh medium.

S. acutus was cultured in WC medium (Guillard & Lorenzen 1972), in 2 l chemostats, with a dilution rate of 0.4 d^{-1}. *Scenedesmus* was present mostly as single cells. PAR intensity was 120 µmol m^{-2} s^{-1} at the vessel surface, with a light:dark cycle of 16:8 h.

The laboratory biotest was performed with *Daphnia magna* feeding on natural water and seston. The experiments started with 3 d old juveniles from one cohort. At the start of each experiment, 15 juveniles from the cohort were dried at 60 °C for at least 4 h, and weighed using a Cahn C-30 microbalance (accuracy 1 µg), to obtain a reliable weight at the start. Twenty juveniles were placed in 1 litre pond water. Water and seston was refreshed daily, and at the same time 3 individuals were removed, dried at 60 °C, and weighed. Each experiment lasted 5 d. Water and seston was stored cool and dark. As a control with each experiment, 20 juveniles were placed in ADAM medium with *S. acutus* at a concentration of 1 mg C l^{-1} and processed in the same way as the field seston experiments.

Growth rate per day as dry weight increase was calculated by plotting the individual weights on natural log scale versus day and fitting a linear line, with the slope of the line

representing the daily dry weight increase. This growth rate for each pond was also compared with the growth rate in the *Scenedesmus* control, resulting in a relative growth rate. This was done to be able to correct for possible differences between the biotest experiments, because the samples were taken in an 8 week period, and in total 12 biotest experiments were performed.

8.2.3 Water analyses

Water samples for DOC analysis were filtered through a 0.2 µm cellulose acetate filter (Sartorius). Samples were acidified with 0.4 ml concentrated phosphoric acid (85-87% W/W) and stored until analysis. DOC concentration was measured using a Shimadzu (Model TOC-5050AXX) carbon analyser connected to a Shimadzu autosampler (Model ASI-5000A) following combustion of the aqueous sample and detection of the CO_2 gas in a non-dispersive infrared gas analyser.

Absorbance was measured on filtered water samples (Whatman GF/C) with a Sargent-Welch Pye Unicam 8-400 UV/VIS spectrophotometer at 250 nm, 365 nm and 390 nm. The absorbance at 250 nm (A250) is usually strongly correlated with the DOC concentration (De Haan & De Boer 1987). The ratio of absorbance at 250 nm and absorbance at 365 nm (A250/A365) gives an indication of the size of the humic acid molecules. A high ratio (around 9) indicates the presence of small molecules, a low ratio (around 5) indicates the presence of large molecules (De Haan & De Boer 1987). The ratio can also be regarded as a proxy for UV attenuation, a higher ratio means that shorter wavelengths are attenuated more rapidly than longer wavelengths. pH was measured with a Fisher Scientific pH meter (model: Accumet 915).

8.2.4 Seston analyses

Duplicate samples for protein, carbohydrate, lipid, and chlorophyll-*a* concentration, and single samples for fatty acid and pigment concentration, were filtered on precombusted GF/C filters (Whatman). The pigment and chlorophyll samples were encased in aluminium foil to protect them from the light. All filters were stored at -70 °C until analysis. 100 ml samples for phytoplankton species analysis were preserved with 1 ml Lugol's solution, and stored in dark bottles.

The size spectra (biovolume) of the seston was determined with an electronic particle counter (Coulter Multisizer II, aperture 100 µm) in the following size fractions: 2-3 µm equivalent spherical diameter (ESD), 3-10 µm ESD, 10-20 µm ESD, 20-30 µm ESD, and 30-40 µm ESD. The phytoplankton species composition and abundance was determined using an inverted microscope (Reichert, Germany), with 500x magnification. Chlorophyll-*a* was

determined by boiling the filter in ethanol. Chlorophyll-*a* fluorescence was then measured on a Turner fluorometer (Model 10-005R) after correction for phaeopigments (Nusch 1980).

Particulate carbohydrate was measured by a phenol-sulphuric acid method (Dubois *et al.* 1956) as described in Pick (1987). The procedure was standardized with reagent grade glucose (Sigma G5767). Total proteins were analysed using a protein assay kit (Sigma, P5656), with an extra extraction with 1 ml 0.5 N NaOH. Absorbance was measured at 750 nm. The procedure was standardized with bovine serum albumin.

Lipids were extracted overnight with 3 ml chloroform:methanol (2:1 v/v). An aliquot of this lipid extract was used for determination of lipid class composition by thin-layer chromatography coupled with flame ionization detection (TLC-FID) using an Iatroscan MK-IV detector (Iatron Labs., Tokyo), as described in Arts *et al.* (1992). Fatty acids were extracted with 4 ml chloroform:methanol (2:1 v/v). Heneicosaenoic acid (C21:0) was added as internal standard. The chloroform contained 0.003% butylated hydroxytoluene as an antioxidant. The extract was vortexed and centrifuged (10 min, 2500 rpm). This procedure was repeated twice, and the supernatants were combined and washed with demineralized water with 0.88% NaCl (Folch *et al.* 1957). Lipid esters were transmethylated at 80 °C for 4 h with 1 ml of 3% H_2SO_4 in dry methanol, and extracted with hexane. The fatty acids were analysed on a Hewlett Packard 5890 gas chromatograph, with a very polar 50 m silica column (OSGE BPX-70). Peaks were identified using retention times of known standards, and calculating equivalent chain length (ECL) values.

Pigments were extracted with acetone:methanol:water (80:15:5 v/v), and analysed with reversed-phase HPLC techniques, as described in Leavitt *et al.* (1989). Particulate organic matter (POM) was calculated as the sum of lipids, carbohydrates, and proteins. Kreeger *et al.* (1997) found a good relation with actual measurement of particulate matter, and using the sum of lipids, carbohydrates and proteins. An estimate of energy value of the seston was made with the following equation (Lemcke & Lampert 1975):

caloric value ($J\ l^{-1}$) = 17.50*carbohydrates ($mg\ l^{-1}$) + 25*proteins ($mg\ l^{-1}$) + 39*lipids ($mg\ l^{-1}$).

8.2.5 Statistical analyses

Multivariate analysis of the phytoplankton species data was performed with CANOCO (Ter Braak 1988, 1990), using direct ordination (CCA). The data were ln transformed, and rare species were downweighted.

Growth rates from the *Daphnia* biotest were compared with the physical and (bio)chemical variables of the pond water, using correlation and (multiple) regression (Genstat 1993). Correlations were calculated with the total seston variables (*e.g.* sum of lipid classes, sum of pigment classes, total number of phytoplankton, total biovolume). Significant product-moment correlation coefficients were determined using sequential Bonferroni techniques, to reduce the type I error (Rice 1989). Linear regressions were calculated with the individual classes of each seston variable (*e.g.* lipid classes, phytoplankton taxa, biovolume classes, fatty

acids classes), with ln-transformed seston variables as the explaining variable and *Daphnia* growth rate as the response variable (equation: $y = a + b*\ln(x)$). This proved to be the best relation to describe the data, and it implies that at increasing food concentration, the growth rate reaches a plateau, which is likely to be the physiological maximum growth rate.

Multiple linear regression models were selected with an iterative procedure in Genstat, selecting the best ln-transformed seston variables to explain *Daphnia* growth (Genstat 1993). In this procedure all possible models are calculated, and the best models are selected on criteria of minimal number of predictor variables, and minimal residual mean sum of squares ($MS_{residual}$) (Montgomery & Peck 1982).

8.3 RESULTS

Table 8.1 gives an overview of the sample sites and the *Daphnia* growth rate as obtained in the laboratory biotest. The average growth rate in the *S. acutus* control was 0.44 d^{-1} (n=12, SE=0.01). Further analysis is done with the actual growth rate for each sample.

Table 8.1: Sample sites and *Daphnia* growth rate (absolute (d^{-1}) and relative (% of control)).

Site		*Daphnia* growth rate (d^{-1})	% of control
St. Denis:	Pond 1	0.07	17
	Pond 15	0.32	65
	Pond 15	0.39	86
	Pond 15	0.48	113
	Pond 20	0.25	59
	Pond 25	0.27	49
	Pond 26	0.15	33
	Pond 37	0.31	72
	Pond 40	0.21	48
	Pond 50	0.20	50
	Pond 60	0.32	69
	Pond 65	0.33	60
	Pond 67	0.41	75
	Pond 86	0.30	76
	Pond 90	0.34	85
	Pond 109	0.28	61
	Pond 120	0.26	57
	Pond 4857	0.32	75
	Pond 4857	0.22	56
	Pond 4857	0.17	39
Lakes:	Constance	0.01	1
	Emerald	0.23	57
	Gursky	0.21	54
	Iroquois	0.09	18
	Last Mountain	0.19	48
	Martin	0.31	78
	Zelma	0.43	88

Multivariate analysis on the phytoplankton composition data was performed with CANOCO. Direct ordination (CCA) showed that absorbance at 250 nm and the ratio A250/A365 are significant variables in the ordination of the different sampling points based on their phytoplankton species composition (Fig. 8.1). The lakes are grouped together in the upper right corner of the graph, with the ratio A250/A365 as ordinating variable. In the upper left corner the three samples of pond 15 are grouped separate from the other ponds, with the absorbance at 250 nm as ordinating variable.

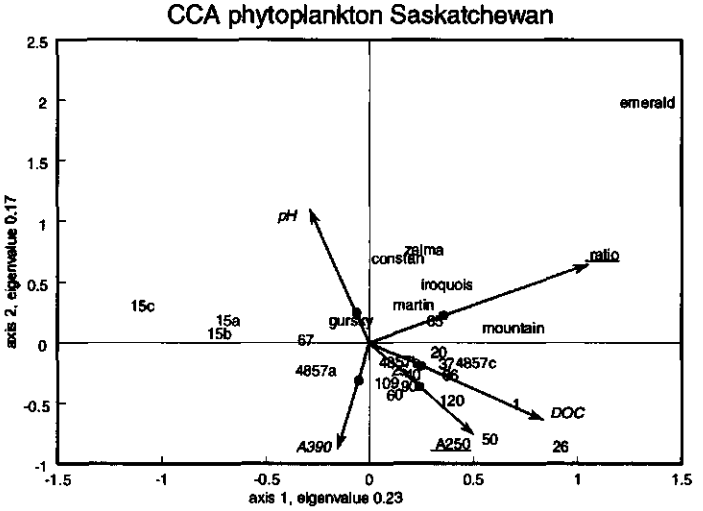


Figure 8.1: CCA plot of the sample points. Arrows in the plot refer to the direction of ordination by the physical variable. The large dot indicates the strength of the physical variable, underlined variables are significant, variables in italics are not significant.

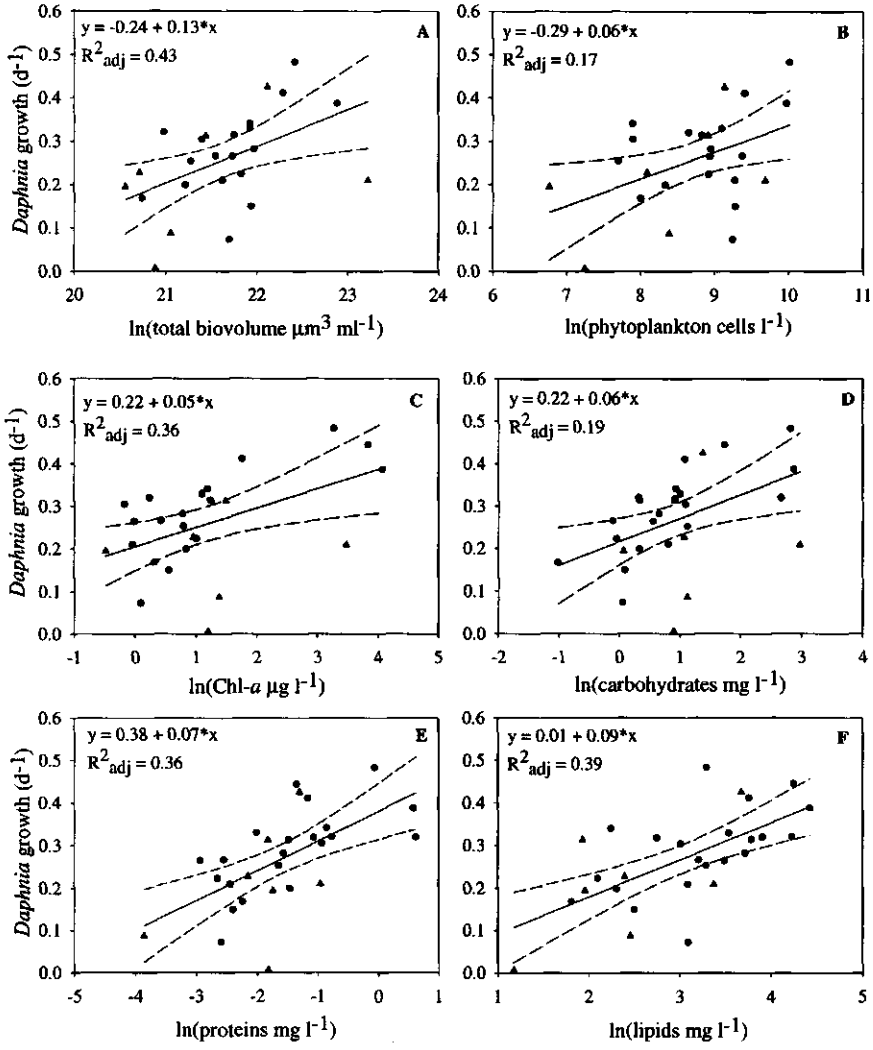
Product-moment correlations were calculated for the physicochemical variables, total seston variables and *Daphnia* growth (Table 8.2). DOC concentration and absorbance at 250 nm were correlated (r=0.84). The ratio A250/A365 was negatively correlated with total lipid concentration (r=-0.73), and with *Daphnia* growth (r=-0.64). Of the seston variables, total lipids (r=0.64) had a significant correlation with *Daphnia* growth. Protein concentration (r=0.62) and chlorophyll-*a* concentration (r=0.62) had nearly significant correlations with *Daphnia* growth.

Complementary to the correlation results, linear regressions were calculated to describe the correlations. The regression between absorbance at 250 nm and DOC gave the following relation: DOC (mg l^{-1}) = 12.88 + 22.21*A250 (cm^{-1}), which explained 61% of the variation in DOC concentration. Fig. 8.2 gives the significant regressions of total (ln-transformed) biochemical seston variables versus *Daphnia* growth. Seston biovolume

(R^2_{adj}=0.43), particulate organic matter (R^2_{adj}=0.40), total lipids (R^2_{adj}=0.39) and caloric value (R^2_{adj}=0.39) gave good explanations for *Daphnia* growth.

Table 8.2: Correlation matrix of physical variables, seston variables (ln transformed) and *Daphnia* growth, bold figure indicates significant correlation coefficient at α=0.05 after sequential Bonferroni technique (n=27, threshold r = 0.626); ratio is A250/A365, Chl-a is chlorophyll-*a*, CHO is carbohydrates, Prot is protein, Lip is lipids, Biov is seston biovolume, FA is fatty acids, Phyto is total phytoplankton abundance, Pigm is pigments.

growth	-																			
pH	-0.15																			
A250	-0.22	**-0.72**	-																	
A365	0.05	**-0.83**	**0.90**	-																
A390	0.11	**-0.81**	**0.84**	**0.95**	-															
ratio	**-0.64**	0.49	-0.13	-0.25	-0.25	-														
DOC	-0.36	-0.32	**0.84**	0.62	0.58	0.39	-													
Chl-a	0.62	0.26	-0.48	-0.30	-0.28	-0.52	-0.42	-												
CHO	0.47	0.11	-0.43	-0.20	-0.12	-0.46	-0.41	**0.82**	-											
Prot	0.62	-0.22	-0.12	0.12	0.26	-0.53	-0.26	**0.65**	**0.66**	-										
Lip	**0.64**	-0.28	-0.15	0.16	0.15	**-0.73**	-0.34	0.47	0.50	0.47	-									
Biov	0.49	-0.13	0.04	0.25	0.30	-0.57	-0.01	**0.78**	**0.67**	0.47	0.60	-								
FA	0.40	-0.09	-0.33	-0.24	-0.19	-0.17	-0.39	**0.72**	0.52	0.38	0.27	0.25	-							
Phyto	0.45	-0.12	0.07	0.28	0.25	-0.52	-0.06	0.62	0.44	0.21	**0.64**	**0.80**	0.11	-						
Pigm	0.31	0.23	-0.26	-0.20	-0.03	-0.15	-0.00	**0.85**	**0.79**	0.59	0.19	0.58	0.55	0.20	-					
	growth	pH	A250	A365	A390	ratio	DOC	Chl-a	CHO	Prot	Lip	Biov	FA	Phyto	Pigm					


y = -0.24 + 0.13*x
R2adj = 0.43
A
Daphnia growth (d-1)
ln(total biovolume µm3 ml-1)
y = -0.29 + 0.06*x
R2adj = 0.17
B
ln(phytoplankton cells l-1)
y = 0.22 + 0.05*x
R2adj = 0.36
C
ln(Chl-a µg l-1)
y = 0.22 + 0.06*x
R2adj = 0.19
D
ln(carbohydrates mg l-1)
y = 0.38 + 0.07*x
R2adj = 0.36
E
ln(proteins mg l-1)
y = 0.01 + 0.09*x
R2adj = 0.39
F
ln(lipids mg l-1)

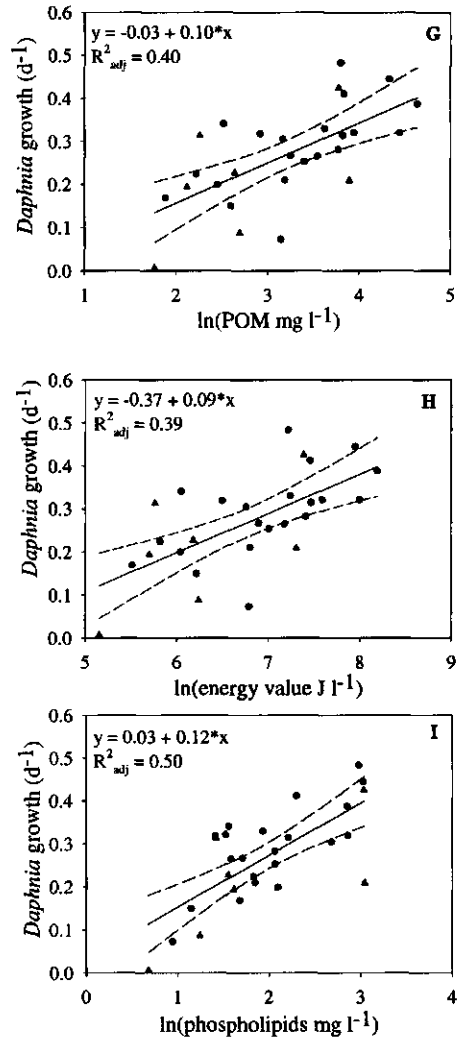


Figure 8.2: (Continued from previous page) Regressions with 95% c.i. of total seston variables analysed from the fraction <40 µm *versus Daphnia* growth rate (d^{-1}): panel A: ln(biovolume); panel B: ln(phytoplankton); panel C: ln(chlorophyll-*a*); panel D: ln(carbohydrates); panel E: ln(proteins); panel F: ln(lipids); panel G: ln(particulate organic matter); panel H: ln(energy value); panel I: ln(phospholipids); symbols used are circles for ponds and triangles for lakes.

From the simple regressions with detailed individual classes (*e.g.* biovolume size fractions, phytoplankton taxa, lipid classes, etc.), the concentration of phospholipids emerged as the best predictor for *Daphnia* growth, explaining 50% of the variation in *Daphnia* growth (Fig. 8.2, panel I). Other variables that gave a reasonable prediction of *Daphnia* growth were Bacillariophyceae, sterol content, and sum of polyunsaturated fatty acids (Table 8.3).

Table 8.3: Significant ($p<0.05$) simple regressions of individual classes of biochemical variables versus *Daphnia* growth (g, d^{-1}), as $g = a + b*\ln(x)$; PUFA is polyunsaturated fatty acids, AMPL is acetone-mobile polar lipids.

Class	x	a	b	R^2_{adj}
lipid classes	phospholipid	0.03	0.12	0.50
	sterol	0.47	0.13	0.39
	AMPL	0.20	0.04	0.24
	hydrocarbon	0.23	0.09	0.11
phytoplankton taxa	Bacillariophyceae	-0.02	0.05	0.40
fatty acids	PUFA	0.39	0.04	0.30
	EPA (20:5ω3)	0.46	0.04	0.22
	total ω3-FA	0.39	0.03	0.20
pigment classes	fucoxanthin	0.34	0.09	0.27
biovolume	3-10 µm ESD	-1.56	0.09	0.21
	10-20 µm ESD	-1.09	0.07	0.19
	20-30 µm ESD	-0.80	0.06	0.14
	30-40 µm ESD	-0.84	0.06	0.19

Table 8.4 gives the results of the multiple regression with the total dataset with detailed individual classes of biochemical variables versus *Daphnia* growth. Three predictors are sufficient to obtain a significant regression. The concentration of acetone mobile polar lipids and phospholipids (both polar lipids) and number of Bacillariophyceae result in a R^2_{adj} of 0.63. Phospholipids and proteins are interchangeable in this 3-predictor model.

Table 8.4: Significant ($p<0.05$) multiple regressions of biochemical variables versus *Daphnia* growth (g, d^{-1}), with $g = a + b_i*\ln(x_i)$; AMPL is acetone-mobile polar lipids, PL is phospholipids.

A	b_i	x_i	R^2_{adj}
-0.08	0.02	AMPL	0.63
	0.08	PL	
	0.03	Bacillariophyceae	
0.15	0.05	protein	0.63
	0.03	AMPL	
	0.03	Bacillariophyceae	

Fig. 8.3 gives the significant regressions of DOC and the ratio A250/A365 versus *Daphnia* growth. There is a negative relation between the ratio and *Daphnia* growth, and also between DOC concentration and *Daphnia* growth.

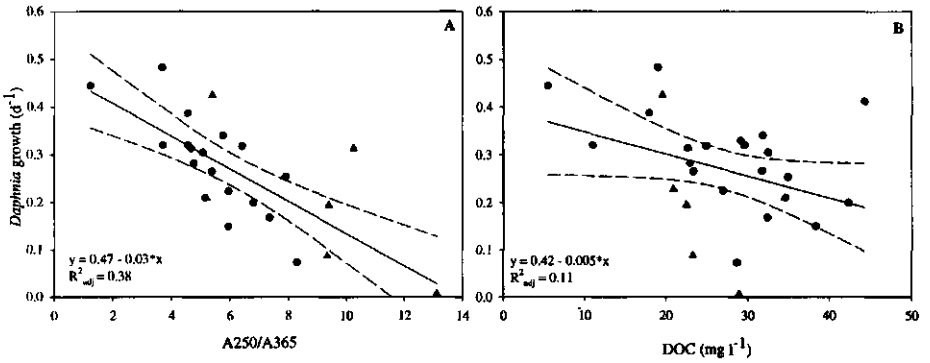


Figure 8.3: Regressions with 95% c.i. of physicochemical variables versus *Daphnia* growth rate (d^{-1}): panel A: A250/A365; panel B: DOC. Symbols as in Fig 8.2.

8.4 DISCUSSION

The multivariate analysis with CANOCO suggested that the phytoplankton species composition was influenced by the light climate of the water. Both A250 and the ratio A250/A365 were significant variables in the ordination of the sample points. The regression analyses confirmed this, with DOC concentration and A250/A365 both correlated with biochemical seston variables. DOC is a highly heterogeneous group of compounds with an absorption spectrum characterized by an exponentially rising absorption towards the blue and ultraviolet part of the solar spectrum (Kirk 1994b). The ratio A250/A365 is an indication of molecular size of DOC, but can also be regarded as proxy for UV attenuation, a higher ratio means that shorter wavelengths are attenuated more strongly than longer wavelengths. The positive relation between A250 and DOC as we found is comparable with literature values (De Haan & De Boer 1987, Rostan & Cellot 1995).

The ratio A250/A365 of the lakes is generally higher than that of the ponds, this indicates that the composition of the organic carbon molecules is different in the lakes, and generally consists of smaller molecules. This might indicate that photolysis of larger molecules has occurred. DOC probably has a longer residence time in the lakes, compared with the ponds. Further, ponds may proportionally have a larger terrestrial input of DOC than lakes, because they are smaller in surface area than the lakes, and are also more immediately affected by agricultural activities and runoff.

From the simple regressions, the concentration of phospholipids emerged as the best predictor, explaining 50% of the variation in *Daphnia* growth. Phospholipids are structural lipids in the cell membrane, and are a good source of eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) (Ahlgren *et al.* 1997, Ahlgren *et al.* 1998). *Daphnia* growth was further explained by both food quality variables (diatom abundance, caloric value, total lipids, sterol esters, polyunsaturated fatty acids), and food quantity variables (total biovolume, particulate organic matter).

In the multiple regression results, protein and acetone-mobile polar lipids also emerge as important predicting variables. Food protein content is a better measure for food quality than N-content, because N cannot be used by daphnids in elemental or inorganic form, and must be metabolized as proteins and amino acids (Brett 1993). The significant negative relation of the ratio A250/A365 versus *Daphnia* growth is most likely through the negative relation of the ratio versus lipid content, and the positive relation of lipid content versus *Daphnia* growth. The DOC concentration also has a negative relation with lipid content and *Daphnia* growth, but with larger variation.

The scatter in the regressions can be explained with the fact that these are field data. Outliers in the graphs can generally be explained by the phytoplankton species. For example, Lake Constance (in this study, Canada) had a very low *Daphnia* growth rate (0.01 d^{-1}). The phytoplankton number was low (*ca.* 1500 cells ml^{-1}), but comparable with the other lake samples. The species composition however was mainly cyanobacteria (*Aphanotece* sp. and *Anabaena* sp.) and chrysophytes (*Kephyrion* sp. and *Syncrypta* sp.) that are hard to ingest or digest. This may explain the much lower growth rate than would be expected from the biochemical composition.

The evidence that Müller-Navarra (1995) found in her study of lake Schöhsee for EPA limiting *Daphnia* growth is not directly confirmed by this study, but indirectly through the phospholipids. In another study by Rothhaupt and co-workers (1998), following Lake Constance (Germany) in one season, linolenic acid (18:3ω3) was found to be the best predictor for *Daphnia* growth. Those two studies followed one system during one season, and each identified a different fatty acid as limiting. This indicates that it is difficult to extrapolate conclusions from one system to another system. Our study describes 23 different systems, and we find phospholipids as the best predictor for *Daphnia* growth. This is a structural lipid that is thought to be more constant relative to energy reserve lipids during the season, and maybe between systems. Phospholipids are high in ω3-PUFA content, so this might be the link between P-content and essential fatty acids defining the food quality of phytoplankton for herbivorous zooplankton.

The ponds of St. Denis are quite similar in appearance (size, hydrology, nutrient loading, seston stoichiometry), and are located close to each other. Still, the DOC concentration varies amongst the ponds, the biochemical composition of the seston from these similar ponds was quite different, as was the resulting *Daphnia* growth in the biotest. Differences in light climate as a result of the differences in DOC might be responsible for the differences in the nutritional quality of the seston (see Sterner *et al.* 1997).

The *Daphnia* growth results can be related to PAR and UV attenuation measurements that were made in 19 of the sites we sampled (Arts *et al.* in prep., Figure 8.4). It is important to note that the K_d measurements for the ponds were not always made at the same time as the seston was sampled (indicated with open symbols), and that these are observational results so it is not possible to determine causal relationships. With these cautions in mind, the following picture can be described. The relation between K_dPAR and *Daphnia* growth is positive and almost significant (linear regression: p=0.052). Sample points with higher K_dPAR values have seston that support *Daphnia* growth better. It is also clear that there is a distinction between the lakes and ponds, with the lakes having lower K_dPAR values. The relation between K_dUVA and K_dUVB versus *Daphnia* growth is more bell-shaped, with an increase in *Daphnia* growth at lower K_d values (which are the lake samples), and a decrease at higher K_d values (pond samples). There seems to be an optimum range of 25 to 55 (m^{-1}) for K_dUVB, and 12 to 22 (m^{-1}) for K_dUVA where *Daphnia* growth is supported best. This apparent optimum range may be explained by the difference in ponds and lakes samples only. It can also be explained by increasing photoprotection against UVA and UVB at low K_d values. Beyond the optimum range higher K_d values and decreased light penetration may result in a decrease in phytoplankton with lower nutritional value, and thus lower *Daphnia* growth. This pattern can also be observed in similar relations between K_d and the phospholipid concentration (Figure 8.5). The highest phospholipid concentrations occur in the range of 30 to 45 (m^{-1}) for K_dUVB, and 12 to 17 (m^{-1}) for K_dUVA.

We hypothesize that in the studied systems, light climate and especially the penetration of ultraviolet radiation is important in determining the phospholipid content of the seston. Because of the high nutritional value of phospholipids, it is the best predictor for *Daphnia* growth. These proposed relationships between light climate, phospholipid content of the seston, and *Daphnia* growth need experimental confirmation.

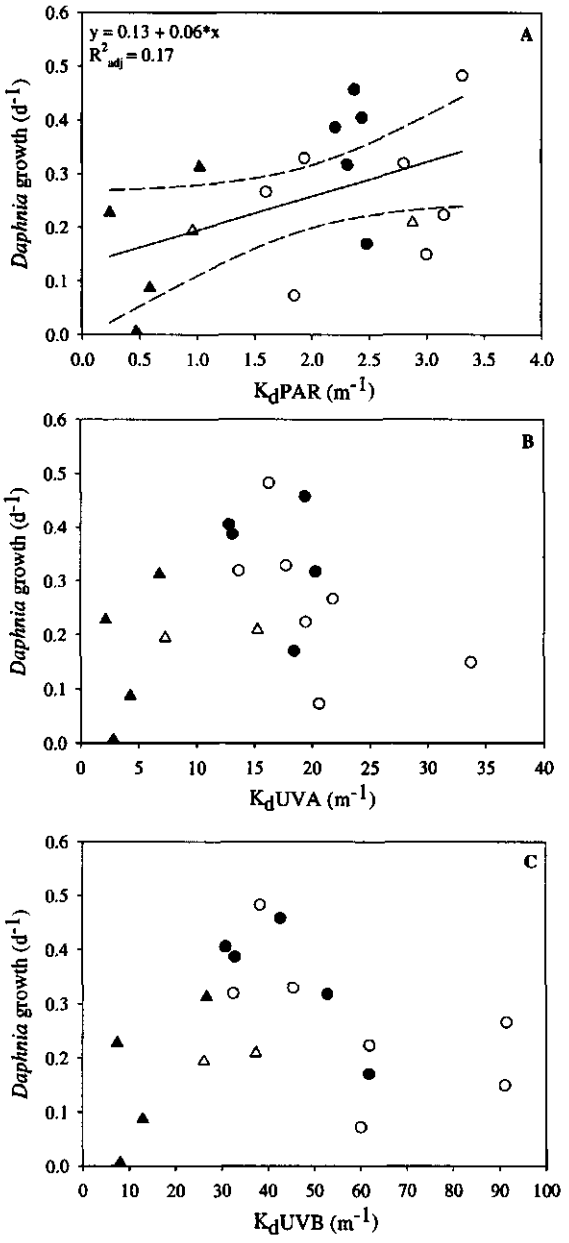


Figure 8.4: K_d values (Arts *et al.* in prep.) versus *Daphnia* growth (g, d^{-1}); panel A = K_dPAR, dashed line indicates the 95% confidence interval of the linear regression; panel B = K_dUVA; panel C = K_dUVB. Symbols as in Fig 8.2. Open symbols indicate that the K_d measurement was not taken at the same day as the seston was sampled.

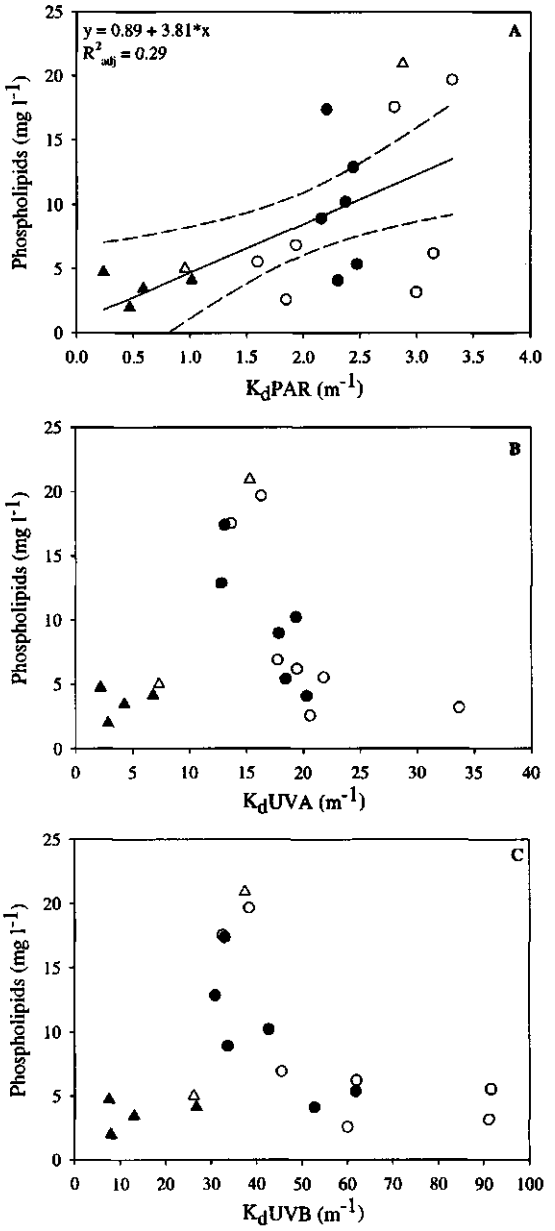


Figure 8.5: K_d values (Arts *et al.* in prep.) versus phospholipid concentration; panel A = K_dPAR, dashed line indicates the 95% confidence interval of the linear regression; panel B = K_dUVA; panel C = K_dUVB. Symbols as in Fig. 8.4.

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CHAPTER 9

CONCLUDING REMARKS

"Where shall I begin, please, your majesty?" he asked.
"Begin at the beginning, " the King said gravely,
"and go on till you come to the end; then stop."

(Lewis Carroll - Alice in Wonderland)

9.1 INTRODUCTION

The aim of this study was to investigate the effects of ultraviolet-B (UVB) radiation on interactions between phytoplankton and zooplankton. The direct reason for this study was the observed decline in stratospheric ozone and concurrent increase in UVB radiation reaching the earth's surface. Particular attention was given to the situation in the Netherlands at present and expected in the near future. The results presented in this thesis can be divided into the following aspects:

- What is the effect of current intensities of UVB radiation at temperate latitude on phytoplankton-zooplankton interactions?
- What is the potential effect of expected increased UVB radiation on phytoplankton-zooplankton interactions?

9.2 UVB RADIATION IN THE NETHERLANDS

Radiation measurements in various lakes, ponds, canals and ditches in the Netherlands showed that the penetration depth of UVB radiation is limited to the upper decimetres (Chapter 2). Even in a clear system such as Lake Maarsseveen, the 1% penetration depth of UVB was only 51 centimetres. The low penetration depth in the studied systems was caused by either high phytoplankton biomass, or high concentrations of DOC.

The impact of UVB radiation on a system is a combination of UVB penetration depth, mixing depth, and total water depth. Mixing processes are important in determining the exposure intensity and duration of an organism to UVB, which in turn determines the balance between damage and repair processes. Reciprocity seems to hold for damage, but not for repair processes. Therefore, mixing processes and the ability for repair are important in determining the impact of UVB on an organism (see Chapter 1).

The majority of aquatic systems in the Netherlands is shallow, *e.g.* ponds, ditches, canals, fens, shallow lakes. One might expect that in well-mixed shallow systems (with mixing depth and euphotic depth both exceeding lake depth) the proportion of phytoplankton exposed to UVB radiation would be larger than in deep systems. Vertical mixing might enhance the inhibition of photosynthesis by UVB, and thus be negative for phytoplankton. For zooplankton, shallow systems will provide little depth refuge. However, in the studied systems the penetration depth of UVB in combination with the water depth does provide some possible refuge in most systems. Mixing processes will also influence the UVB exposure of zooplankton. For species capable of photoreactivation, vertical mixing might enhance survival (*e.g.* Zagarese *et al.* 1998).

The different mechanisms of photoreactivation and adaptation to UVB and the role these mechanisms play in UVB damage in phytoplankton and zooplankton are still not fully

understood. These are however very important in determining the UVB effect, and this will need further investigation.

9.3 *IN SITU* EFFECTS OF UVB ON PLANKTON

UVB stress on phytoplankton and zooplankton is widely studied. Negative effects of UVB radiation can be easily demonstrated in laboratory experiments and static field incubations. The magnitude of damage is however dependent on the dose, low intensities of UVB may be even beneficial to phytoplankton, *e.g.* an increase in growth rate. Current intensities of UVB radiation in the Netherlands and Norway do affect phytoplankton and zooplankton (see Chapter 7), but not dramatically. In the Antarctic current UVB radiation intensities present a significant environmental stress, and UVB effects are enhanced by ozone depletion (Neale *et al.* 1998). Chemical ozone depletion in the Arctic may be even greater than the Antarctic ozone depletion, but is usually compensated for by atmospheric dynamics which differ from the Antarctic (Müller *et al.* 1997, Chapter 1). However, the situation in the Netherlands is quite different. Even if stratospheric ozone depletion occurs at temperate latitude, the humid sea climate and tropospheric pollution are likely to counteract stratospheric ozone depletion, and prevent increases in UVB radiation reaching the earth's surface.

The impact of UVB on a system is also determined by light attenuation properties (Chapter 2). An important aim of water management in the Netherlands is an increase in water clarity. Management is usually aimed to achieve a transition from an algal dominated (turbid) system to a macrophyte dominated (clear) system (*e.g.* Scheffer 1998). This would increase the UVB penetration in a system, which would likely be larger than the UVB increase as result of ozone depletion expected at temperate latitude. There are however two important differences in UVB increases as result of ozone depletion or as result of increases in water clarity. Increased UVB radiation as result of ozone depletion has in potential a stronger effect.

1) Wavelength specific differences: Ozone depletion causes an increase in UVB radiation only, longer wavelengths (UVA and PAR), which are important in photoenzymatic repair processes, are not affected. Ozone depletion will thus result in a higher ratio of UVB to PAR at the water surface, and, hence, also below the surface.

When water clarity increases, the radiation above the water surface will be the same, with the same ratio of UVB to PAR. The attenuation properties will be affected, resulting in increased penetration depth for all wavelengths. The important difference with increase in UVB radiation as result of ozone depletion is that the ratio of UVB to PAR will not increase.

2) Time scale differences: Variations in ozone concentration may occur within days, variations in water clarity are more likely to occur on longer timescales, weeks or seasons. This will influence light adaptation processes, shorter timescales might result in stronger effects. More information on adaptation and repair processes is however necessary to estimate these timescale effects.

9.4 Potential Effects of Increased UVB Radiation on Phytoplankton-Zooplankton Interactions

As described in Chapter 3, there is a wide range of potential mechanisms how UVB radiation may affect phytoplankton-zooplankton interactions. Morphological changes in phytoplankton cells induced by UVB radiation may influence ingestion and digestion by herbivorous zooplankton. The potential negative effect of UVB on the biochemical composition of phytoplankton cells will result in reduced food quality for herbivorous zooplankton. The potential negative effect of UVB on phytoplankton growth will result in reduced food quantity for herbivorous zooplankton. These potential effects were studied in this thesis.

Ingestion and incorporation rates of 3 different zooplankton species grazing on UVB-irradiated algae were affected in different ways (Chapter 4). Grazing rates were both increased and decreased. Based on these results, generalizations to predict effects of UVB stressed algae on grazing rates of zooplankton are difficult to make.

Reduced food quality effects in UVB-irradiated phytoplankton were tested in life history experiments with *Daphnia* (Chapter 5). UVB-irradiated phytoplankton resulted in a slightly smaller *Daphnia* body length. Length at maturity was significantly reduced for *D. pulex* and *D. magna* feeding on UVB-irradiated *Cryptomonas pyrenoidifera*, but not in the experiments with other species of UVB-irradiated phytoplankton. The population growth rate of *Daphnia* feeding on UVB-irradiated phytoplankton was not significantly affected.

It is interesting to compare these results with results of nutrient-limited phytoplankton. In life history experiments with the same clone of *D. pulex* feeding on 5 different species of nutrient-limited phytoplankton, the length at maturity was significantly reduced for all species. The population growth rate was significantly reduced for 3 of the 5 phytoplanton species tested (Lürling & Van Donk 1997). These comparisons suggest that the effect of nutrient-limited algae on *Daphnia* may be stronger than the effect of UVB-stressed algae. Generally, P-limited algae are poorest in quality, N-limited algae are intermediate, and non-limited algae are highest in quality as food for *Daphnia* (*e.g.* Lürling & Van Donk 1997, Mitchell *et al.* 1992, Sterner *et al.* 1993). UVB-irradiated phytoplankton is of poorer food quality than non-stressed phytoplankton, but not as poor as nutrient-limited phytoplankton.

Results from the microcosm experiment (Chapter 6) showed some negative effects of the UVB treatment on the phytoplankton-zooplankton interaction tested in a bio-assay. These results indicate that the transfer of energy from phytoplankton to zooplankton can be negatively influenced by UVB radiation. However, phytoplankton and zooplankton species compositions in the microcosms were not affected by the UVB stress. The explanation for this different effect may be found in the multitude of zooplankton species in the microcosms, compared with only one species in the bio-assay.

Summarizing the different experiments described in this thesis, the effects of UVB on phytoplankton-zooplankton interactions were present: effects were detectable, and effects were mostly negative. The magnitude of the UVB effects was not large. However, these experiments were short-term, and it may be erroneous to make long-term predictions based on these results. For example, in the life history experiments with *Daphnia*, a smaller number of juveniles of poorer quality was born in the UVB treatment. How this will affect long-term zooplankton dynamics is difficult to predict. But it may mean that even the subtle differences found in this study may be important for ecosystem functioning.

This study was initially started because of the expected effects of increased UVB radiation resulting from thinning of the ozone layer. However, our data showed that current UVB levels already had an effect on aquatic ecosystems, therefore UVB is already a factor controlling ecosystem functioning.

The potential effect of increased UVB radiation on the phytoplankton-zooplankton interactions is negative, caused by a combination of quantity and quality constraints. The most important mechanisms will be: 1) Altered biochemical composition of phytoplankton that will reduce food quality. 2) Reduced phytoplankton growth that will reduce food quantity.

9.5 Implications for Future Research

Increases in UVB radiation as result of decrease in stratospheric ozone has a potential negative effect on aquatic ecosystem functioning. Smith *et al.* (1992) clearly showed a reduction in primary production under the Antarctic ozone hole. However, for temperate latitudes, reservations can be made about potential effects of ozone decreases. Several enclosure and mesocosm experiments have shown that the UVB effects on phytoplankton are not always negative (see Chapter 6). The general conclusion might be that phytoplankton communities are able to resist to increases in UVB. Zooplankton might have refuge possibilities and thus be protected against UVB. Also in terrestrial ecology, reservations are made on the potential threat of increased UVB radiation to photosynthesis in higher plants (Allen *et al.* 1998).

The tremendous amount of research initiated by the discovery of the ozone hole does clearly show that UVB radiation is already a factor affecting ecosystem functioning. Light and nutrients are two important factors determining (aquatic) ecosystem functioning (*e.g.* Sterner *et al.* 1997). The results described in Chapter 8 indicate that there might be a link between UVB, UVA and PAR penetration depth, and the phospholipid content of the seston. Because of the high nutritional value of phospholipids, it was the most important factor predicting *Daphnia* growth in a laboratory biotest. More information is however necessary to integrate photobiology and ecology of phytoplankton and zooplankton organisms.

The penetration depth of ultraviolet radiation also plays a role in other ecosystem functions, *e.g.* the spawning behaviour of fish (yellow perch). Large spawning depths in a lake

with high UVB penetration suggest that yellow perch can avoid direct UVB damage (Williamson *et al.* 1997).

The results presented in this thesis show that UVB radiation is a negative factor controlling ecosystems. UVB has negative effects on the phytoplankton-zooplankton interactions, reducing the transfer of energy from phytoplankton to zooplankton. The mechanisms of these effects are still not fully understood. The results in this thesis show that it is not possible to make generalizations for an ecosystem based on experiments with single species of phytoplankton and zooplankton. UVB radiation interacts with other environmental variables (such as temperature, nutrient concentrations, mixing processes), and is thus playing a role in determining the functioning of an ecosystem. The exact role of UVB radiation in aquatic ecosystems needs further research.

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SUMMARY

The decrease in stratospheric ozone concentration has received wide attention because the ozone layer protects the earth from harmful ultraviolet-B radiation (UVB, 280-320 nm). UVB radiation is harmful for organisms, and therefore scientific research into how UVB radiation affects organisms and ecosystems receives great interest. This thesis describes the effects of UVB radiation on interactions between phytoplankton (algae) and zooplankton (waterfleas) in freshwater ecosystems. The underlying hypothesis in this thesis is that phytoplankton is directly affected by UVB radiation because it needs visible light (PAR, 400-700 nm) for photosynthesis, and is consequently also exposed to UVB. Zooplankton on the other hand is not dependent on light, and is able to move actively through the water. This capability of vertical migration, and the possible ability to detect UV radiation may allow zooplankters to regulate their exposure to UVB. Therefore, indirect effects on zooplankton through changes in phytoplankton (its food) may play a more important role than direct UVB effects.

UVB, UVA (320-400 nm) and PAR radiation were measured in 19 aquatic systems in the Netherlands (Chapter 2). In most systems the penetration of UVB radiation was limited to the upper decimetres. High phytoplankton biomass or high concentrations of humic acids caused the limited light penetration. Lake Maarsseveen was the clearest system in this study with a vertical attenuation coefficient (K_d) for UVB of 9.1 (m^{-1}). This corresponded to a 1% UVB penetration depth of 51 cm. The effect of UVB radiation on a system will be a combination of penetration depth, mixing processes, and depth of the system.

There is a number of UV-mediated qualitative changes in phytoplankton that may affect herbivorous zooplankton (Chapter 3). Changes in phytoplankton species composition, increase in cell size and increase in cell wall thickness will negatively affect ingestion and digestion by zooplankton. Phytoplankton cell biochemistry is also affected by UVB radiation, namely a decrease in lipids and proteins, and an increase in carbohydrates. This will strongly influence the cells nutrient quality. The potential negative effect of UV on fatty acids (FA) in phytoplankton call for special attention. FA play a major role in nutrition for most animals, and as such UV-mediated lipid peroxidation or reduced biosynthesis of essential FA could be a major determinant of food quality for aquatic herbivores.

Experiments with zooplankton grazing on UVB-stressed phytoplankton showed varying effects on grazing rates (Chapter 4). Grazing rates of the large waterflea *Daphnia* were not affected by UVB-stressed phytoplankton. The small waterflea *Bosmina* and small rotifer *Brachionus* had both increased and decreased grazing rates on UVB-stressed phytoplankton. These experiments suggest that possible effects of enhanced UVB radiation on phytoplankton-zooplankton interactions are not straightforward predictable from grazing experiments. Moreover, extrapolation of data to predict ecosystem response to UVB stress seems unjustified.

Life history experiments with *Daphnia* feeding on several species of UVB-stressed phytoplankton showed that life history traits were negatively affected (Chapter 5). Effects of UVB-stressed phytoplankton on the population growth rate of *Daphnia* were not significant. Effects on clutch sizes and quality of offspring were significant. In general, a smaller number of juveniles of poorer quality was produced in the UVB treatments. This may have implications for the food web functioning. The UVB effect was dependent on the phytoplankton species. The UVB effects may be caused by a change in food quality combined with possible reduced digestibility of UVB stressed phytoplankton.

No UVB effects were found in the experiments with indoor model ecosystems (Chapter 6). UVB radiation had no significant effect on the phytoplankton, zooplankton, periphyton or macro-invertebrate communities in these model ecosystems. A bio-assay with *Daphnia* feeding on phytoplankton from the model ecosystems showed that phytoplankton from the UVB treatments had a negative effect on *Daphnia* growth and survival, and to a lesser extent on fecundity. These results indicate that the transfer of energy from phytoplankton to zooplankton can be negatively influenced by UVB radiation. Overall, the model ecosystems were not affected by the UVB stress. From these results it can be concluded that a natural ecosystem with similar penetration of UVB can be resistant to UVB radiation presently occurring at temperate latitude.

Field experiments at 3 different latitudes (in the Netherlands, Norway and Spitsbergen) showed that present levels of UVB radiation may negatively affect phytoplankton and zooplankton (Chapter 7). The different phytoplankton species showed different responses in growth rate to UVB, UVA and PAR radiation. This implies that increased levels of UVB radiation could lead to shifts in phytoplankton community structure. *Chlamydomonas* (a green algae with flagella) responded to UVB radiation with a loss of flagella, whereas the growth rate was not affected. *Daphnia* in Lake Zwemlust (the Netherlands) responded to UVB with a decreased grazing rate and smaller body size, whereas the survival was not affected. *Daphnia* grazing rates were affected in all three locations, though variance was large and differences marginal. This shows that solar UVB radiation in potential can reduce the transfer of energy from phytoplankton to zooplankton. UVB effects were comparable between the three locations. The magnitude of the UVB effects was quite different, due to very different weather conditions.

A field study was done in the province of Saskatchewan, Canada, comparing seston (suspended live and dead particles) quantity and quality of ponds and lakes with different light penetration properties (Chapter 8). Multivariate analysis suggested that the phytoplankton species composition was influenced by the light climate of the studied system. A standardized laboratory experiment with *Daphnia* showed that the phospholipid content of the seston was the best predictor of *Daphnia* growth, because of the high nutritional value of phospholipids. It was hypothesized that in the studied systems, light climate and especially the penetration of ultraviolet radiation was important in determining the phospholipid content of the seston. The proposed relationships between light climate, phospholipid content of the seston, and *Daphnia* growth need experimental confirmation.

Summarizing the different experiments described in this thesis, the effects of UVB on the phytoplankton-zooplankton interactions were present, detectable, and mostly negative (Chapter 9). The magnitude of the UVB effects was not large. It was not possible to make generalizations because of the species-specific reactions to UVB. The experiments were all short-term, and it may be erroneous to make long-term predictions based on these results. However, the subtle differences found in this study may be important in determining ecosystem functioning. UVB radiation interacts with other environmental variables (such as temperature, nutrient concentrations, and vertical mixing), and is already playing a role in the functioning of an ecosystem.

SAMENVATTING

De afname van de ozonconcentratie in de stratosfeer heeft grote belangstelling omdat de ozonlaag de aarde beschermt tegen schadelijke ultraviolet-B straling (afgekort tot UVB, golflengten van 280 tot 320 nm). UVB is schadelijk voor organismen en daarom is er veel wetenschappelijke aandacht voor de effecten van UVB op organismen en ecosystemen. Dit proefschrift beschrijft de effecten van UVB op de interacties tussen fytoplankton (algen) en zoöplankton (watervlooien) in zoetwaterecosystemen. De hypothese in dit proefschrift is dat fytoplankton direct beïnvloed wordt door UVB, omdat het zichtbaar licht (PAR, 400-700 nm) nodig heeft voor de fotosynthese en daardoor ook blootgesteld wordt aan UVB. Zoöplankton is niet afhankelijk van licht en kan actief door de waterkolom bewegen. Dit vermogen tot verticale migratie en de mogelijkheid tot waarnemen van UVB geeft zoöplankton de mogelijkheid om hun blootstelling aan UVB te reguleren. Daarom zouden indirecte effecten op herbivoor zoöplankton door veranderingen in fytoplankton (hun voedsel) belangrijker kunnen zijn dan directe effecten.

In negentien oppervlaktewatersystemen in Nederland is UVB-, UVA- (320-400 nm) en PAR-straling gemeten (Hoofdstuk 2). In de meeste systemen was de doordringing van UVB beperkt tot de bovenste decimeters. Hoge concentraties van algen of humuszuren veroorzaakten deze beperkte lichtpenetratie. De Grote Maarsseveense Plas was het helderste systeem in dit onderzoek, de diepte tot waar 1% van de UVB-straling doordrong was 51 cm. Het effect van UVB op een ecosysteem zal een combinatie zijn van lichtpenetratie, verticale menging en waterdiepte.

UVB kan een aantal kwalitatieve veranderingen in fytoplankton veroorzaken die invloed kunnen hebben op herbivoor zoöplankton (Hoofdstuk 3). Veranderingen in fytoplanktonsoortensamenstelling, toegenomen grootte van de cel en toegenomen dikte van de celwand van fytoplankton zullen de opname en vertering door zoöplankton negatief beïnvloeden. UVB kan de biochemische samenstelling van een fytoplanktoncel veranderen: de hoeveelheden vetten en eiwitten zullen afnemen en koolhydraten toenemen. Deze veranderingen zullen de voedselkwaliteit van de cel zeer sterk beïnvloeden. Vooral het potentiële effect van UVB op vetzuren vraagt aandacht. Vetzuren in het voedsel zijn zeer belangrijk voor de meeste dierlijke organismen en daardoor kan de lipide-oxidatie door UVB of verminderde biosynthese van essentiële vetzuren een belangrijke factor zijn die de voedselkwaliteit van fytoplankton voor herbivoor zoöplankton bepaalt.

Experimenten met verschillende soorten zoöplankton grazend op UVB-bestraald fytoplankton lieten verschillende effecten op de graassnelheid zien (Hoofdstuk 4). De UVB-dosis was hoger dan de huidige intensiteit in Nederland. De graas van de grote watervlo *Daphnia* werd niet beïnvloed door UVB-bestraald fytoplankton. De kleine watervlo *Bosmina* en het raderdiertje *Brachionus* hadden beiden zowel een toegenomen als een afgenomen graassnelheid op UVB-bestraald fytoplankton, afhankelijk van de fytoplanktonsoort.

Gebaseerd op deze experimenten is het niet mogelijk om effecten van verhoogde UVB-straling op fytoplankton-zoöplanktoninteracties eenvoudig te voorspellen op basis van graasexperimenten. Voorts is extrapolatie van deze gegevens om ecosysteemreacties op UVB-straling te voorspellen niet gerechtvaardigd.

Experimenten met *Daphnia* gevoerd met UVB-bestraald fytoplankton lieten zien dat de life-historykarakteristieken van *Daphnia* negatief beïnvloed werden (Hoofdstuk 5). De UVB-dosis was hoger dan de huidige intensiteit in Nederland. Het effect van UVB-bestraald fytoplankton op de populatiegroeisnelheid van *Daphnia* was niet significant. Effecten op hoeveelheid en kwaliteit van het nageslacht waren significant. In het algemeen werden er minder juvenielen geboren in de UVB-behandeling, terwijl de geboren juvenielen van inferieure kwaliteit waren. Dit zal gevolgen hebben voor het functioneren van het ecosysteem. Mogelijke oorzaken van deze UVB-effecten zijn een veranderde voedselkwaliteit en een verminderde vertering van UVB-bestraald fytoplankton.

Er werden geen UVB-effecten gevonden in experimenten met kunstmatige ecosystemen (Hoofdstuk 6). De behandeling met een range van UVB-doses vergelijkbaar met en hoger dan de huidige intensiteit in Nederland, had geen significant effect op de fytoplankton-, zoöplankton-, perifyton- en macrofaunagemeenschappen. Een bio-assay met *Daphnia* liet zien dat het fytoplankton afkomstig uit de UVB-behandelde ecosystemen een negatief effect had op de groei en overleving van *Daphnia* en in mindere mate op de voortplanting. Deze resultaten tonen aan dat de doorgifte van energie van fytoplankton naar zoöplankton negatief beïnvloed kan worden door UVB-straling. Over het geheel genomen werden de ecosystemen niet beïnvloed door UVB-straling. Uit deze resultaten kan geconcludeerd worden dat een natuurlijk ecosysteem waarin UVB tot op een vergelijkbare diepte doordringt, weerstand kan bieden tegen UVB-straling met intensiteiten die nu voorkomen in Nederland.

Veldexperimenten uitgevoerd op drie verschillende breedtegraden (Nederland, Zuid-Noorwegen en Spitsbergen) lieten zien dat de huidige UVB-intensiteit invloed heeft op fytoplankton en zoöplankton (Hoofdstuk 7). De verschillende fytoplanktonsoorten lieten een verschillende respons in groei zien op UVB-, UVA- en PAR-straling. Dit houdt in dat een verhoogde UVB-straling kan leiden tot een verschuiving in fytoplanktonsoortensamenstelling en -gemeenschapsstructuur. *Chlamydomonas* (een groenalg met flagellen) reageerde op UVB met een verlies van beide flagellen, terwijl de groei niet door UVB beïnvloed werd. In het plasje Zwemlust (Nederland) reageerde *Daphnia* op UVB-straling met een lagere graassnelheid, terwijl de overleving niet door UVB beïnvloed werd. In alle drie de locaties werd de graassnelheid van *Daphnia* beïnvloed door UVB, hoewel de variantie groot was en verschillen marginaal. In potentie kan UVB uit het zonlicht een effect hebben op de fytoplankton-zoöplanktoninteractie. UVB-effecten waren vergelijkbaar tussen de drie locaties. De grootte van de UVB-effecten was verschillend doordat de weersomstandigheden sterk verschillend waren.

Een veldstudie naar de kwantiteit en kwaliteit van het seston (zwevende levende en dode deeltjes) uit poeltjes en meren met verschillende lichtuitdovingseigenschappen werd

uitgevoerd in de provincie Saskatchewan in Canada (Hoofdstuk 8). Het lichtklimaat was een belangrijke factor voor de fytoplanktonsoortensamenstelling. Een laboratoriumexperiment liet zien dat de fosfolipideconcentratie in het seston de beste voorspellende variabele was voor *Daphnia*-groei, vanwege de hoge voedselkwaliteit van fosfolipiden. Een verklarende hypothese kan zijn dat in de onderzochte systemen het lichtklimaat, vooral de diepte tot waar UVB doordringt, belangrijk is in het bepalen van de fosfolipideconcentratie van het seston. Deze hypothetische relaties tussen lichtklimaat, fosfolipideconcentratie van het seston en *Daphnia*-groei moeten nog experimenteel bevestigd worden.

Uit de vergelijking van de verschillende experimenten blijkt dat effecten van de huidige intensiteit van UVB-straling op fytoplankton-zoöplanktoninteracties aanwezig zijn, waarneembaar zijn en voornamelijk negatief zijn (Hoofdstuk 9). Het UVB effect was echter niet groot. Voorts was het niet mogelijk om generaliserende conclusies te trekken vanwege de soortsafhankelijke reacties op UVB-straling. De experimenten zijn op een korte tijdsschaal uitgevoerd en het kan foutief zijn om lange-termijnvoorspellingen te maken gebaseerd op deze experimenten. De subtiele verschillen kunnen wel belangrijk zijn in het bepalen van het functioneren van het ecosysteem. UVB-straling zal samen met andere omgevingsvariabelen (zoals temperatuur, nutriëntenconcentraties en verticale menging) het functioneren van een ecosysteem bepalen.

CURRICULUM VITAE

Hendrika Joacomina de Lange werd op 29 augustus 1970 te Zeist geboren. In 1988 behaalde zij het V.W.O. diploma aan het Christelijk Lyceum te Zeist, aansluitend begon zij in september 1988 aan de studie Milieuhygiëne aan de Landbouwuniversiteit te Wageningen.
In deze studie koos zij de afstudeerrichting Waterkwaliteitsbeheer. Twee afstudeervakken werden in Wageningen gedaan bij de (toenmalige) vakgroep Natuurbeheer, een afstudeervak Waterkwaliteitsbeheer en een afstudeervak Aquatische Oecologie . Daarna liep zij 5 maanden stage bij het Plymouth Marine Laboratory, Engeland. Aansluitend volgde een laatste afstudeervak Waterkwaliteitsbeheer, uitgevoerd bij het Institute of Hydrology in Wallingford, Engeland.
Na in augustus 1994 afgestudeerd te zijn, volgde in januari 1995 de aanstelling als Assistent in Opleiding bij de (toenmalige) Vakgroep Waterkwaliteitsbeheer en Aquatische Oecologie, Landbouwuniversiteit. De resultaten van dit onderzoek hebben tot dit proefschrift geleid. In het kader van haar onderzoek bracht zij 1 maand door op het Høyfjellsøkologisk Forskningsstasjon te Finse, Noorwegen, en 3 maanden bij het National Hydrology Research Institute te Saskatoon, Saskatchewan, Canada.
Vanaf april 1999 zal zij werkzaam zijn als post-doc aan de Lehigh University, Bethlehem PA, Verenigde Staten.

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