Ministerie van Verkeer en Waterstaat
Directoraat-Generaal Rijkswaterstaat

Rijksinstituut voor Kust en Zee/*RIKZ*

# The impact of marine eutrophication on phytoplankton, zooplankton and benthic suspension feeders

## Progress report: Effects of stratification on plankton dynamics

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NIOO/CEMO-1998.06
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# The impact of marine eutrophication on phytoplankton, zooplankton and benthic suspension feeders

## Progress report: Effects of stratification on plankton dynamics

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# Samenvatting


## KADER VAN HET ONDERZOEK

Er bestaat veel aandacht voor de effecten van nutriënten op de productiviteit van fytoplankton en hogere trofische niveau's in verband met eutrofiëring en het optreden van plaagalgenbloeien in de Noordzee. Anthropogene eutrofiëring is in het algemeen ongunstig voor diatomeëen en bevorderlijk voor flagellatenbloeien, omdat de toevoer van silicaat gelijk blijft, terwijl de toevoer van N en P verhoogd is. Het terugdringen van de nutriëntenbelasting van de Noordzee is succesvol voor P, maar heeft nog niet geleid tot vermindering van de N-belasting. De hoge N:P ratios die daar het gevolg van zijn, kunnen bevorderlijk zijn voor de toxiciteit van bepaalde soorten dinoflagellaten. Naast de abiotische factoren licht en nutriënten, is ook de turbulentie in de waterkolom een belangrijke sleutelfactor voor het fytoplankton. Lage turbulentie in combinatie met hoge instraling en/of hoge zoetwaterafvoer kan leiden tot stratificatie. Deze condities leiden op de Oestergronden tot langdurige en nabij de Hollandse kust tot kortdurende stratificaties. Stratificatie bevordert flagellatenbloeien, terwijl diatomeëen onder die condities verhoogde sedimentatie vertonen. De combinatie van stratificatie met een hoge N-belasting vormt derhalve een risico voor het ontstaan van (potentiëel toxische) dinoflagellatenbloeien.
Voor een goed begrip van de condities die tot algenbloeien leiden, is het noodzakelijk ook graas als controlerende factor te bestuderen. Graas door zoöplankton kan leiden tot verschuivingen in soortensamenstelling en -productie van het fytoplankton. Graas door macrozoobenthos kan van sterke invloed zijn op het fytoplankton, maar wordt verhinderd wanneer stratificatie optreedt.
In eerder mesocosm onderzoek, door RIKZ in samenwerking met NIOO-CEMO, is de invloed van nutriëntenbelasting op fytoplanktonsamenstelling en -productie onderzocht, waarbij tevens aandacht werd geschonken aan interacties met graas door zoöplankton en macrozoobenthos. Dit eerder verrichtte onderzoek is uitgevoerd in volledig gemengde systemen. In dit rapport worden de resultaten gepresenteerd van mesocosm-onderzoek in gestratificeerde systemen, dat in 1997 is uitgevoerd op het veldstation van RIKZ. Het onderzoek vond plaats in het kader van een aantal verschillende projecten. Vanuit het RIKZ-project PenP ('Productiviteit en Plaagalgen') en het BEON-project EUPRO ('Invloed van eutrofiëring op productiviteit van mariene ecosystemen') was de vraag geformuleerd in hoeverre eutrofiëring, resulterend in hoge N:P ratios, in combinatie met het optreden van stratificatie, kan leiden tot het optreden van plaagalgen. Daarnaast vond onderzoek plaats voor het EU-MAST project PHASE ('Physical forcing and biogeochemical fluxes in shallow coastal ecosystems'). Dit laatste project richt zich met name op de vraag wat de effecten zijn van stratificatie op de koppeling tussen benthische grazers en het plankton.

## DOELSTELLING

Twee experimenten zijn uitgevoerd in 1997.
De doelstelling van het eerste experiment was een kwantificering van de relatie tussen benthische graas en productiviteit en structuur van het plankton, en de invloed van stratificatie op deze relatie.

De doelstelling van het tweede experiment was een kwantificering van de relatie tussen stikstofbelasting en fytoplanktonsamenstelling onder invloed van stratificatie en begrazing door zoöplankton.

## SAMENVATTING DEELRAPPORTAGES

In hoofdstuk 2 wordt een beschrijving gegeven van de gebruikte mesocosms en van de experimentele en analyse methoden.

Hoofdstuk 3 beschrijft de effecten van schelpdiergraas, onder verschillende hydrodynamische omstandigheden, op de planktonbiomassa en -structuur.
Tijdens de eerste periode van dit experiment was de stratificatie in een aantal mesocosms zwak; na opnieuw aanbrengen van de stratificatie bleef deze in drie van de vier mesocosms stabiel. Gedurende de tweede stratificatie periode daalde de hoeveelheid silicaat tot beperkende concentraties in alle mesocosms. De fytoplankton biomassa werd gedomineerd door flagellaten, hoofdzakelijk *Cryptophyceae* en *Phaeocystis* sp., met een tendens tot een hoger aantal flagellaten in de gestratificeerde mesocosms.
Binnen het microzoöplankton werd de biomassa vooral bepaald door aloricate (naakte) ciliaten en de heterotrofe dinoflagellaat *Oxyrrhis marina*. Er was geen effect van stratificatie op de verticale verdeling van de biomassa. Evenmin waren er verschillen in biomassa tussen de experimentele behandelingen. De biomassa van het grotere mesozoöplankton werd aanvankelijk gedomineerd door zeepoklarven. Na het verdwijnen van deze larven namen de calanoide copepoden in belangrijkheid toe, al bleef de biomassa laag. Ook hier was geen verschil in biomassa tussen de experimentele behandelingen waarneembaar.
Na het instellen van de stratificatie nam de filtratiesnelheid van de mossels af, waarschijnlijk als gevolg van lage zuurstofgehaltes in de onderlaag. In de behandeling met de hoogste mosselbiomassa was de mosselfiltratie het hoogst. Echter, als gevolg van de hogere groeisnelheden en hogere individuele pompsnelheden van de mossels in de mesocosms met een lage mosselbiomassa, was het verschil in filtratiesnelheid tussen hoge en lage mossel biomassa veel kleiner dan het initiële verschil in biomassa.
De verschillen in biomassa en productie van het fytoplankton tussen de gestratificeerde en gemengde mesocosms, en tussen de behandeling met lage en hoge mossel biomassa's waren klein en statistisch niet significant. De turnover snelheden van het fytoplankton waren zeer hoog bij een laagblijvende biomassa, hetgeen erop duidde dat er een belangrijke verliesterm was waardoor de biomassa laag bleef. De geschatte graas door het microzoöplankton bleek veel hoger te zijn dan de mosselgraas, en het is daarom aannemelijk dat de ontwikkeling van het fytoplankton in hoge mate door het microzoöplankton gecontroleerd werd.

In hoofdstuk 4 worden de effecten beschreven van verschillende niveau's van stikstofbelasting op de ontwikkeling en samenstelling van het plankton onder gemengde en gestratificeerde omstandigheden. Gedurende dit tweede experiment werd de stratificatie 30 dagen gehandhaafd, waarna de mesocosms tot het einde van het experiment weer volledig gemengd werden.
Tijdens de stratificatie waren de stikstof- en fosfaatconcentraties in de bovenlaag lager dan in de onderlaag van de mesocosms. De verschillen waren meer uitgesproken in de mesocosms met de laagste stikstofbelasting. De silicaatconcentraties in zowel boven- als onderlaag

van de gestratificeerde mesocosms stegen tijdens de stratificatie; gedurende deze periode namen de silicaat concentraties in de gemengde mesocosms ook toe, maar in veel mindere mate.
De fytoplanktonbiomassa bestond vooral uit flagellaten, met uitzondering van een grote bloei van de diatomee *Leptocylindrus danicus,* in één van de gemengde mesocosms in de tweede helft van het experiment. *Phaeocystis* sp. bereikte de hoogste biomassa in de gemengde mesocosms. In de gestratificeerde mesocosms was er geen eenduidig effect van de stikstofbelasting op de fytoplankton-soortensamenstelling waarneembaar. De als "modelsoort" geënte dinoflagellaat *Prorocentrum micans* domineerde de fytoplanktonbiomassa in één van de gestratificeerde mesocosms met hoge stikstofbelasting, terwijl *Phaeocystis* sp. in de duplo mesocosm domineerde. Dit verschil werd verklaard door lagere microzoöplankton-graas in de laatste mesocosm. In een monster uit één van de gestratificeerde mesocosms met lage stikstof-belasting was de flagellaat *Chattonella* sp. aanwezig met een twee keer zo hoge concentratie als ooit in de Nederlandse zoute wateren waargenomen.
Microzoöplankton bereikte de hoogste biomassa in de gestratificeerde, hoogbelaste mesocosms, en de laagste in de gestratificeerde, laagbelaste mesocosm. Het grootste aandeel in het microzoöplankton had de heterotrofe dinoflagellaat *Oxyrrhis marina*, naast aloricate (naakte) ciliaten. De gemiddelde mesozoöplankton biomassa was laag, en verschilde niet tussen de verschillende behandelingen.

In de appendices zijn de resultaten van specifieke metingen aan microzoöplanktongraas en fotosynthese van het fytoplankton gerapporteerd.

## CONCLUSIES

Uit de resultaten van deze experimenten, gecombineerd met de resultaten van eerdere mesocosm experimenten, komen de volgende conclusies.
De schuimalg *Phaeocystis* sp. groeit goed onder omstandigheden waarbij licht en nutriënten in overvloed aanwezig zijn.
Microzoöplankton graas lijkt het ontstaan van *Phaeocystis*-bloeien te kunnen verhinderen.
Stratificatie is gunstig voor bepaalde soorten flagellaten (de dinoflagellaat *Prorocentrum micans* in dit experiment), maar andere flagellatensoorten (*Phaeocystis* sp. in dit experiment) groeien goed in een volledig gemengde waterkolom. De resultaten geven geen uitsluitsel over het effect van hoge N-belasting, omdat ook bij lage N-belasting hoge concentraties flagellaten (*Chattonella* sp.) zijn opgetreden.
De resultaten van het experiment met mosselgraas zijn minder eenduidig door de dominantie van microzoöplankton. Stratificatie heeft effect op de graascontrole door het macrobenthos op fytoplankton, enerzijds doordat een deel van de waterkolom niet meer 'bereikbaar' is voor het benthos, anderzijds doordat verlaagde zuurstofconcentraties een negatief effect hebben op de activiteit van het benthos. Dit laatste effect kan ook na opheffing van de stratificatie nog doorwerken.

# Summary


## BACKGROUND

The effects of nutrient loading of the North Sea on the production of phytoplankton and higher trophic levels and on the risk of harmful algal blooms are still a matter of concern. Anthropogenic eutrophication generally favours flagellates over diatoms, as a consequence of the increased N- and P-loadings relative to Si. The level of P-loading to the North Sea has decreased, due to the implementation of sanitation programmes, while N-loading has remained at a high level. This has resulted in high N:P ratios, which may promote the toxicity of certain dinoflagellate species.
In addition to light and nutrients, turbulence is an important abiotic factor affecting the phytoplankton. Low turbulence, combined with high freshwater runoff, can lead to stratification. These conditions result in short periods of stratification in the coastal waters off the Dutch coast, in the river Rhine plume. Stratification promotes the dominance of motile algal species (flagellates), as diatoms suffer from nutrient limitation and increased sedimentation rates under these circumstances. Consequently, the combination of stratification with high N-loads imposes a risk of potentially toxic dinoflagellate blooms.
Grazing is an important factor in controlling algal blooms, and should be taken into consideration in the study of the conditions that lead to algal blooms. Grazing by zooplankton can result in changes in phytoplankton species composition and biomass. Grazing by mesozooplankton is important in shallow waters, but stratification prevents effective grazing control by uncoupling part of the water column from the benthic system.
Cooperative research by RIKZ and NIOO-CEMO in earlier years focused on the effects of nutrient loading on phytoplankton production and composition. In this research, interactions with grazing by zooplankton and macrozoobenthos were included. The experiments were done in experimental ecosystems (mesocosms) mimicking a well-mixed coastal North Sea water column.
In this report, the results are presented of two mesocosm experiments in stratified systems, that were conducted in 1997 at the field station of RIKZ.

## OBJECTIVES

Two experiments were carried out in 1997,
The objectives of the first experiments was to quantify the relation between bivalve grazing on plankton biomass and structure, under mixed versus stratified conditions
The second experiment aimed at a quantification of the relation between nitrogen loading and plankton development and composition under mixed versus stratified conditions.

## SUMMARY EXPERIMENTAL RESULTS

In chapter 2 a description is given of the mesocosms and of the experimental and analytical methods.

In chapter 3 the effects of bivalve grazing on plankton biomass and structure under different hydrodynamic conditions are presented.

During the first period of this experiment, stratification in some of the mesocosms was weak, but after stratification was induced again a stable stratification was realised in three of the four mesocosms. Silicate concentrations dropped to limiting levels in all mesocosms during the second half of the experiment. Total phytoplankton biomass was dominated by flagellates, mainly *Cryptophyceae* and *Phaeocystis* sp., with a tendency for a higher abundance of flagellates in the stratified mesocosms.
Microzooplankton biomass was dominated by aloricate ciliates and the heterotrophic dinoflagellate *Oxyrrhis marina*. There were no significant differences in vertical distribution of microzooplankton biomass. Biomass showed no significant differences between treatments either.
Initially mesozooplankton biomass was dominated by cirriped larvae. After their disappearance, mesozooplankton biomass was dominated by calanoid copepods, but values were low. No vertical distribution of biomass was observed. Differences in average biomass between treatments were not observed.
After the induction of stratification mussel filtration rates declined, probably due to reduced oxygen concentrations in the Bottom compartments. Overall, mussel filtration was highest in the treatments with high mussel biomass. However, due to higher mussel growth rates and higher individual clearance rates in mesocosms STRAT-L, the difference in filtration rates between low and high mussel biomass was much smaller than the initial difference in biomass.
Overall, differences in phytoplankton biomass and production between stratified and mixed mesocosms, and between the low and high mussel biomass treatments were low and not statistically meaningful. Phytoplankton turnover rates were very high and biomass values remained low, indicating the presence of a major loss term leading to the observed low biomass values. Estimates of microzooplankton grazing rates appeared to be much higher than mussel grazing rates and it is likely that microzooplankton controlled phytoplankton biomass development to a large extent.

In chapter 4 the effects of different nitrogen loads on plankton development and composition under mixed versus stratified conditions are described.
During this second experiment stratification was successfully maintained for 30 days, after which period the mixing configuration was restored again until the end of the experiment.
Under stratified conditions DIN and DIP concentrations in the Top compartments were lower than in the Bottom compartments. These concentration differences were more pronounced in the mesocosms with the lowest nitrogen loadings. Silicate concentrations in Top and Bottom compartments increased after the induction of stratification; during this stratified period silicate concentrations in the mixed mesocosms also increased, but to a much lower extent than in the stratified mesocosms.
Phytoplankton biomass was dominated by flagellates, with the exception of a large chlorophyll-a peak resulting from a bloom of the diatom *Leptocylindrus danicus* in one of the mixed mesocosms, developing during the second half of the experiment. *Phaeocystis* sp. reached highest biomass levels in the mixed mesocosms. In the stratified mesocosms, the effects of differences in nitrogen loading on phytoplankton species composition were not unequivocal. The added 'model species', the dinoflagellate *Prorocentrum micans*, dominated phytoplankton biomass during stratification in one of the stratified mesocosms with high nitrogen loading, but in the replicate mesocosm

*Phaeocystis* sp. dominated. These differences were explained by lower microzooplankton grazing in the latter mesocosm. In one sample taken in one of the stratified mesocosms with low nitrogen loading, the concentration of the raphidophycean flagellate *Chattonella* sp. was more than twice as high as the largest concentration ever recorded in Dutch marine waters.
Microzooplankton biomass differed significantly between treatments, with highest biomass in the stratified mesocosms with high nitrogen loading, and lowest biomass in the stratified mesocosms with low nitrogen loading. Aloricate ciliates and especially the heterotrophic dinoflagellate *Oxyrrhis marina* dominated microzooplankton biomass. Mesozooplankton biomass did not reach high values and showed no significant treatment effects.

The appendices present the results of microzooplankton grazing experiments, and of measurements of diurnal changes in photosynthetic rates of the phytoplankton.

## CONCLUSIONS

The following conclusions were drawn from the experiments presented in this report, and from earlier results.
Blooms of *Phaeocystis* sp. occur under conditions with high irradiance and absence of nutrient limitation. Grazing by microzooplankton may prevent the formation of *Phaeocystis* sp. blooms.
Stratification promotes the blooming of some flagellate species (*Prorocentrum micans* in this study), but other flagellate species thrive under well-mixed conditions (*Phaeocystis* sp. in this study). The effects of N-loading on the abundance of flagellates is less clear, as high concentrations of flagellates (*Chattonella* sp.) were observed during stratification with low N-loading.
The results of the mussel grazing experiments were not conclusive, The high abundance of microzooplankton overshadowed the effects of mussel grazing. Stratification may decrease benthic grazing control of the phytoplankton, by uncoupling the water column and the benthos, and because low oxygen tension reduces the activity of the benthos. The latter effect may last even after the stratification has ended.

# 1 Introduction

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In this report, the results are presented of two mesocosm experiments, carried out in 1997 at the field station of RIKZ. The experiments were conducted to study the effects of benthic grazing and of nitrogen loading, in combination with different degrees of physical dynamics in the water column, on plankton development.

## 1.1 GENERAL BACKGROUND

In order to prevent harmful effects of marine eutrophication, it was decided during the Second (1987) and Third (1990) International Ministers Conference on the Protection of the North Sea that by 1995 the anthropogenic input of nutrients to the North Sea should be reduced to half of the inputs in 1985. Much effort has been put into various measures to achieve the required nutrient loading reduction. This has resulted in a 50% decline in phosphorus loadings by riverine transport to the Dutch coastal zone, and an immediate and proportional response of dissolved inorganic phosphorus concentrations in the water column (De Vries et al., 1998). For nitrogen, sanitation measures have been less effective and no significant changes in inorganic nitrogen concentrations in the Dutch coastal zone have been observed yet (De Vries et al., 1998). These changes in nutrient concentrations in the coastal zone resulted in increased DIN:DIP ratios. High DIN:DIP ratios are a potential risk, as there seems to be a positive effect of high DIN concentrations on growth rates and toxin production of dinoflagellates (Anderson et al., 1990; Reguera & Oshima, 1990; Peperzak, 1994).
In the Dutch coastal zone, haline stratification is sometimes observed in the plume of the river Rhine (Klein & Van Buuren, 1992; Peperzak et al., 1996). Stratification in general favours motile phytoplankton species (flagellates), whereas non-motile phytoplankton species (diatoms) suffer from increased sedimentation rates. The combination of periodic stratification and high DIN:DIP ratios, therefore, may increase the chances of toxic dinoflagellate blooms in the coastal zone of the North Sea.
The development of phytoplankton is regulated by both bottom-up factors (light, nutrients) and by top-down factors (grazing). In estuarine and shallow coastal areas, grazing by benthic suspension-feeders can be an important factor in structuring the planktonic community. The strength of this benthic-pelagic coupling depends on the degree of vertical mixing. Periods of stratification will lead to an uncoupling between the benthos and the pelagic system, and the reduced grazing mortality of the phytoplankton may therefore result in massive algal blooms. Grazing by zooplankton is another important factor, affecting biomass and composition of the phytoplankton. Microzooplankton grazing on small algae can induce a shift towards a dominance of larger algae, like diatoms or dinoflagellates (Riegman et al., 1993). When evaluating the response of phytoplankton biomass and species composition to nutrient loading and physical processes, the effects of grazing have to be taken into account.

## 1.2 OBJECTIVES

Two mesocosm studies were carried out in 1997. The mesocosms are designed to mimic physical conditions in the Dutch coastal zone, and have been used in extensive studies on the effects of nutrient loading on phytoplankton development and productivity in well-mixed systems (Escaravage et al., 1995, 1996; Smaal et al., 1997; Prins et al., 1998). The mesocosms have also been used in experiments to study the interactions between bivalve grazing and phytoplankton development Prins et al., 1995a, b). A pilot experiment with stratified mesocosms was carried out in 1996 (Escaravage et al., 1997).
The first experiment was designed to quantify the effects of bivalve grazing on plankton biomass and structure, under mixed versus stratified conditions. During this experiment, a more detailed study of zooplankton grazing on the phytoplankton was carried out as well.
The second experiment was designed to test the hypothesis that high levels of DIN loading, in combination with stratification, increase the chance of (potentially toxic) dinoflagellate blooms. In this experiment, phytoplankton development and composition under mixed conditions was compared to growth and species composition under stratified conditions.
As a follow-up to the two experiments presented in this report, a long-term experiment was carried out in 1998, running from April through August 1998. In the latter experiment, the development of phytoplankton in systems with a high bivalve suspension feeder biomass is compared to systems without bivalves and hence a predominantly pelagic community. In the systems without bivalves, high- versus low-nitrogen loading treatments are contrasted. In two periods during the experiment the mesocosms had a haline stratification. Results of this experiment will be reported in 1999.

## 1.3 OUTLINE OF THE REPORT

Chapter 2 gives a general description of the mesocosms and of the methods used. Chapter 3 describes the results of the first experiment, where plankton development was studied as a function of bivalve grazing under different physical conditions. A description is given of the changes in phytoplankton biomass, production and composition, and the responses in micro- and mesozooplankton. In chapter 4 the results of the second experiment are presented. In this experiment the effects of differences in DIN loading on plankton development were studied under different physical conditions. Appendix I describes the results of zooplankton grazing experiments, carried out during the first experiment. Appendix II presents the results of measurements of diurnal changes in phytoplankton photosynthetic activity.

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# 2 Description of mesocosms and methods

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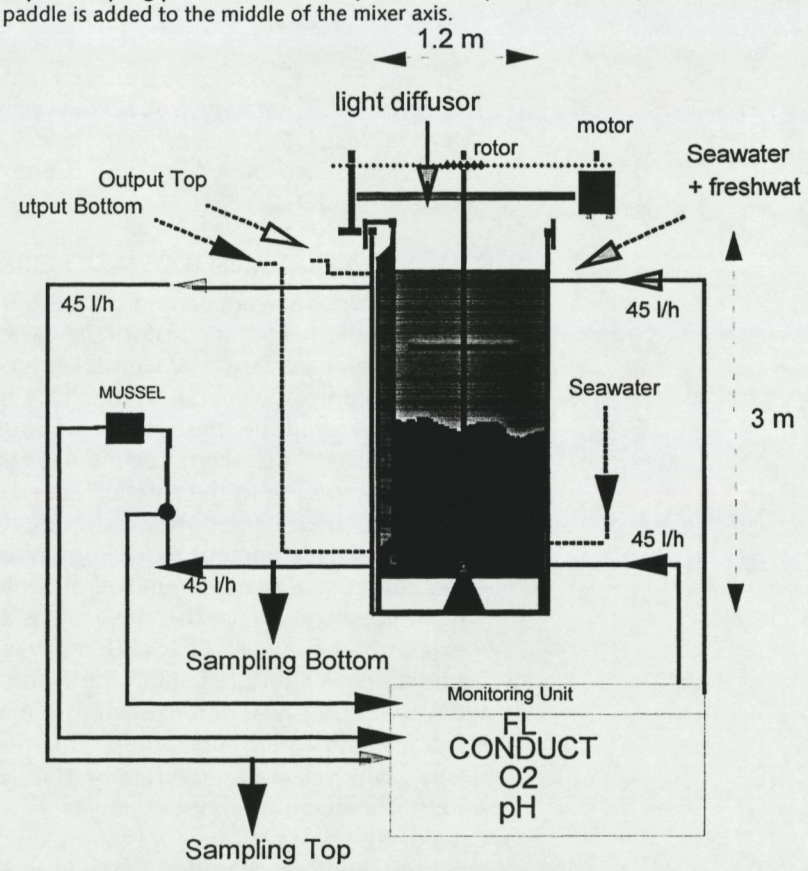
## 2.1 GENERAL MESOCOSM DESCRIPTION

Two experiments were carried out with 6 land-based mesocosms, located at the field-station of RIKZ near the mouth of the Oosterschelde estuary (SW Netherlands). The mesocosms consist of black solid polyethylene tanks (height 3 m, diameter 1.2 m, volume 3000 l). Water in the mesocosm was continuously mixed with a rotating mixer. The mixing regime used in the mesocosms could be adjusted according to the rotation speed and configuration of a central mixer. A scraper, coated with a strip of abrasive material, was used to prevent the development of fouling organisms on the walls of the tanks. Additional manual removal of fouling organisms was carried out when necessary. Above the mesocosms an optical diffuser of structured Plexiglas (Groenendijk, Pl 20070 TK) was installed to ensure a homogeneous light gradient in the water column. Inorganic nutrients were continuously added to each of the mesocosms from stock solutions with a peristaltic pump. The mesocosms were continuously flushed with sea water at a rate of 100 $l \cdot day^{-1}$, resulting in a residence time of the water of about 30 days.
Each of the 'pelagic' tanks was connected to a 16 l Perspex benthos chamber. Benthos chambers were designed especially to contain a benthic compartment with macrofauna, and to enable measurements of the exchange of particulate and dissolved matter between the pelagic system and the benthos. The chambers were shielded from light. A 5 cm sand layer was added to each chamber. The benthos chambers were flushed with mesocosm water at a rate of 45 $l.h^{-1}$ with a 701 VB/R Watson & Marlow tubing pump. With an automated system in- and outflow of the benthos chambers were alternately pumped through a bypass containing a Turner fluorometer and a Stork-Servex Datasonde 3 with multi parameter water quality data logger for the registration of fluorescence, oxygen, temperature, conductivity and pH. Data were stored on a personal computer.
Heating of the mesocosms by solar radiation was diminished by spraying sea water on the outer wall of the tanks and by shelling the tanks with foil.
A sediment container of 150 l was placed on the bottom of the tanks and filled with artificial sediment (azoic sand with a median grain size of 210 µm). Figure 1 gives a schematic view of a mesocosm unit. An extensive description of the mesocosms is given in Prins *et al.* (1995).

**Figure 2.1**
Schematic view of a stratified mesocosm unit with benthos chamber and the continuous measurement unit providing monitoring for fluorescence, conductivity, oxygen and pH. Sampling position (for lab analysis) of the top and bottom layers are indicated. In not stratified mesocosms, a third mixing paddle is added to the middle of the mixer axis.




## 2.2 DESCRIPTION OF THE EXPERIMENTS

### 2.2.1 Experimental design

The design of the mesocosm as used during the years 1992 to 1995 was slightly modified to allow the establishment and maintenance of a salinity stratification. New connections were made to allow an independent flushing of the top and the bottom layer of the water column (Figure 2.1). In order to allow the maintenance of the salt stratification, the top layer was flushed with sea water diluted with fresh water to a final salinity of about 27 ‰ whereas the bottom layer was flushed with undiluted sea water (*ca* 32‰). During the second part of the first experiment and during the second experiment, extra additions of salt (artificial seawater salt) were made to the bottom layer to strengthen stratification. In both cases the flushing rate induced a 30 days turn over of the compartments. Connections to the peristaltic pump and the monitoring unit were adapted to allow independent measurements for each compartment. Water from the bottom layer of each mesocosm was circulated at the rate of 45 $l.h^{-1}$ through a benthos chamber containing 40 mussels (17 to 20 mm long).
Stratification was established by injecting 150 l of demi water at 1,50 m depth by using a PVC T-tube immersed at desired depth in the mesocosm. The rotation speed of the mixer was then set from 10 rpm (default) to 3 rpm to allow the maintenance of the pycnocline
Vertical profiles of salinity were recorded each day with a portable conductivity meter (WTW™).

**Table 2.1**
Treatments used during the mesocosm experiment. The table gives the abbreviations used in this document for the different treatments and corresponding mesocosm number (#). The stratification period lasted about four weeks in both experiments. In the first experiment 60 and 12 mussels were introduced in the benthos chambers of mesocosms with a respectively High and Low mussel biomass. In the second experiment, 15 mussels were placed in each mesocosm, two nitrogen loadings (High and Low) were used in duplicate (1 and 2).

| | | EXPERIMENT 1 | | | EXPERIMENT 2 | | |
|---|---|---|---|---|---|---|---|
| **Mixing regime** | | periodically stratified | periodically stratified | not stratified | periodically stratified | periodically stratified | not stratified |
| **Mussel biomass** | | High | Low | High | Low | Low | Low |
| **Average loading actual (nominal) µmol.l$^{-1}$.d$^{-1}$** | DIN= | 4.62 *(4.40)* | 4.62 *(4.40)* | 4.62 *(4.40)* | 2.36 *(2.45)* | 0.96 *(0.80)* | 2.47 *(2.45)* |
| | DIP= | 0.11 *(0.10)* | 0.11 *(0.10)* | 0.11 *(0.10)* | 0.08 *(0.07)* | 0.09 *(0.07)* | 0.08 *(0.07)* |
| | Si= | 0.97 *(0.93)* | 0.97 *(0.93)* | 0.97 *(0.93)* | 0.42 *(0.51)* | 0.42 *(0.51)* | 0.42 *(0.51)* |
| **Mesocosm name (#)** | | STRAT-H1 (1) STRAT-H2 (6) | STRAT-L1 (2) STRAT-L2 (4) | NOSTRAT-H1 (3) NOSTRAT-H2 (5) | STRAT-H1 (1) STRAT-H2 (4) | STRAT-L1 (2) STRAT-L2 (5) | NOSTRAT-H1 (3) NOSTRAT-H2 (6) |

Two experiments were run from 15/04 to 17/06 (Experiment 1) and from 08/07 to 09/09 (Experiment 2). The first experiment combined two mussel grazing regimes with two mixing regime whereas one single nutrient loading regime was used for all mesocosms. The second experiment combined two nutrient loading regime with two mixing regime whereas one single mussel grazing regime was used for all mesocosms. Mesocosms were either mixed (three paddles mixer set to 10 rpm) or stratified (halocline+two paddles mixer set to 3 rpm). In both experiments two mesocosms remained continuously mixed (NOSTRAT) whereas a stratification period of about 4 weeks was induced in the four other mesocosms (STRAT). The three treatments used in each experiment were applied in duplicate (Table 2.1).

### 2.2.2 Nutrient loadings

The used nutrient loadings were representative for actual loadings to the Dutch coastal zone. The high nitrogen regime (also used for the mussel experiment) corresponded to the present decade with nitrogen and phosphorus loadings equalling respectively 90% and 50% of the loadings of the 1980's. The low nitrogen regime corresponded to a scenario in which nitrogen loadings are 25% of the loadings in the 1980's. These loadings were estimated from the DYNAMO simulation model (M. van der Tol, pers. comm.) including the *in situ* seasonal variation. Planned and actual nutrient loadings in both experiments are shown in Figures 2.2 to 2.7.
Phosphate was added as $NaH_2PO_4.H_2O$, nitrate as $NaNO_3$, and silicate as $Na_2SiO_3.5H_2O$. In order to prevent any micronutrient limitation for phytoplankton growth, trace metals (Fe, Mn, Zn, Co and Mo in molar proportions: 100:10:1:1:1) and vitamins (thiamin, vitamin B12 and biotin in molar proportions 1:1:1) were added together with the inorganic nutrients (± 0.1 mmol Fe and 10 nmol vitamins per mmol N). Treatments were randomly assigned to the mesocosms. The differences measured between the actual and the planned loadings were mainly due to varying concentrations in the sea water continuously flushing the mesocosms. The nutrient loadings were not uniform over the span of the experiments but were aimed to reproduce natural seasonal patterns. In Experiment 1 all nutrient loadings showed a sharp decrease in the first month of experiment corresponding to the *in situ* decline of the freshwater input to the North Sea during this period. In accordance with field patterns, the nutrient loadings used during the summer period (Experiment 2) were lower (-50% for N and Si) and less variable than during the vernal period (Experiment 1).

**Figure 2.2**
Planned (broken lines) and actual loadings (full lines) of dissolved inorganic and total N during Experiment 1.

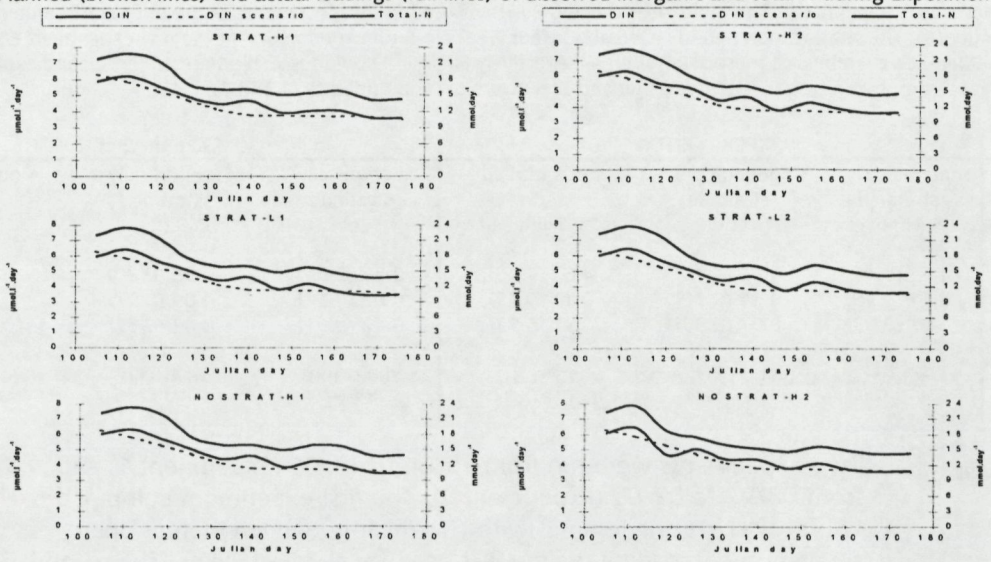



**Figure 2.3**
Planned (broken lines) and actual loadings (full lines) of dissolved inorganic and total P during Experiment 1.

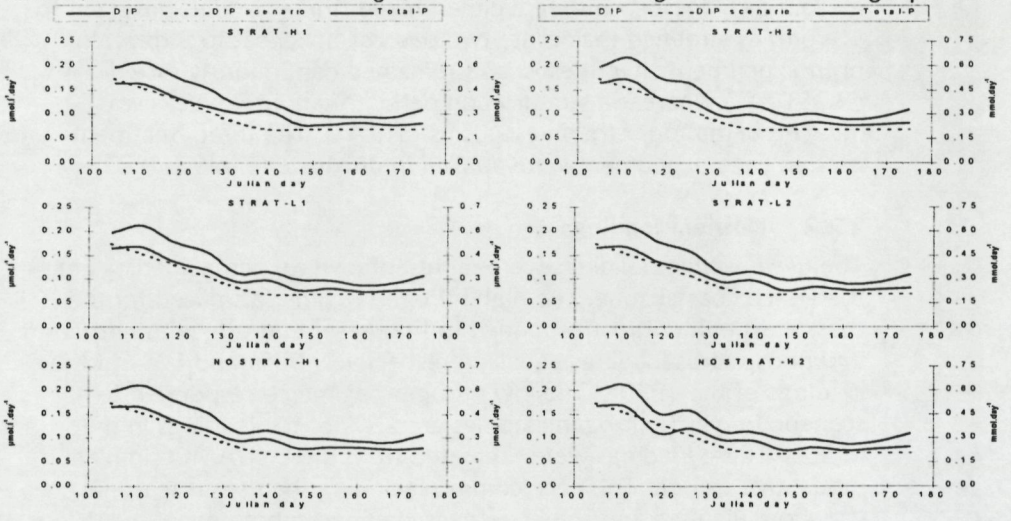



**Figure 2.4**
Planned (broken lines) and actual loadings (full lines) of dissolved inorganic Silicate during Experiment 1.

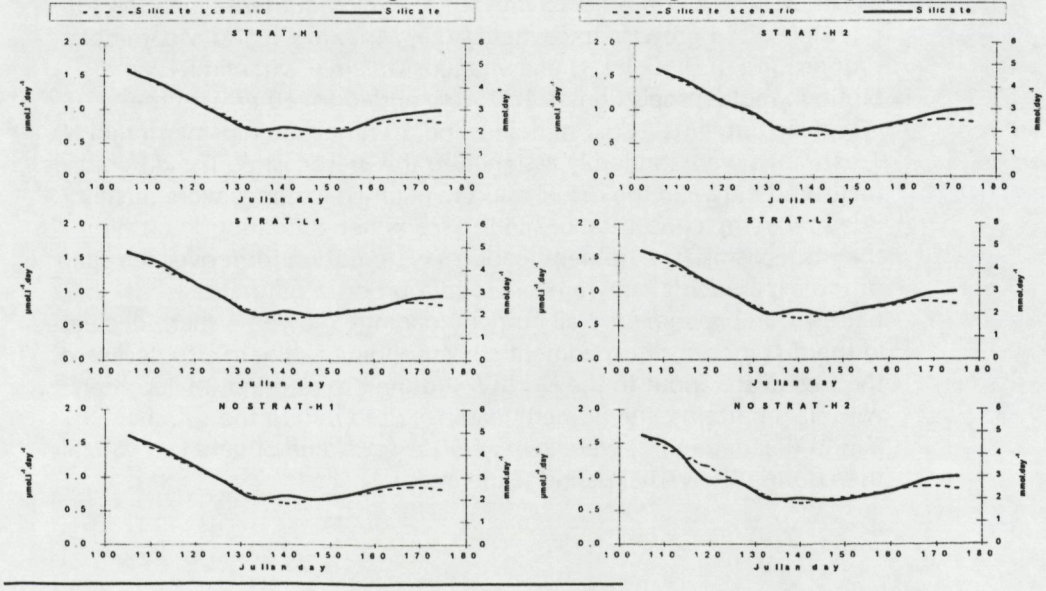


**Figure 2.5**
Planned (broken lines) and actual loadings (full lines) of dissolved inorganic and total N during Experiment 2.

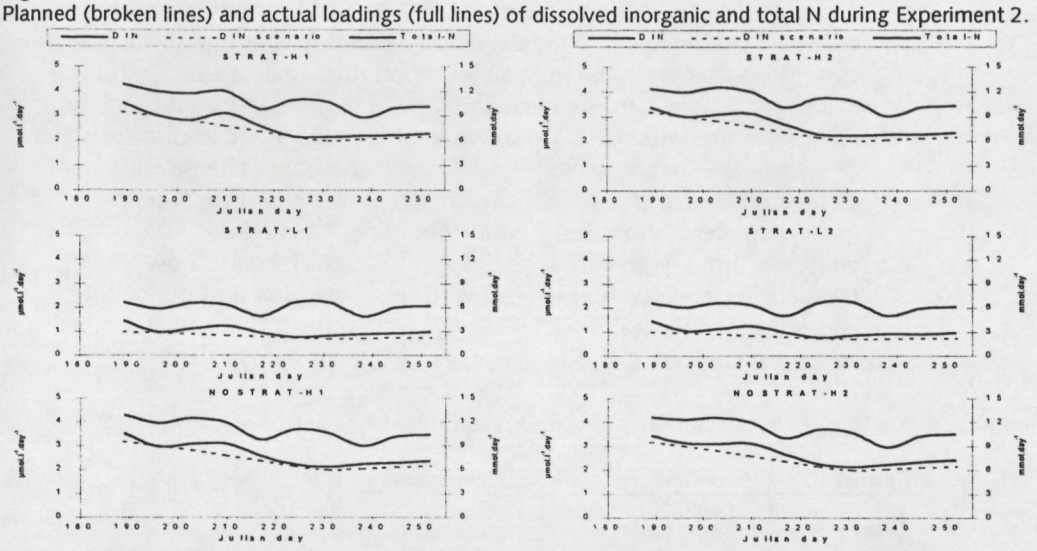



**Figure 2.6**
Planned (broken lines) and actual loadings (full lines) of dissolved inorganic and total P during Experiment 2.

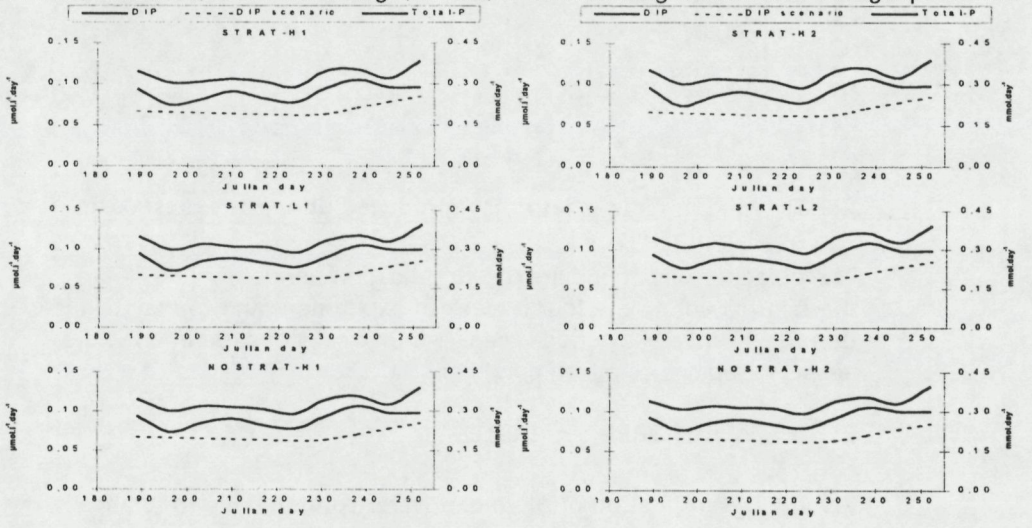
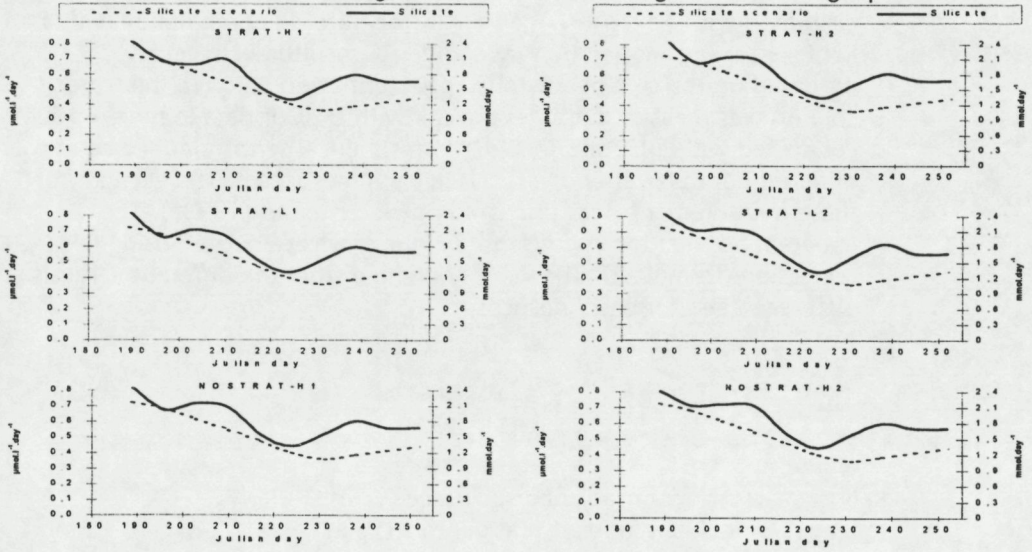



**Figure 2.7**
Planned (broken lines) and actual loadings (full lines) of dissolved inorganic silicate during Experiment 2.



### 2.2.3 Inoculation of Prorocentrum micans (dinoflagellate model)

A dinoflagellate (*Prorocentrum micans*) was added to the mesocosm water and served as a "model species" aimed to identify environmental conditions that could be profitable to potential toxic algae. Culture of *Prorocentrum micans* were run in 8 l polyethylene bags with medium PEP-Si. Temperature in culture was kept close to value in outside water by allowing seawater to run on surface of the bags. The development of the culture was followed by microscope. When *Prorocentrum micans* concentrations reached a stationary phase (about 10 days after dilution), three quarter (6 l) of the culture was distributed over the mesocosms, the rest was diluted with fresh medium and the culture was left to grow again. Table 2.2 gives the date and the final concentration of the inocula in cells per litre mesocosm.

**Table 2.2**
*Prorocentrum micans* inoculation (in cells added per l mesocosm water) to all mesocosms during both experiments.

| Experiment | Julian day | Inocula (cells/l mesocosm) |
|---|---|---|
| 1 | 109 | 963 |
| 1 | 125 | 1001 |
| 1 | 134 | 1482 |
| 1 | 140 | 1295 |
| 1 | 147 | 1130 |
| 1 | 157 | 966 |
| 1 | 164 | 1077 |
| 2 | 206 | 1611 |
| 2 | 210 | 1787 |
| 2 | 217 | 966 |
| 2 | 238 | 1464 |

### 2.2.4 Mixing regime patterns reproduced during the mesocosm experiments

The objective was to initiate the stratification period in the STRAT mesocosms during the fourth week of experiment and to maintain it during a four weeks period. The last four weeks of experiment were aimed to reproduce mixed conditions.

## 2.3 MONITORED VARIABLES

### 2.3.1 Determination of the mean water column daily irradiance

Daily irradiance was recorded directly under the optical diffuser with a LiCor Quantum SR sensor connected with the data acquisition system described by Peeters *et al.* (1993a). The LiCor Quantum SR sensor was calibrated with a Kipp & Zonen Solar Integrator in combination with a light sensor (developed by Wageningen Agricultural University) mounted on top of a nearby building. Light attenuation in the water column was measured 3 times a week with a LiCor data logger LI-1000 connected with a LiCor SPA-Quantum spherical sensor, immersed at different depths over two verticals in each mesocosm. The accuracy of these measurements was discussed in Peeters *et al.* (1993a). The apparent attenuation coefficient ($K_d$ in $m^{-1}$) was calculated using linear regression (formula 1). The values obtained from the different verticals were averaged for each mesocosm.

$$\mathrm{Ln}\left(\frac{I_z}{I_0}\right) = K_d \,.\, z \quad (1)$$

where:
$I_0$ = incident irradiance at surface (W $m^{-2}$)
$I_z$ = incident irradiance at depth z (W $m^{-2}$)

The daily irradiance averaged over the entire water column follows from:

$$\overline{I} = \frac{\overline{I_0} \cdot (1 - e^{K_d \cdot z})}{K_d \cdot z} \quad (2)$$

where:

$\overline{I}$ = Mean water column daily irradiance (mol $m^{-2}$ $d^{-1}$)
$\overline{I_0}$ = daily irradiance at surface (PAR in mol $m^{-2}$ $d^{-1}$)
$z$ =depth of the water column (m)
$K_d$ = irradiance attenuation coefficient ($m^{-1}$)

### 2.3.2 Particulate and dissolved nutrient concentrations

Suspended particulate matter (SPM) was determined after filtration of 1 litre through a pre-weighed Whatman GF/C filter, rinsing with distilled water and drying for 48 hours at 50 °C. Particulate organic carbon (POC) was determined after filtration of 1 litre through a Whatman GF/C filter. The filter was treated with HCl gas to remove inorganic carbon, put into a tin cup and burned at 1380 °C in a Carlo-Erba Elementary Analyser. $CO_2$ formed was detected by a Katarometer. Particulate nitrogen (PN) was determined by filtration of 0.5 litre through a Whatman GF/C filter, followed by an alkaline persulphate destruction and detection of the nitrogen as nitrate with an Autoanalyzer. Particulate phosphorus (PP) was determined by filtration of 0.5 litre through a Whatman GF/C filter, an acid persulphate destruction and determination of the phosphorus as inorganic phosphate with an Autoanalyzer (Grasshoff *et al.*, 1983).
Dissolved substances were analysed in the filtrate after filtration of 0.25 litre through a Whatman GF/C filter. DOC was determined by a colorimetric method (Schreurs, 1978). Dissolved inorganic nutrients (DIN: $NH_4^+$, $NO_3^-$, $NO_2^-$), $PO_4^{3-}$ and $H_4SiO_4$ were determined with an Autoanalyzer. Total dissolved nitrogen (TDN) was determined as nitrate after an alkaline persulphate destruction. Total dissolved phosphorus (TDP) was determined as inorganic phosphate after an acid persulphate destruction (Grasshoff *et al.*, 1983). Dissolved organic nitrogen (DON) was calculated from the difference between DIN and TDN, dissolved organic phosphorus (DOP) from the difference between TDP and $PO_4^{3-}$.

### 2.3.3 Phytoplankton abundance, biomass and production

Chlorophyll-*a* and phaeophytin-*a* were extracted on GF/C filters according to Gieskes & Kraay (1984) and analysed with a HPLC method using a 85-100% acetone/water-water gradient and a reversed phase RP18 Novopack column (Waters) in a Spectra Physics Chromatography station. Chlorophyll-*a* was detected with a Perkin Elmer LS-2B fluorometer (excitation: 410-430 nm; emission: >530 nm). A standard chlorophyll-*a* solution was used for calibration. The observed chlorophyll-*a* values were used to convert the continuously measured fluorescence data to chlorophyll-*a*.
Phytoplankton samples were fixed with acid Lugol's iodine solution. Phytoplankton cell numbers and species composition were determined by the Utermöhl technique (Utermöhl, 1958). Hydrobios 10 ml sedimentation counting chambers were filled with 5 to 10 ml mesocosm water or 1 to 5 ml of concentrated (factor x10) samples. Samples were examined at 200x and 500x magnification with an inverted microscope. Carbon biomass was estimated from appropriate

biovolume geometric approximations and standard allometric equations as in Smayda (1976).
P/I curve parameters were determined by $^{14}C$-incubations twice a week. Water samples were incubated for 2 hours with 185 kBq $^{14}C$-bicarbonate (Amersham) at irradiances of 0, 2.3, 5.3, 11.7, 29.6, 61.1, 144.3 and 332.3 W $m^{-2}$ in a thermostated incubator. Samples were processed according to Peeters *et al.* (1991a). Irradiance (I, in W $m^{-2}$) and production (P), in mgC $mg^{-1}$Chl $h^{-1}$) were fitted to the model (Eilers & Peeters, 1988):

$$P(I) = \frac{I}{aI^2 + bI + c} \qquad (3)$$

Maximum production ($P_{max}$), irradiance half saturation constant ($I_k$) and daily integrated production were estimated by non-linear regression. Daily primary production was estimated by combining daily values of irradiance and chlorophyll-*a* concentration with P/I curve characteristics. As $^{14}C$-incubations were carried out once a week only, intermediate values for the photosynthetic parameters were estimated by trapezoidal interpolation.

### 2.3.4 Characterisation of the light limitation

Irradiance and nutrients are the main factors governing primary production. The mean irradiance available for the primary production was expressed as $I_{av}$, the daily average irradiance in the water column (in mol photons $m^{-2}$ $d^{-1}$). Peeters *et al.* (1991b) compared the photosynthetic characteristic irradiance $I_k$ with the mean daily irradiance $I_{av}$ to scale the light limitation of natural phytoplankton populations over extended geographical areas in the North Sea. The photosynthetic characteristic irradiance $I_k$ is derived from the P/I curve (equation 3) as $I_k = P_{max} / \alpha$, where $P_{max}$ is the peak and $\alpha$ the initial slope of the P/I curve. At low light intensity, equation 3 can be simplified to $P(I) = \alpha I$. Combined with $P_{max} = \alpha * I_k$, this gives: $P/P_{max} = I_{av}/I_k$. The ratio $I_{av}/I_k$ was used as an index of daily mean light limitation in the mesocosms. In their typography of the limiting factors for the primary production on the Dutch continental shelf, Peeters *et al.* (1993b) considered phytoplankton with an $P/P_{max}$ ratio of less then 0.1 as severely light limited. The same convention was used in this experiment.

### 2.3.5 Determination of potential nutrient limitation

Whether a nutrient limits phytoplankton production depends on the nutrient concentration and on the uptake efficiency of the algae at low nutrient concentrations. As the phytoplankton in the mesocosms consisted of a mixture of species, it was not possible to unequivocally determine nutrient limitation for phytoplankton growth. As an index of potential nutrient limitation, nutrient concentrations were compared to literature values of the half saturation constant for nutrient uptake, by calculating the $V:V_{max}$ ratio according to Zevenboom (1986):

$$\frac{V}{V_{max}} = \frac{S}{(S + K_s)} \qquad (4)$$

where:
V = potential nutrient uptake rate.
$V_{max}$ = maximum nutrient uptake rate.
S = concentrations of dissolved nutrients.
$K_s$ = half saturation constant for nutrient uptake.

The degree of nutrient limitation was estimated from the water column nutrient concentrations using the following $K_s$ values for nutrient uptake: N= 2 µM; P= 0.1 µM; Si = 2 µM (Peeters & Peperzak, 1990; Peeters *et al.*, 1993b). Nutrients were considered to be potentially limiting at $V:V_{max}$ ratios below 0.5.

### 2.3.6 Microzooplankton sampling and analysis

The phytoplankton samples were also used to analyse microzooplankton (20-200 µm, mainly heterotrophic dinoflagellates and ciliates). Samples (1 l) were taken twice a week (Monday and Thursday) from the top and bottom layers (see Figure 2.1), always between 7 and 8 a.m. Only samples, taken simultaneously with the mesozooplankton samples, were analysed; this resulted in a frequency of on average once a week.
Samples were fixed with acid Lugol's iodine solution (Throndsen, 1978) to a final concentration of ca. 0.3 %.
After concentration (ca. 10x) by sedimentation 5 ml was taken from the residue, decolorised with sodium thiosulphate, and stained with Bengal rose. Microzooplankton species composition and dimensions were determined using an inverted microscope. The used magnifications were 400x and 500x. Depending on organism abundance between 75 and 200 fields of view were counted. Microzooplankton dimensions were measured with a video image analysing system. From aloricate ciliates like Mesodinium rubrum and Strombidium spp. the diameter was measured and from loricate ciliates (tintinnids) length and width of the animals inside the lorica. For a number of species/groups the following coded size categories were used: sp.1 = 0-20 µm, sp.2 = 20-40 µm, sp.3 = 40-60 µm and sp.4 = >60 µm. Unidentifiable ciliates without a lorica are referred to as Ciliate sp.1-4.
The smallest naupliar stages belong to the microzooplankton, but because the majority of the nauplii were > 200 µm and counting was more reliable in the formalin samples, these smaller stages were counted and measured in the mesozooplankton samples (see 4.2.3). For the same reason Egg sp. 59 µm was counted in the mesozooplankton samples.
Carbon biomass was estimated from appropriate geometric approximations, using a conversion factor of 0.19 pg C mm-3 for the ciliates (Putt & Stoecker, 1989) and 0.14 pg C mm-3 for the heterotrophic dinoflagellates (Lessard, 1991).
To correct for cell shrinkage in acid Lugol's solution, a volume (biomass) correction factor of 1.33 for ciliates was estimated from literature data in Choi & Stoecker (1989), Ohman & Snyder (1991) and Putt & Stoecker (1989); for heterotrophic dinoflagellates a correction factor of 1.18 was estimated from Montagnes et al. (1994).
In general, sample analysis was done within 6 months after sampling. An overview of all procedures, used to estimate biovolumes and biomasses, conversion factors and used literature for species identification, is given in Wetsteyn & Vink-Lievaart (1995) and Wetsteyn et al. (1996).

### 2.3.7 Mesozooplankton sampling and analysis

Mesozooplankton (200-2000 µm, mainly copepods and cirriped larvae) samples were taken with a Zenit submersible pump at ca. mid-depth of the top (0.75 m during Experiment 1 and 0.50 m during Experiment 2) and the bottom (2.25 m) layers. Under mixed conditions only one sample was taken at mid-depth (1.5 m) of the mesocosm. Each sample was well over 50 l.

The pumped water was filtered through a 55 µm zooplankton net; in this way also the smallest naupliar stages and eggs were retained. The filtered water, originating from the bottom layer, was gently pumped back (using an aquarium pump) through a PVC-pipe (diameter 20 mm) to the depth from where it originated; the filtered water from the top layer was gently pumped back (also using an aquarium pump) to the surface of the top layer of each mesocosm. A sample from the inlet water, used to flush the mesocosms, was also filtered through a 55 µm zooplankton net. During former experiments under stratified conditions, depth-integrated samples were taken using a long PVC-tube, provided with a ball valve at the lower end (see Kuiper, 1977). This sampling strategy might have disturbed the pycnocline and was not appropriate for routine sampling during stratification. Therefore, the PVC-tube was used only once in the continuously mixed mesocosms NOSTRAT at the end of the experiment. In general, samples were taken once a week, always between 10 and 12 a.m. Each sample was fixed with borax-buffered formalin (Steedman, 1976) to a final concentration of ca. 4 % in 50 ml glass tubes with snap-caps. After staining the samples with Bengal rose, zooplankton species composition and dimensions (small amounts) were determined with a stereomicroscope (magnification 40-50x). The sedimented zooplankton from the 50 ml samples was suspended in demi to a volume of 40 ml. Nauplii were counted in a subsample of 5 ml in a 10 ml cuvet; copepodites and other (large) animals were counted in the remaining 35 ml. All calanoid copepod stages from each species in samples from Experiment 1 were counted; from samples taken during Experiment 2 copepodite stages I-III and IV-V were pooled in two copepodite clusters. This was done after a thorough check, leading to similar biomass values using both approaches on a series of sample data. For the adult copepod stages distinction was made between males and females.
The smallest naupliar stages belong to the microzooplankton, but because the majority of the nauplii were > 200 µm and counting was more reliable in the formalin samples, these smaller stages were counted and measured in the mesozooplankton samples. For the same reason Egg sp. 59 µm was counted in the mesozooplankton samples. Beginning at copepodite stage IV distinction was made between males and females. From naupliar stages body-length and from calanoid copepodite and adult stages cephalo-thorax length was measured. Length of harpacticoid and cyclopoid copepodites and adults was measured as body-length. From other groups like larval stages of Mollusca and Annelida body-length was measured; from nematodes length (if possible) and width were measured.
To calculate copepod (all stages) carbon biomass the length-weight equations given by Klein Breteler et al. (1982) were used.
In general, sample analysis was done within 6 months after sampling. An overview of all procedures, used to estimate biovolumes and biomasses, conversion factors and used literature for species identification, is given in Wetsteyn & Vink-Lievaart (1995) and Wetsteyn et al. (1996).

### 2.3.8 Mussel biomass and growth

A random sample of 40 mussels was used to determine initial length, dry weight and ash-free dry weight. Dry weight was determined after drying the mussels for 48 hours at 70 °C. Ash-free dry weights (ADW) were determined from weight loss after incineration for 4 hours at 540 °C in a muffle furnace. To determine growth rates, number and shell lengths of the living mussels were measured at 4-5 week intervals. At

the end of the experiment dry weights and ash-free dry weights of the mussels were determined.
Growth was determined as increase in shell length, and instantaneous growth rates were calculated from the increase in ADW:

$$\mu_w = \frac{\ln(W_t) - \ln(W_0)}{\Delta t} \qquad (5)$$

with
$\mu_w$ = growth rate in $day^{-1}$
$W_0$, $W_t$ = ADW at begin and end of the experiment.

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# 3 Effects of water column structure and mussel grazing on plankton development

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## 3.1 INTRODUCTION

Phytoplankton development is determined by bottom-up and top-down factors. In estuarine and coastal marine systems, the most important bottom-up factors are light availability and nutrient supply, while grazing represents the major top-down factor. The relative strength of bottom-up versus top-down factors can vary widely both in time and space. In shallow marine systems, interactions between the pelagic system and the benthos can be very important, and grazing control of the phytoplankton can therefore be exerted not only by zooplankton but by benthic suspension feeders as well.
Benthic suspension feeders, mainly bivalves and tunicates, have been shown to be dominant grazers in many shallow coastal areas, with the ability to control phytoplankton biomass (see e.g. Petersen & Riisgård, 1992; Dame, 1993, 1996; Prins, 1996). Bivalve suspension feeders have a lower turnover than zooplankton, which means that nutrient turnover rates in a benthos-dominated system could differ from pelagic ecosystems. It was hypothesised that the presence of benthic suspension feeders may thus stabilise coastal ecosystems and can mitigate the effects of eutrophication (Herman & Scholten, 1990).
The control of the planktonic community depends on the feeding activity of the benthic suspension feeders. This activity may be reduced in the presence of harmful algal blooms, like *Phaeocystis* sp. (Prins et al., 1994; Smaal & Twisk, 1997), and this adverse effect on filtration rates can lead to a breakdown of the grazer control of phytoplankton biomass. Physical factors may also interfere with the grazing control by the benthos. Under conditions with reduced mixing or stratification, part of the water column is not exposed to benthic suspension feeding, and massive algal blooms can occur under these conditions (Koseff et al., 1993; Møhlenberg, 1995).
The physical structure of the water column, in addition to interfering with benthic grazing effects, is an important factor in determining the composition of the phytoplankton community. Reduced mixing favours flagellate species above diatoms in general, and in combination with high nutrient availability, these conditions may be very favourable for the development of dinoflagellate blooms (Taylor & Pollingher, 1987). This may include toxic species, and thus poses an additional destabilising factor for grazer control of phytoplankton biomass.
In earlier experiments, we have investigated the relations between mussel grazing and plankton development in experimental marine ecosystems with a well-mixed water column. These experiments showed that bivalve grazing can significantly reduce phytoplankton biomass. At the same time, grazing may affect phytoplankton growth rates, by causing shifts towards a dominance of faster growing algal species and by increasing inorganic nutrient availability. The experiments also indicated that microzooplankton abundance may be

**Table 3.1**
Main features of experimental design. Within each treatment, duplicate mesocosms were used.

| Treatment | STRAT-H (periodically stratified) | STRAT-L (periodically stratified) | NOSTRAT-H (no stratification) |
|---|---|---|---|
| **Initial mussel biomass (g ADW $m^{-3}$)** | 2.9 | 0.6 | 2.9 |
| **Inorganic nutrient loading: (mmol m-2 d-1)** | | | |
| **DIN** | 13.2 | 13.2 | 13.2 |
| **DiP** | 0.33 | 0.33 | 0.33 |
| **Si** | 2.79 | 2.79 | 2.79 |

affected, due to either food competition or predation (Prins et al., 1995b, c). In the present study, we tested the hypothesis that stratification in systems dominated by benthic suspension feeders, results in massive algal blooms due to an uncoupling between the benthic and the pelagic system. In the experiment we contrasted the effect of bivalve grazing on plankton development in well-mixed mesocosms versus periodically stratified mesocosms. In addition, plankton development was studied in systems with stratification in combination with low mussel biomass as well.

## 3.2 MATERIAL AND METHODS

In this experiment, two treatments with a high mussel biomass were used. In one treatment the water column was well-mixed during the entire experiment, in the other treatment a stratification was induced. A third treatment combined a low mussel biomass with stratification. Within each treatment duplicate mesocosms were used. Nutrient loadings were identical in each treatment (Table 3.1). During stratification, nutrients were added to the upper mixed layer (Top) and bottom mixed layer (Bottom) at similar rates.
At the start of the experiment at day 104 the mesocosms were filled with water from the Oosterschelde estuary, and the continuous nutrient loading was started. The mesocosms were flushed with seawater at a rate of 100 l $day^{-1}$, resulting in a residence time of 30 days. At day 119 a salinity stratification was induced in 4 mesocosms by adding demineralized water, removing the mixer at mid depth and adjusting rotation speed of the mixers. As this stratification was unstable, mixing was increased again at day 126. At day 147 salinity stratification was induced for the second time. The salinity gradient between the upper mixed layer and the bottom mixed layer was maintained by addition of seawater with 27 $^{0}/_{00}$ S to the top layer, and addition of 37 $^{0}/_{00}$ S seawater to the bottom layer. At day 171 standard mixing conditions were restored in the 4 mesocosms.
General methods are described in Chapter 2.

## 3.3 RESULTS

### 3.3.1 Salinity gradients

The observed vertical distributions of salinity reflect the experimentally changed mixing conditions (Fig. 3.1). The first initiated stratification period showed a weak stratification in some of the mesocosms between days 119 and 126, as indicated by the density differences (Fig. 3.2). The second period showed a stable stratification in 3 mesocosms, and a slightly weaker stratification in STRAT-H2. In the 2 mesocosms with permanent mixing no stratification was observed.

**Figure 3.1**
Vertical distribution of salinity during the experiment in all mesocosms

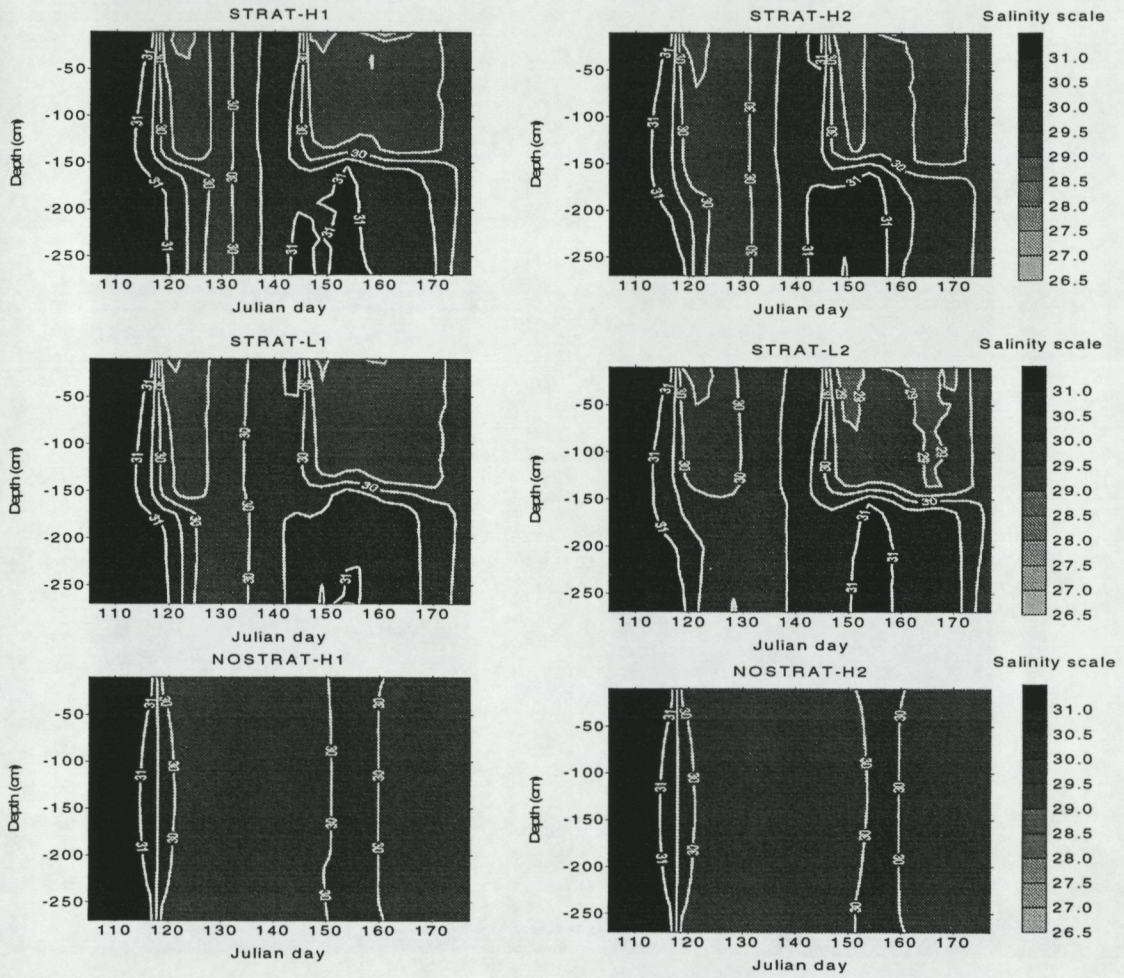



**Figure 3.2**
Strength of stratification in the mesocosms, as indicated by $\sigma_{t,\,Bottom}$-$\sigma_{t,\,Top}$. Narrow line: <0.5; grey bar: 0.5-1; black bar:>1

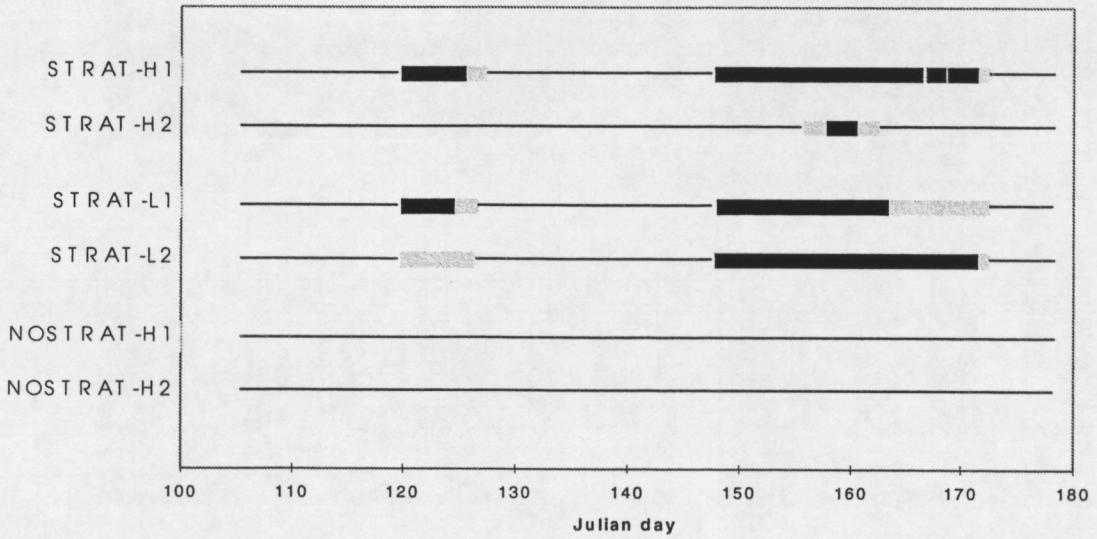


**Figure 3.3**
A: Daily average surface irradiance (PAR). The smoothed curve shows the 15-day moving average. Arrows indicate stratification period.
B: Daily average water temperatures. Arrows indicate stratification period.

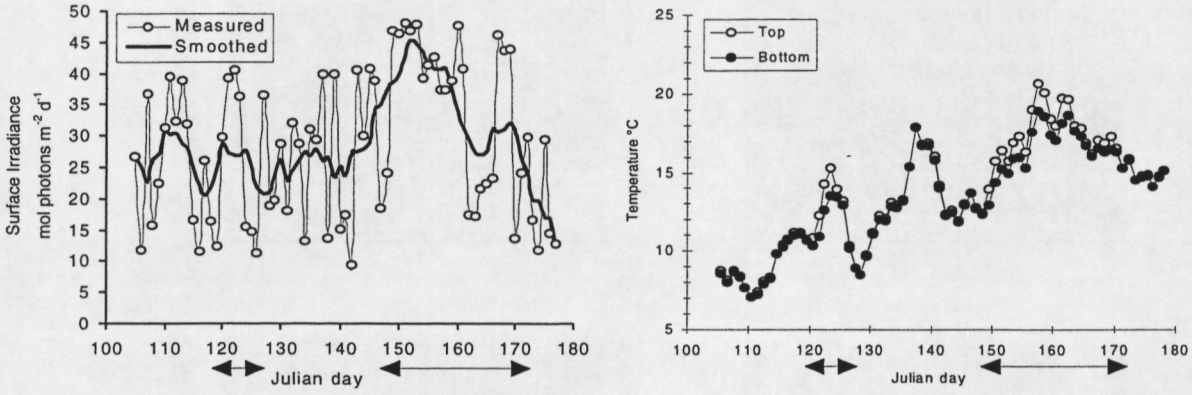



**Figure 3.4**
Vertical distribution of the light limitation index ($I/I_k$) during the present experiment. I: mean daily irradiance, $I_k$: photosynthetic characteristic irradiance ($I_k = P_{max}/a$, $P_{max}$ highest point and a slope of the P/I curves obtained by the $^{14}C$ incubations). When $I/I_k$ is between 0.1 and 0.5, light limitation is considered to be moderate; when $I/I_k$<0.1, light limitation is considered to be severe (See Chapter 2 for further explanation).

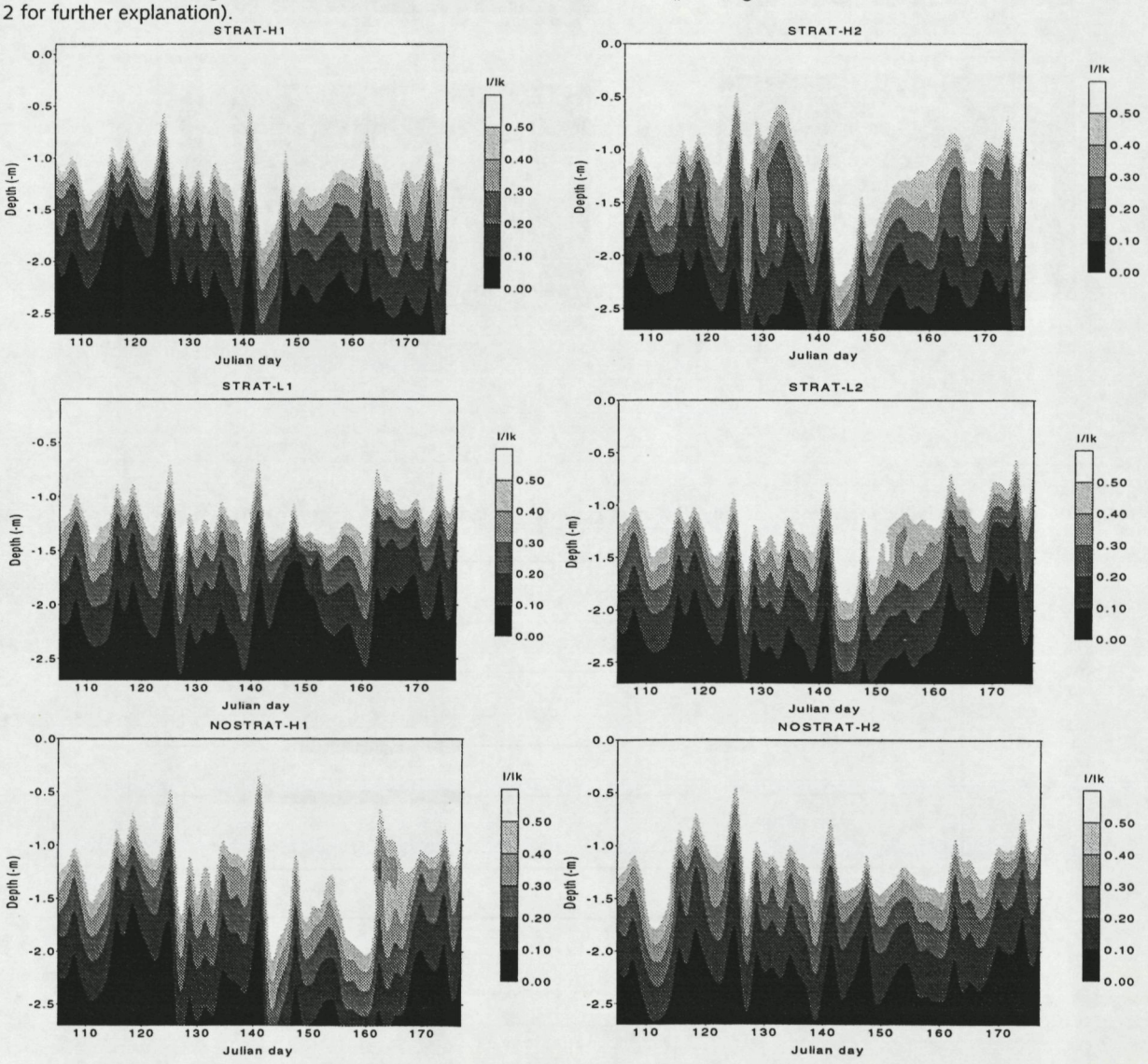
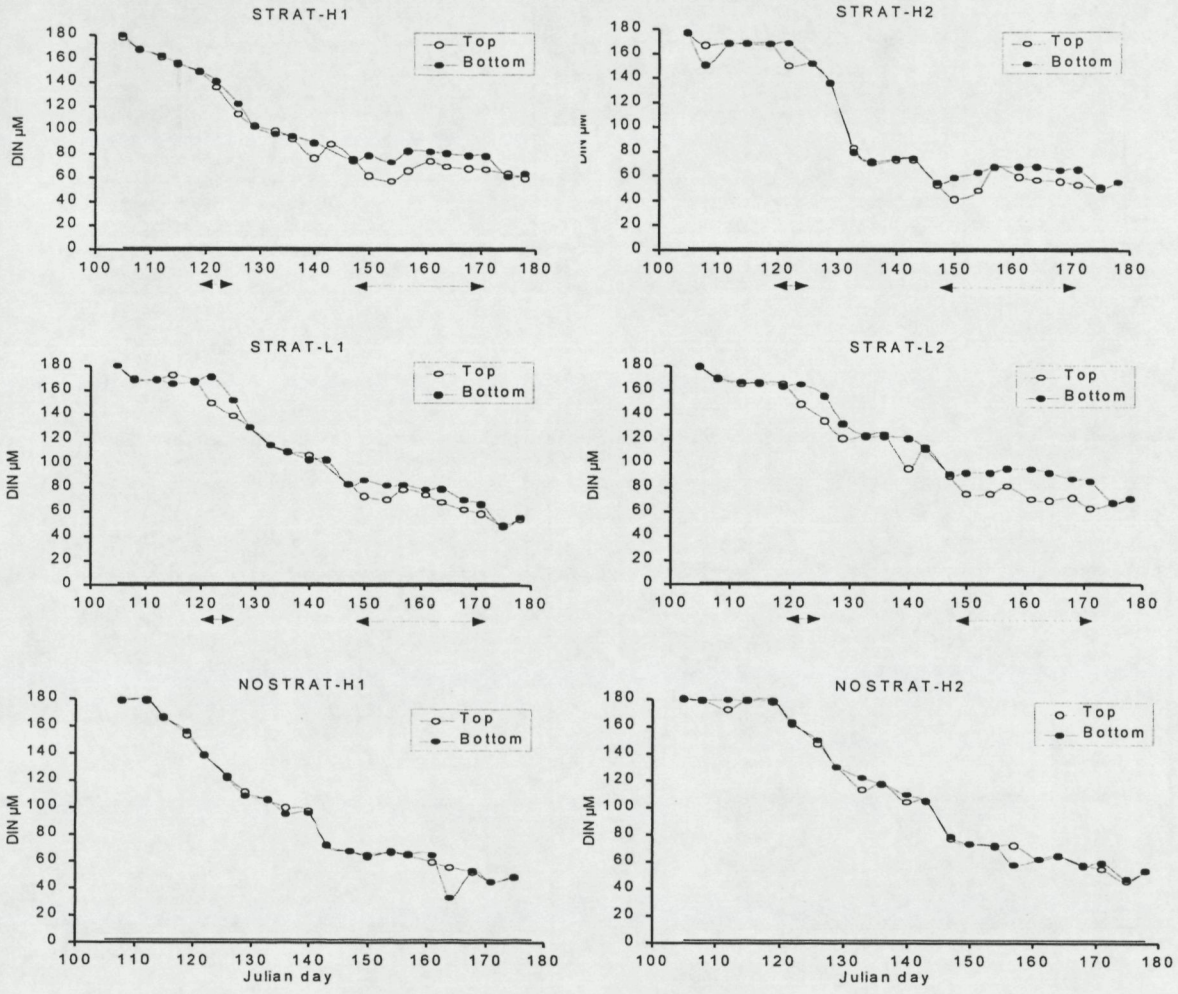


**Figure 3.5**
DIN concentrations in the six mesocosms in the upper mixed layer (Top) and the bottom mixed layer (Bottom). When concentrations decrease below 2 µM (level line plotted on graphs), DIN becomes potentially limiting for the primary production (Ks value from literature sources, see Chapter 2). Stratification periods are indicated below the X-axis (↔)




### 3.3.2 Irradiance and water temperatures

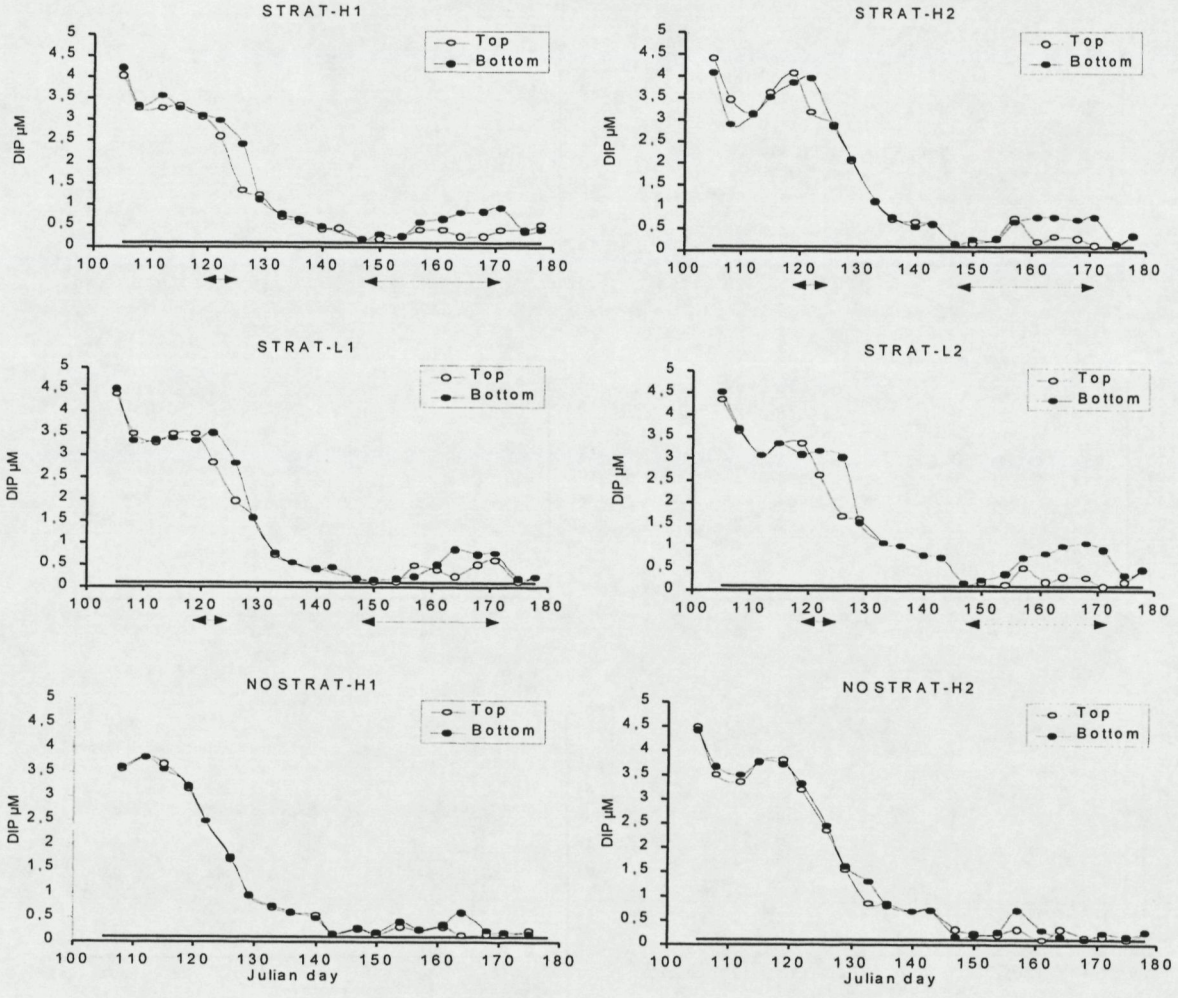
The stratification period between day 147 and day 171 coincided with a period of higher irradiance (Figure 3.3a). Water temperatures showed a gradual increase, with highest temperatures during the second stratification period.
An analysis of light limitation, based on measured P-I curves and the underwater light climate in each mesocosm, showed that light limitation was limited to the deepest layers of the mesocosms, and consequently phytoplankton in the bottom mixed layer was occasionally light limited in the stratified mesocosms (Figure 3.4).

### 3.3.3 Inorganic nutrient concentrations and potential nutrient limitation of phytoplankton

#### 3.3.3.1 DIN

At the start of the experiment, inorganic nutrient concentrations were adjusted to the steady state concentrations related to the experimental nutrient loading. DIN concentrations showed a steady decrease during the entire experiment, but remained much higher than limiting levels.

**Figure 3.6**
DIP concentrations in the six mesocosms in the upper mixed layer (Top) and in the bottom mixed layer (Bottom). When concentrations decrease below 0.1 µM (level line plotted on graphs), DIP becomes potentially limiting for the primary production (Ks value from literature sources, see Chapter 2). Stratification periods are indicated below the X-axis (↔).




During the stratification period, concentrations in the bottom mixed layer were slightly elevated compared to the upper mixed layer.

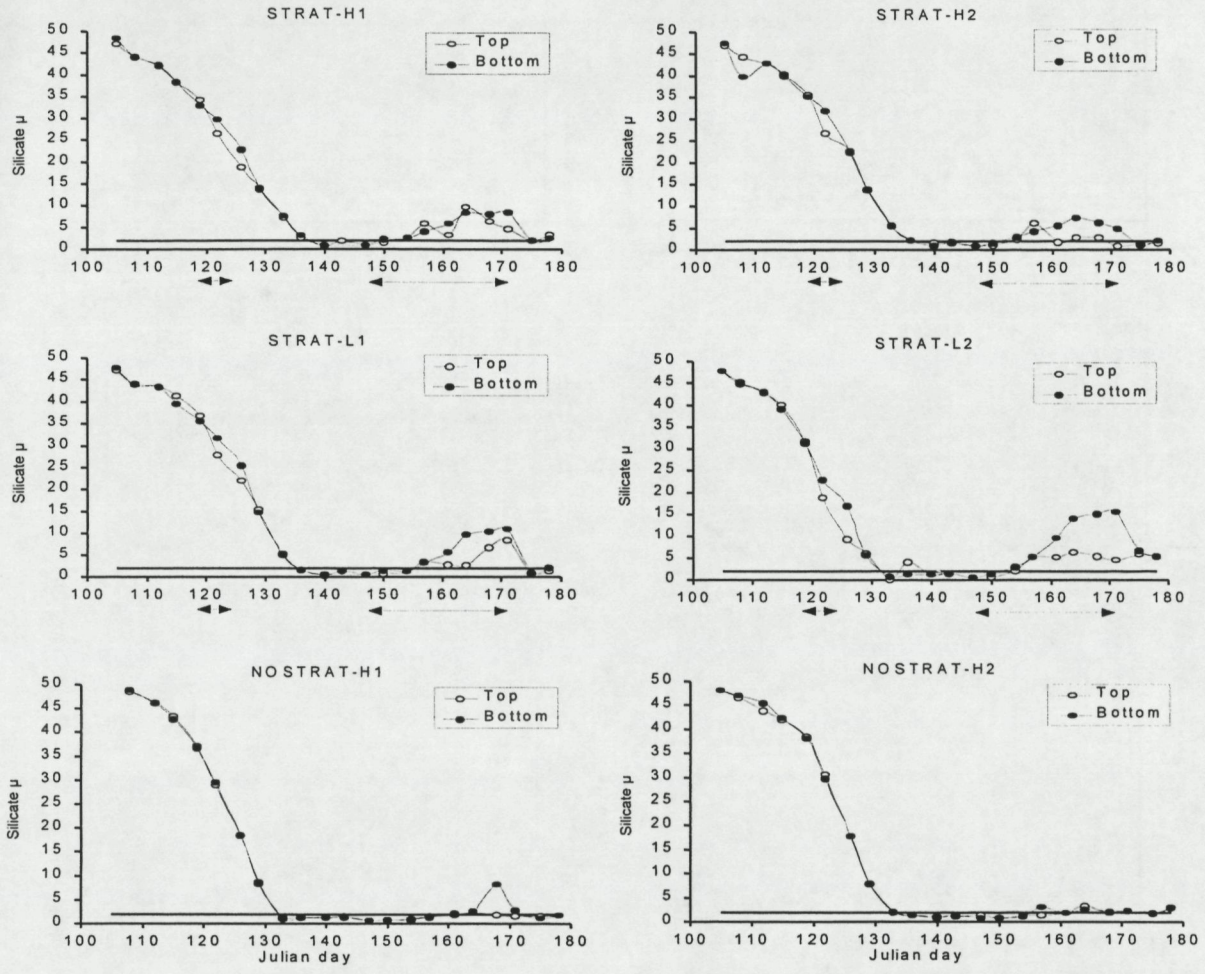
### 3.3.3.2 DIP

Concentrations of DIP decreased from steady state concentrations at the start of the experiment, to levels close to limiting concentrations around day 150. During the stratification period, DIP concentrations were lower in the top layer than in the bottom layer, but were always above limiting levels. DIP concentrations in the mixed mesocosms were slightly lower than in the stratified mesocosms (Figure 3.6).

### 3.3.3.3 Silicate

Silicate concentrations dropped to limiting levels in all mesocosms between day 130 and day 140. During the following stratification period, silicate concentrations increased to non-limiting levels in the 4 mesocosms that were stratified, with generally slightly higher concentrations in the bottom layer. In the mixed systems, silicate concentrations remained low during the rest of the experiment (Figure 3.7). Consequently, silicate was potentially limiting diatom growth during the second half of the experiment in the well-mixed

**Figure 3.7**
Silicate concentrations in the six mesocosms in the upper mixed layer (Top) and in the bottom mixed layer (Bottom). When concentrations decrease below 2 µ(level line plotted on graphs), DIP becomes potentially limiting for the primary production (Ks value from literature sources, see Chapter 2). Stratification periods are indicated below the X-axis (↔).




mesocosms, while silicate limitation occurred during a short interval only in the mesocosms with a stratified water column.

### 3.3.4 Phytoplankton

#### 3.3.4.1 Chlorophyll-a concentrations and primary production

Initial chlorophyll-a concentrations were high, due to a bloom of *Phaeocystis* sp. in the sea water that was incubated in the mesocosms. This bloom settled within a few days, and then a small bloom occurred with a peak around day 130. During the stratification period between days 147-171, blooms were observed in the top layer of the mesocosms with a stratified water column, whereas chlorophyll-a levels remained low in the well-mixed systems (Figure 3.8).
Table 3.2 summarises the results. The first 5 days of the experiment, when initial chlorophyll-a concentrations were decreasing due to settlement of the phytoplankton, were excluded from the analysis. Similarly, the period before day 147 is treated as one period because the first period of stratification that was initiated, was very short (6 days) and did not show clear responses in the chlorophyll-a concentrations.

**Figure 3.8**
Chlorophyll-*a* concentrations in the Top and the Bottom compartments of the six mesocosms. Stratification periods are indicated below the X-axis (↔).

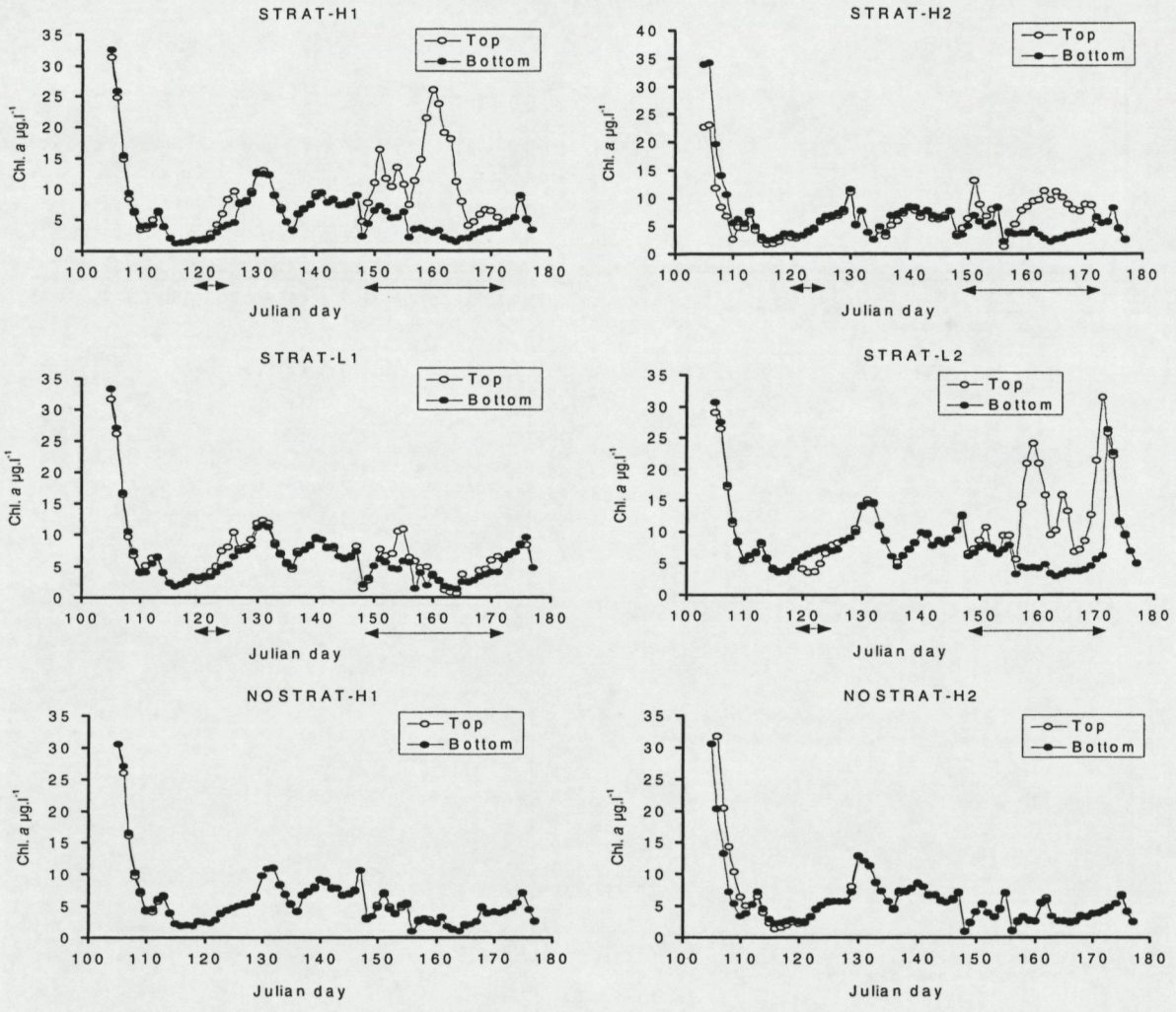
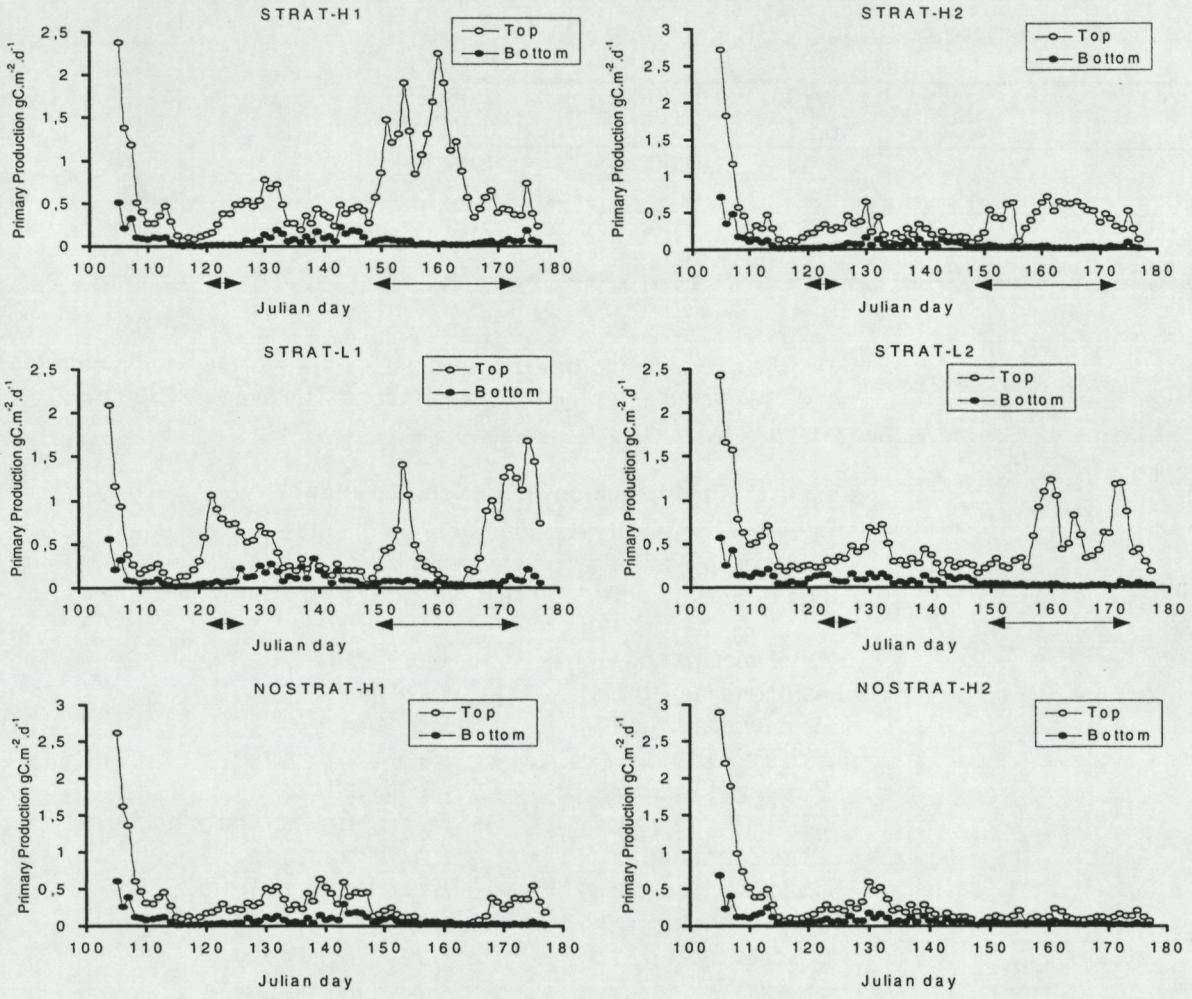



**Table 3.2.**
Average chlorophyll-*a* concentration in the Top and Bottom layer of each mesocosm during 3 periods of the experiment. Significant differences between Top and Bottom (paired t-test) are indicated.

| Treatment | Location | Day 105-146 | | Day 147-171 | | Day 172-177 | |
|---|---|---|---|---|---|---|---|
| STRAT-H1 | Top | 7.50 | | 11.53 | *** | 5.20 | |
| | Bottom | 7.23 | | 3.86 | | 5.36 | |
| STRAT-H2 | Top | 6.29 | ** | 7.90 | *** | 5.49 | |
| | Bottom | 7.68 | | 4.18 | | 5.56 | |
| STRAT-L1 | Top | 7.91 | * | 4.81 | ** | 6.94 | |
| | Bottom | 7.61 | | 3.61 | | 7.12 | |
| STRAT-L2 | Top | 8.82 | | 12.69 | *** | 13.50 | |
| | Bottom | 9.15 | | 5.47 | | 13.67 | |
| NOSTRAT-H1 | Top | 7.15 | | 3.62 | | 4.78 | |
| | Bottom | 7.22 | | 3.58 | | 4.77 | |
| NOSTRAT-H2 | Top | 7.73 | | 3.63 | | 4.56 | * |
| | Bottom | 7.03 | | 3.65 | | 4.66 | |

**Figure 3.9**
Primary production in the Top and the Bottom compartments of the six mesocosms. Stratification periods are indicated below the X-axis (↔).




In period 1 (day 110-146), 4 of the mesocosms showed no differences between chlorophyll-a levels in the top layer and those in the bottom layer of the water column. STRAT-L1 had slightly lower levels, STRAT-H2 had slightly higher levels near the bottom. During the stratification period, chorophyll-a levels in the top layer were significantly higher than those in the bottom layer in all stratified mesocosms. This difference was smallest in STRAT-L1. No differences were observed in the well-mixed mesocosms. After the end of the stratification the differences between top and bottom layers disappeared again.
During the first period, chlorophyll-a levels were slightly higher in the mesocosms with low mussel biomass (STRAT-L), but differences were small and not statistically significant. During the stratification period blooms occurred in the top layer of all stratified mesocosms, but the maximum concentrations showed large differences between mesocosms. As a consequence of the large variation within treatments, differences in chlorophyll-a concentrations between treatments were not significant for any of the three periods.
Primary production rates showed patterns similar to chlorophyll-a concentrations (Figure 3.9). The average turnover rates of the phytoplankton were calculated using a C:chlorophyll ratio of 30, which

**Table 3.3**
Average chlorophyll concentrations , primary production rates and P/B ratios during the experiment. Turnover rates of the phytoplankton were calculated assuming a C:chl ratio of 30.

| Treatment | Mesocosm # | Chl in $\mu g\ l^{-1}$ | PP in $g\ C\ m^{-2}\ d^{-1}$ | P/B in $day^{-1}$ |
|---|---|---|---|---|
| STRAT-H | 1 | 7.30 | 0.707 | 1.08 |
| | 2 | 6.54 | 0.469 | 0.80 |
| STRAT-L | 1 | 6.48 | 0.605 | 1.04 |
| | 2 | 9.40 | 0.590 | 0.70 |
| NOSTRAT-H | 1 | 5.76 | 0.462 | 0.89 |
| | 2 | 5.87 | 0.365 | 0.69 |

was the average ratio based on estimates of phyto-plankton biomass and observed chlorophyll-a concentrations. The average P/B ratios were high (Table 3.3).

### 3.3.4.2 Phytoplankton species composition

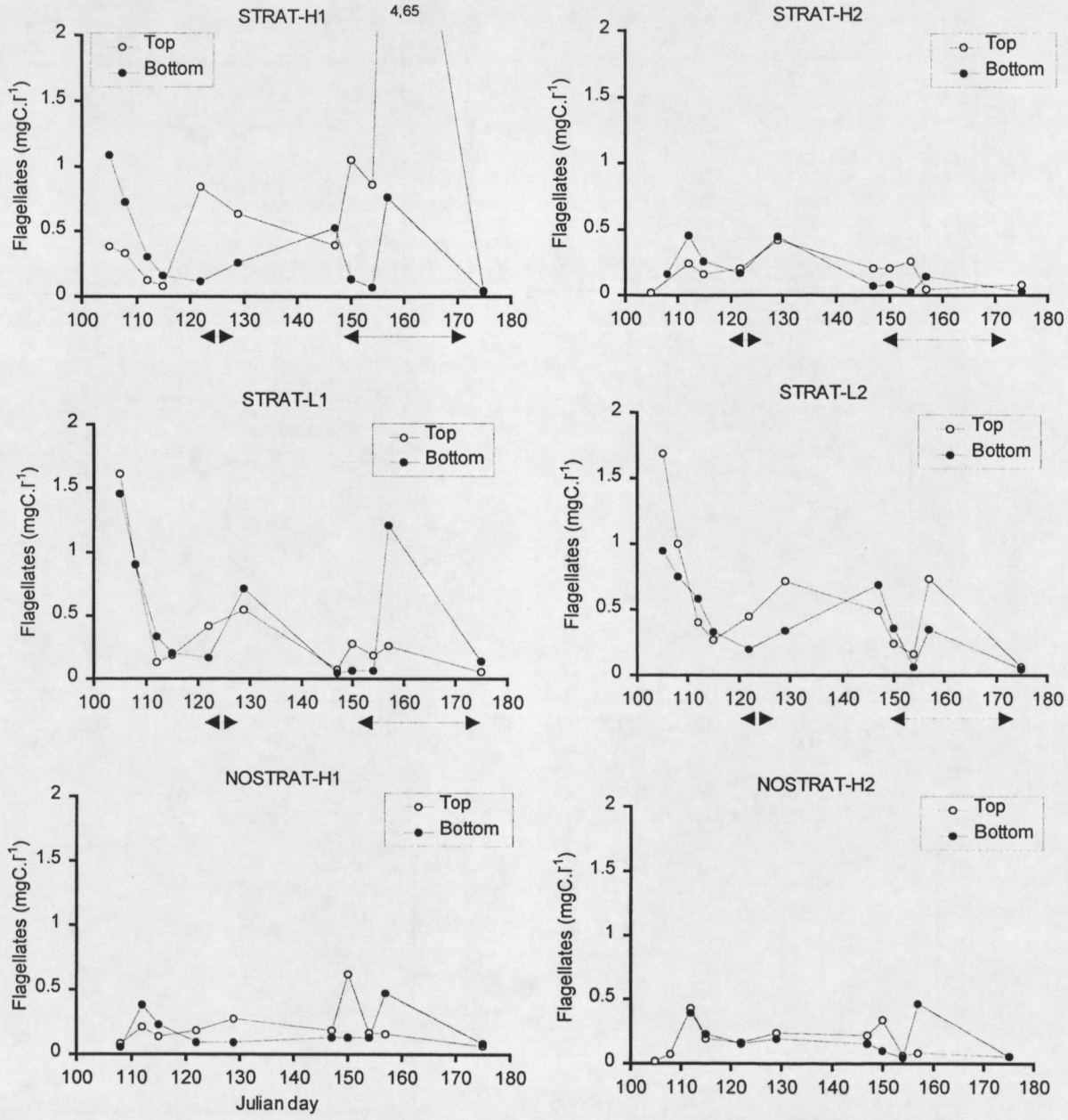
Taxonomic groups encountered during the present experiment are listed below. Identification at species level was not always possible. In such cases, phytoplankton cells were classified according to their size.

*Amphidinium* sp.
*Asterionella glacialis*
*Asterionella kariana*
*Biddulphia aurita*
*Biddulphia sinensis*
*Cerataulina bergonii*
*Chaetoceros* sp.
*Chaetoceros socialis*
*Chrysochromulina* sp.
*Coscinodisceae*
*Cryptophyceae*
*Cyanophyceae*
*Ditylum brightwellii*
*Eutreptiella* sp.
*Leptocylindrus danicus*
*Lycmophora* sp.
*Nanoflagellates*
*Nitzschia closterium*
*Nitzschia delicatissima*
*Nitzschia longissima*
*Nitzschia seriata*
*Pennates*
*Phaeocystis* sp.
*Picoflagellates*
*Plagiogramma brockmanni*
*Pyramimonas* sp.
*Rhizosolenia delicatula*
*Rhizosolenia pungens*
*Rhizosolenia setigera*
*Rhizosolenia shrubsolii*
*Skeletonema costatum*
*Tetraselmis marina*
*Thalassionema nitzschioides*
*Thalassiosira decipiens*
*Thalassiosira levanderi*
*Thalassiosira nordenskioldii*
*Thalassiosira rotula*

### 3.3.4.3 Main components of the phytoplankton biomass

Two different functional groups were distinguished, viz. flagellates and diatoms (respectively non-silicon and silicon using phytoplankton). Flagellates consisted mainly of three sub-groups: autotrophic nanoflagellates (2 to 20 µm), Cryptophyceae and *Phaeocystis* sp. The initial biomass of autotrophic flagellates was high due to a bloom of *Phaeocystis* sp. in the Oosterschelde estuary at the time the experiment started (Figure 3.10). Flagellate biomass remained relatively high during the entire experiment in comparison to diatom biomass (Figure 3.11). Total phytoplankton biomass was dominated by flagellate species (Figure 3.12), mainly *Cryptophyceae* and *Phaeocystis* sp. There was a tendency for a higher abundance of flagellates in the

**Figure 3.10**
Total Flagellate (non diatom) biomass measured in the Top and Bottom compartments of the six mesocosms. Stratification period is indicated below the X-axis (↔). Note the different Y-scale used for NOSTRAT-H1 and NOSTRAT-H2.




stratified mesocosms. However, consistent patterns with blooms of flagellates in the top layer, as was observed with chlorophyll-a, were not apparent. Diatom biomass was generally somewhat higher in the bottom layer during the stratification period, but a similar distribution was observed in the non-stratified mesocosms.
The average biomass of diatoms and flagellates during the experiment showed no significant differences between treatments. A comparison of phytoplankton composition in terms of the ratio between flagellate and diatom biomass, showed that treatments were not significantly different either.

**Figure 3.11**
Total diatom biomass (mgC.l$^{-1}$) measured in the Top and Bottom layer of the six mesocosms. The stratification period is indicated below the X-axis (↔). Note the different Y-scale used for NOSTRAT-H2.

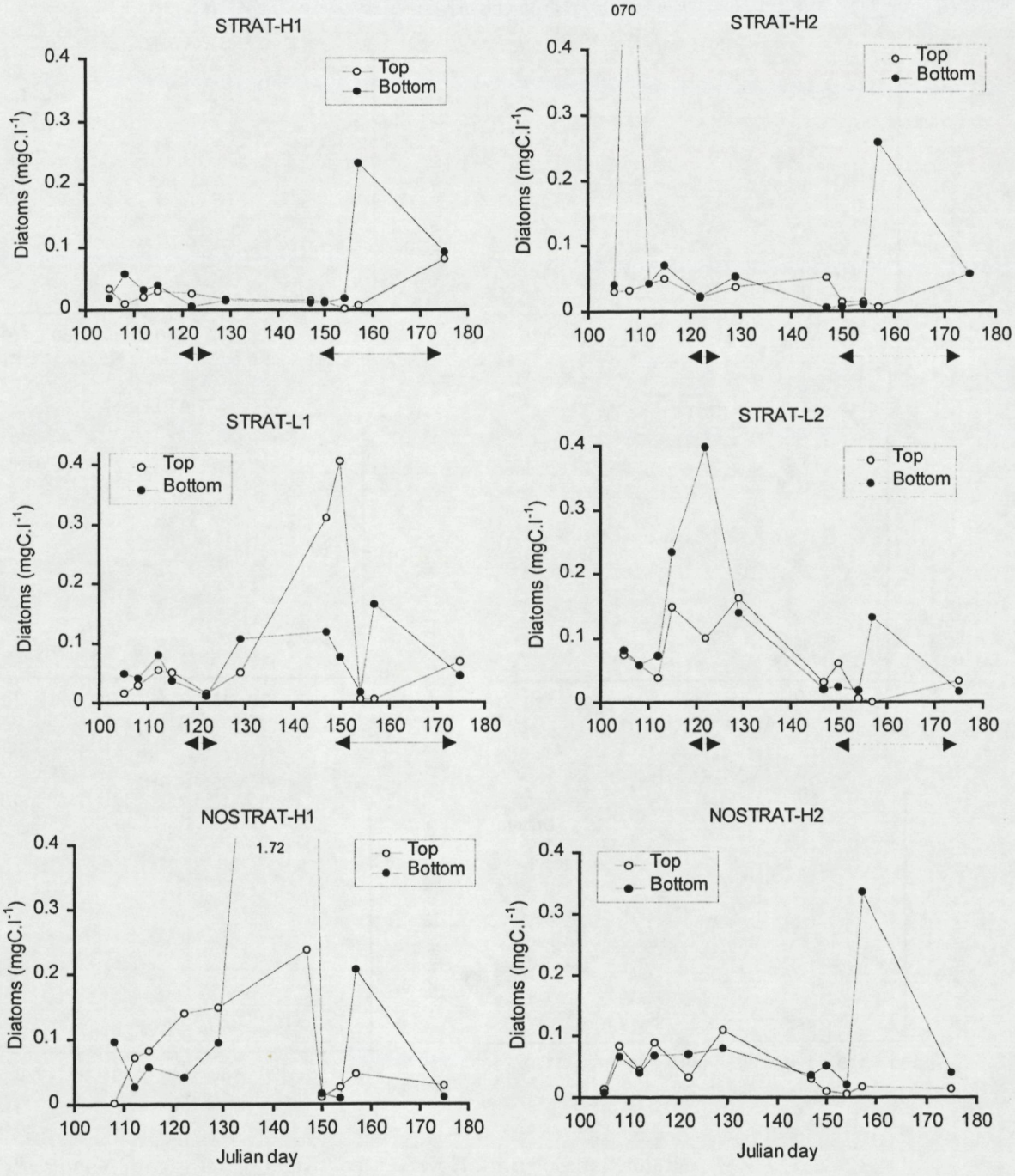


**Figure 3.12**
Contribution of autotrophic flagellate biomass to total phytoplankton biomass.

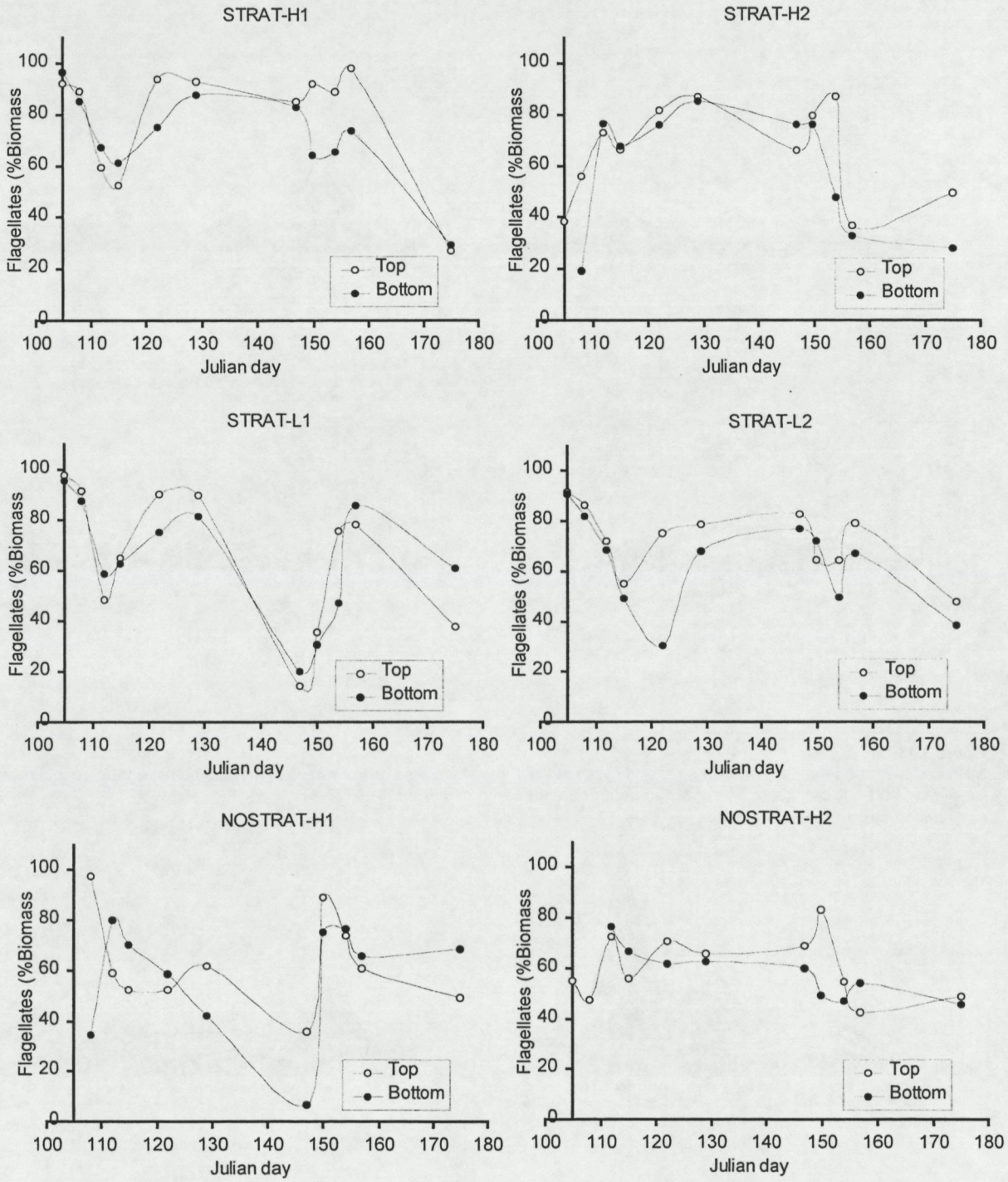


**Figure 3.13-a**
Microzooplankton biomass (without eggs and cysts) in the two mesocosms of treatment STRAT-H. Biomass data are given separately for the top and bottom layers. Stratification periods are indicated below the X-axis (↔).

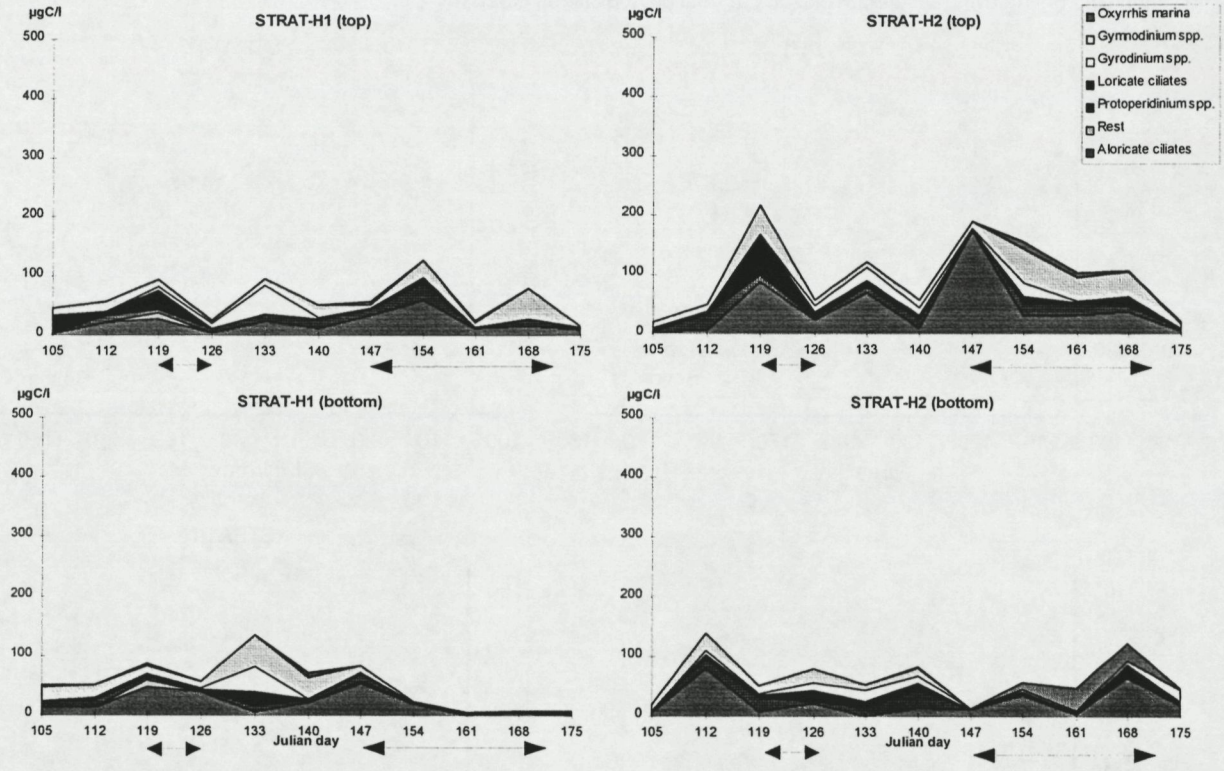



**Figure 3.13-b**
Microzooplankton biomass (without eggs and cysts) in the two mesocosms of treatment STRAT-L. Biomass data are given separately for the top and bottom layers. Stratification periods are indicated below the X-axis (↔).

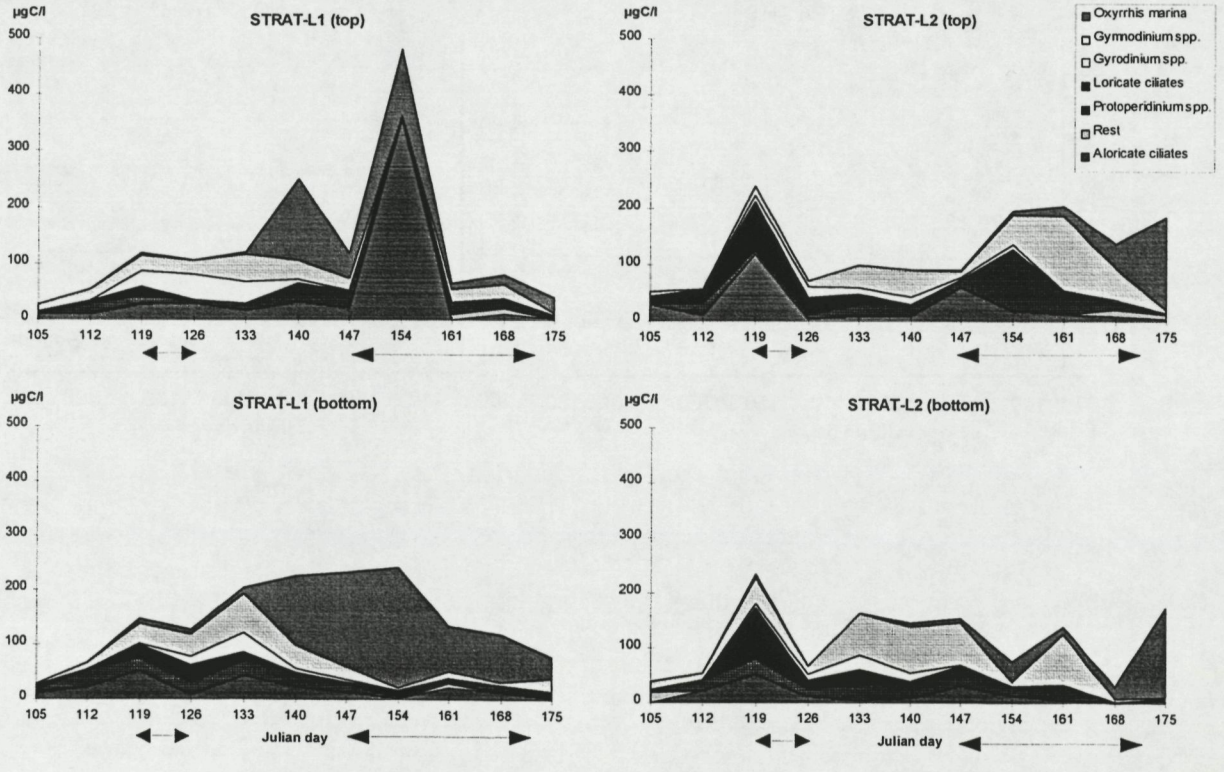
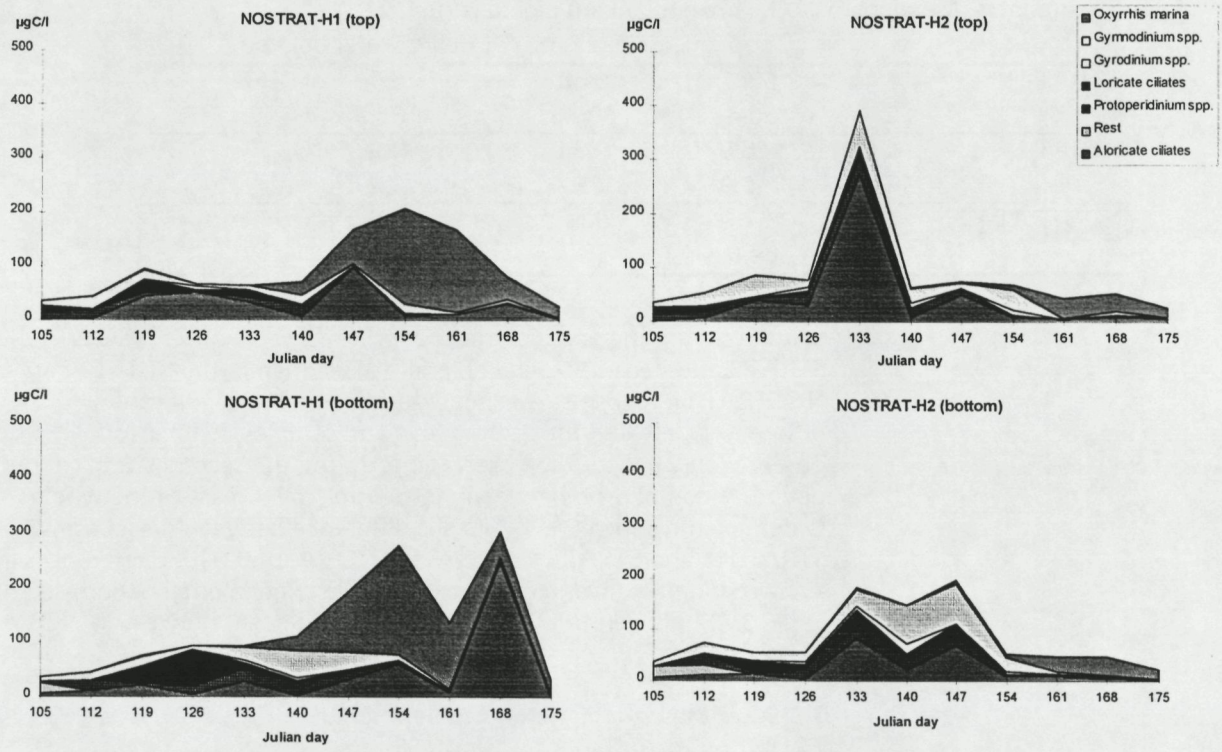


**Figure 3.13-c**
Microzooplankton biomass (without eggs and cysts) in the two mesocosms of treatment NOSTRAT. Biomass data are given separately for the top and bottom layers. Stratification periods are indicated below the X-axis (↔).




### 3.3.5 Zooplankton

#### 3.3.5.1 Microzooplankton

The development of microzooplankton biomass (without eggs and cysts) is shown in Figures 3.13a-c.
In mesocosm STRAT-H1 (Top and Bottom) and mesocosm STRAT-H2 (Bottom) biomass ranged between ca. 5 and 130 µgC $l^{-1}$; in mesocosm STRAT-H2 (Top) biomass reached somewhat higher values of ca. 200 µgC $l^{-1}$. In both mesocosms (Figure 3.13a) half of the biomass was formed by aloricate ciliates; all other distinguished groups contributed more or less equally to microzooplankton biomass.
In the STRAT-L mesocosms (Figure 3.13b) higher biomass values were measured, generally with peak values of 240 µgC $l^{-1}$; however, in mesocosm STRAT-L1 (Top) the highest value was ca. 480 µgC $l^{-1}$. All distinguished groups contributed more or less equally to microzooplankton biomass with the exception of mesocosm STRAT-L1 (Top) where aloricate ciliates were responsible for the high peak value at day 154 and mesocosm STRAT-L1 (Bottom) where biomass during the second half of the experiment was dominated by the heterotrophic dinoflagellate *Oxyrrhis marina*.
Biomass values in the NOSTRAT-H mesocosms (Figure 3.13c) reached values between those of the STRAT-H and STRAT-L mesocosms. In mesocosm NOSTRAT-H1 (Top and Bottom) biomass reached higher values during the second half of the experiment due to the contribution of *Oxyrrhis marina*. Also aloricate ciliates formed an important fraction of microzooplankton biomass.
Table 3.4 gives the microzooplankton biomass, averaged over the entire experimental period, for the Top and Bottom compartments of the mesocosms, and also the average biomass in the whole water

**Table 3.4**
Microzooplankton biomass (averaged over the entire experimental period) in the Bottom and Top compartments of the 6 mesocosms and in the whole water column (in italics).

| Treatment | Mesocosm | Microzooplankton biomass (µgC $l^{-1}$) | | |
|---|---|---|---|---|
| | | Top | Bottom | Entire water column |
| STRAT-H | 1 | 53.2 | 61.9 | *57.5* |
| | 2 | 65.4 | 100.5 | *82.9* |
| STRAT-L | 1 | 146.9 | 133.0 | *139.9* |
| | 2 | 115.9 | 129.4 | *122.6* |
| NOSTRAT-H | 1 | 127.9 | 93.2 | *110.5* |
| | 2 | 84.0 | 88.7 | *86.3* |

column during the experimental period. Average biomass values in the STRAT-L mesocosms reached higher values than in the STRAT-H and NOSTRAT mesocosms. A paired t-test showed no significant differences between the Bottom and Top samples, neither for all observations, nor for the observations during the second stratification period. A comparison between mesocosms of biomass of the whole water column showed a significant difference between mesocosms STRAT-H1 and STRAT-L1 (one-way ANOVA, p=0.015). Differences in average biomass (averaged over the whole experimental period, see Table 3.4) between treatments were not significant however (ANOVA, p=0.07).

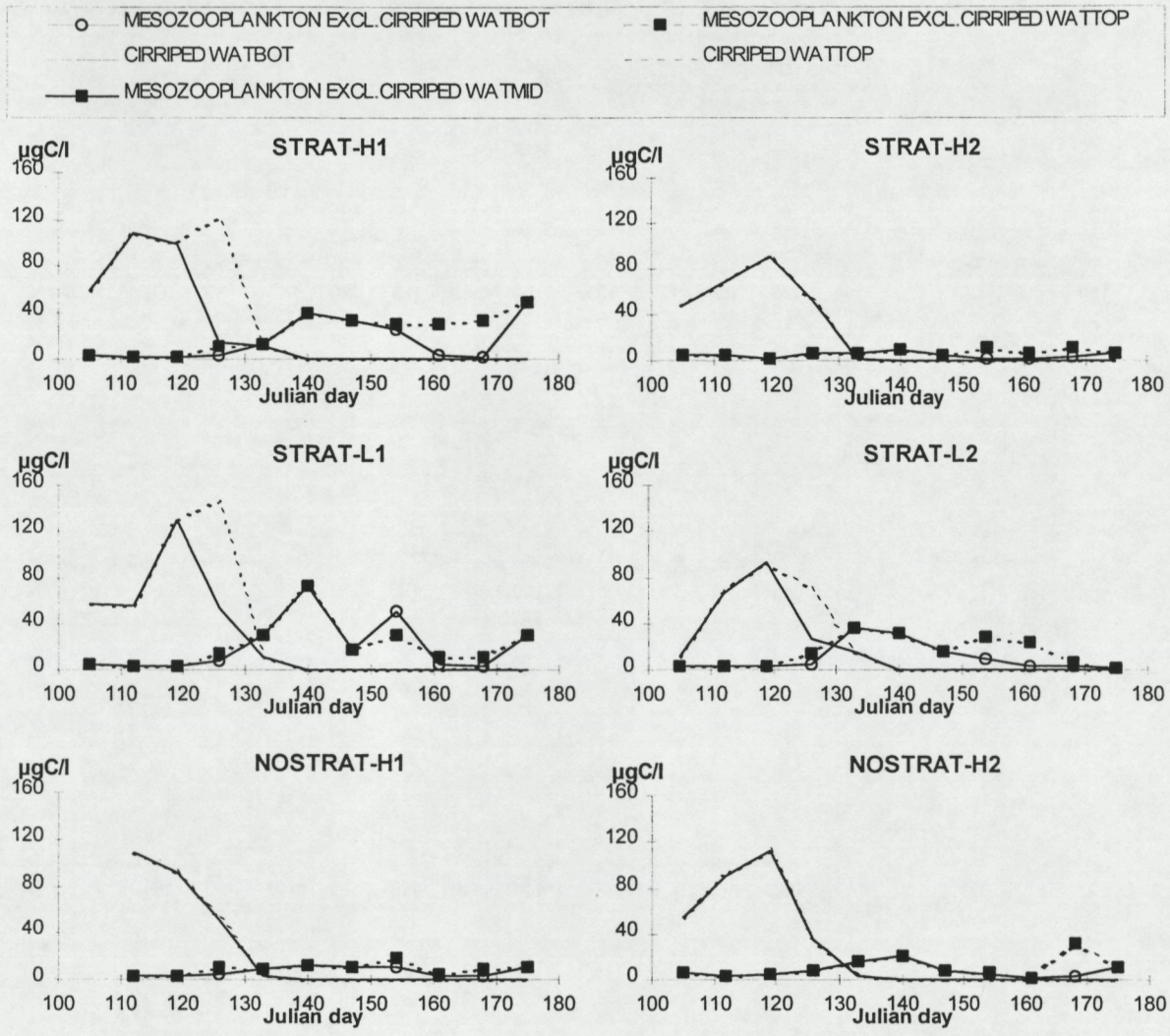
#### 3.3.5.2 Mesozooplankton development

The development of mesozooplankton biomass (without eggs) is shown in Figures 3.14-3.16. Initially, mesozooplankton biomass (maximum values between ca. 80 and 140 µgC $l^{-1}$) was dominated by cirriped larvae (Figure 3.14). After day 120 their numbers dropped rapidly and at day 130 cirriped larvae had disappeared from the plankton samples. From that day on mesozooplankton biomass in 5 of the 6 mesocosms was characterised by an increase in the biomass of calanoid nauplii, copepodites and adults, most of them belonging to *Temora longicornis*. In mesocosm STRAT-H2 biomass remained rather low as a consequence of the poor development of copepodites and adults.
The total biomass of nauplii was relatively low and maximum biomass values varied between ca. 2 and 7 µgC $l^{-1}$ (Figure 3.15). During the two stratification periods biomass of nauplii in the Top samples of the stratified mesocosms was greater than in the Bottom samples. Before the end of the experiment nauplii disappeared from the plankton samples.
Calanoid copepodites and adults reached maximum biomass values

**Table 3.5**
Calanoid copepod (all stages) biomass (averaged over the entire experimental period) in the Bottom and Top compartments of the 6 mesocosms and in the whole water column (in italics).

| Treatment | Mesocosm | Calanoid copepod biomass (µgC $l^{-1}$) | | |
|---|---|---|---|---|
| | | Top | Bottom | Entire water column |
| STRAT-H | 1 | 18.8 | 13.2 | *16.0* |
| | 2 | 4.3 | 2.9 | *3.6* |
| STRAT-L | 1 | 18.8 | 18.4 | *18.6* |
| | 2 | 13.2 | 8.7 | *11.0* |
| NOSTRAT-H | 1 | 6.3 | 4.9 | *5.6* |
| | 2 | 5.7 | 5.7 | *5.7* |

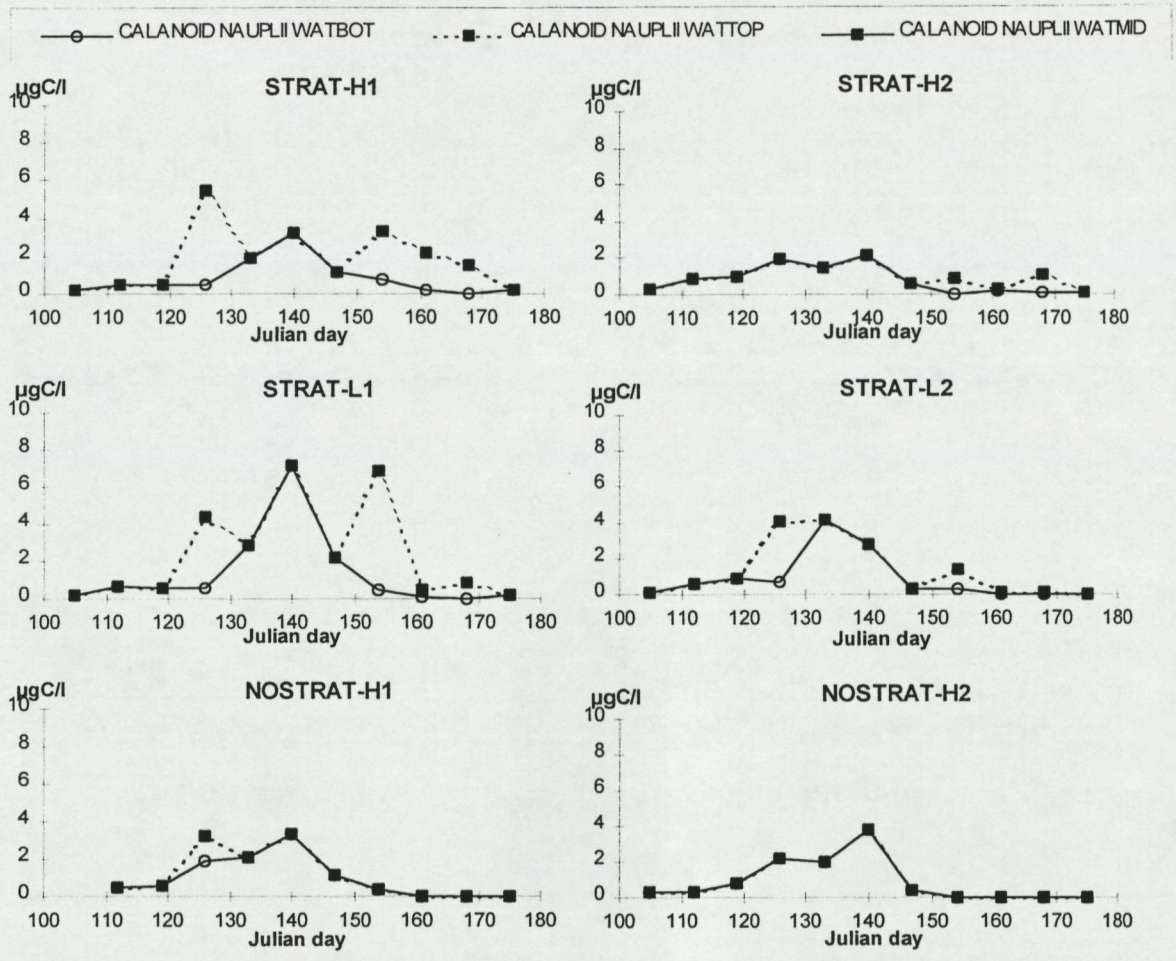
**Figure 3.14**
Total mesozooplankton biomass (without eggs) in the 6 mesocosms. WATBOT = bottom layer of water column; WATMID = mid depth of water column; WATTOP = top layer of water column.




between ca. 10 and 65 µgC $l^{-1}$ (Figure 3.16). During the second stratification period biomass of copepodites and adults in the Top samples of the stratified mesocosms was greater than in the Bottom samples. In four mesocosms copepodites and adults had disappeared from the plankton samples while in the two other mesocosms (STRAT-H1 and STRAT-L1) biomass still had values between ca. 20 and 30 µgC.$l^{-1}$. Biomass of other groups, cirriped larvae excluded, was negligible.

Table 3.5 gives the calanoid copepod (all stages) biomass, averaged over the entire experimental period, for the Top and Bottom compartments of the mesocosms, and also the averaged biomass in the whole water column during the experimental period. The highest average values were measured in mesocosms STRAT-H1 and STRAT-L1. A paired t-test showed that there were no significant differences between the Bottom and Top samples, neither for the entire experiment, nor for the second stratification period. With respect to the biomass in the whole water column, STRAT-L1 appeared to be significantly higher than STRAT-H2 ($p=0.004$) and NOSTRAT-H2

**Figure 3.15**
Biomass development of calanoid nauplii in the 6 mesocosms. WATBOT = bottom layer of water column; WATMID = mid depth of water column; WATTOP = top layer of water column.




(p=0.021), and STRAT-H1 had higher biomass than STRAT-H2 (p=0.029) (one-way ANOVA). Differences in average biomass (averaged over the whole experimental period, see Table 3.5) between treatments were not significant however (ANOVA, p= 0.42).

**Figure 3.16**
Biomass development of calanoid copepodites and adults in the 6 mesocosms. WATBOT = bottom layer of water column; WATMID = mid depth of water column; WATTOP = top layer of water column.

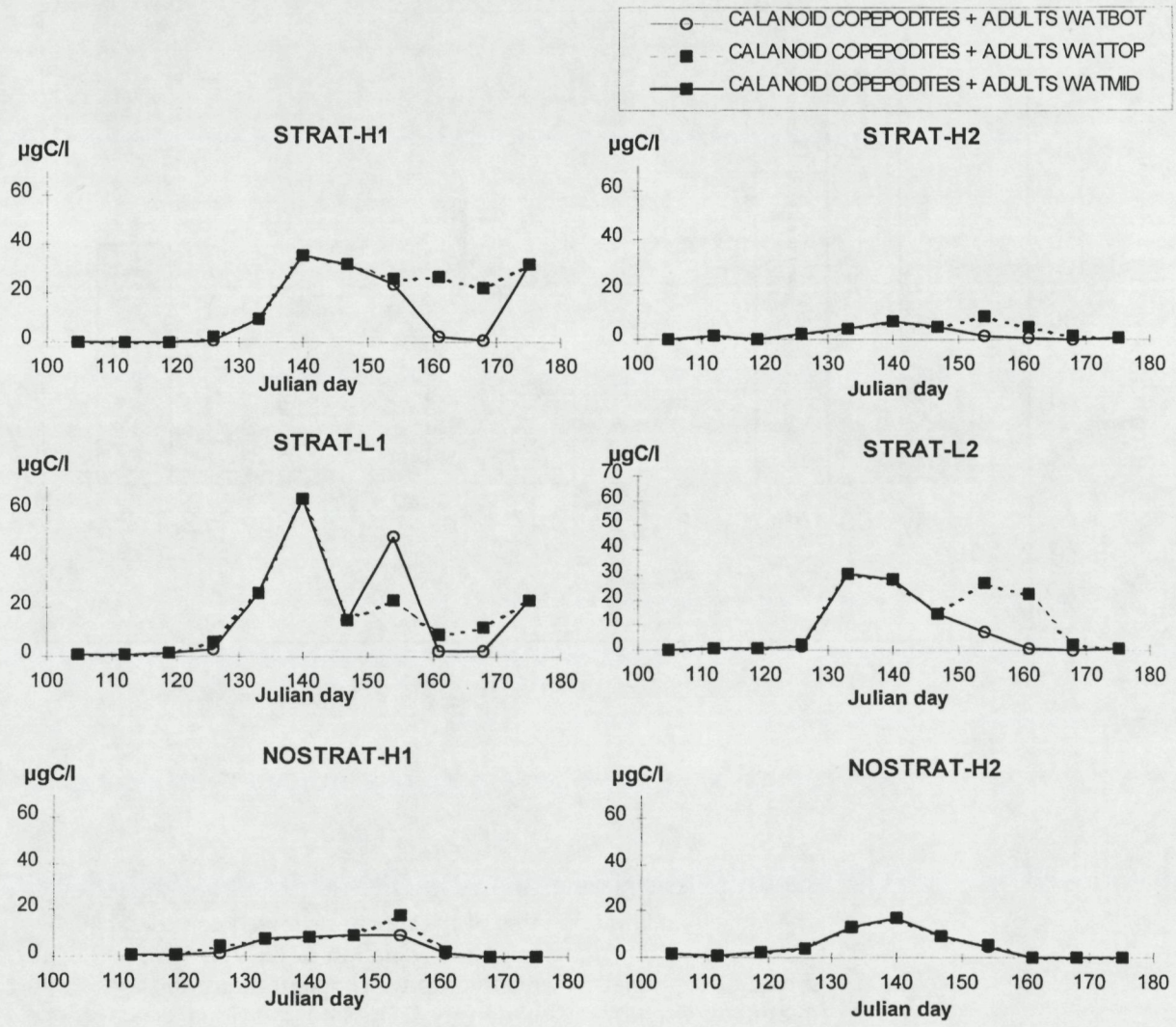
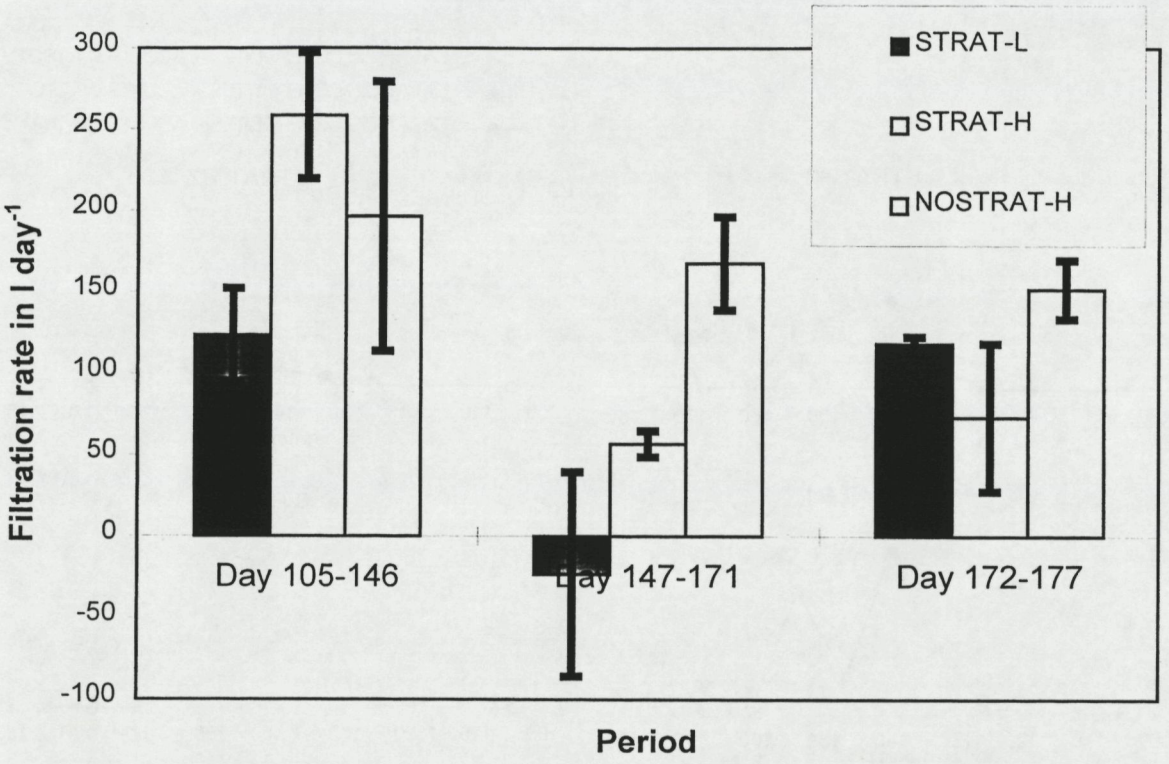


**Figure 3.17.**
Filtration rates of the mussels during the pre-stratification period, the stratification period and the period after stratification. The bars show the average for each treatment (n=2), the error bars show the standard error.




### 3.3.6 Mussel grazing

The individual filtration rates of the mussels showed considerable variation over time (coefficients of variation 67-200%). This variation showed no correlation with chlorophyll concentrations. In the treatment with low mussel density (STRAT-L), individual clearance rates of the mussels were twice as high as in the other two treatments (ANOVA, $p<0.001$). Due to the much higher mussel density, total grazing rates of the mussels in the treatments with high mussel biomass were significantly higher than in STRAT-L (ANOVA, $p<0.001$). The difference in filtration rates between low and high mussel biomass was, on average, a factor 2.5.

During the stratification period, clearance rates decreased considerably in the mesocosms with stratification, whereas mussels in the mixed mesocosms maintained clearance rates at the same levels (Figure 3.17). During this period, oxygen concentrations in the stratified systems dropped to values of less than 5 mg $l^{-1}$, and reached minimum values of less than 3 mg $l^{-1}$, while the mixed systems had minimum concentrations of 6.5 mg $l^{-1}$. Chlorophyll-a concentrations in the bottom mixed layer were low as well, but concentrations were low in the both the stratified and mixed mesocosms with high mussel biomass, and slightly higher in the stratified mesocosms with low mussel biomass.

**Table 3.5.**
Mussel shell length and ash-free dry weights at the start and the end of the experiment

| Treatment | # | Shell length in mm (mean ± s.e.) | ADW in g (mean ± s.e.) | n |
|---|---|---|---|---|
| start | | 23.0 ± 0.35 | 0.146 ± 0.006 | 30 |
| STRAT-L | 2 | 35.1 ± 0.61 | 0.307 ± 0.011 | 12 |
| | 4 | 33.3 ± 1.81 | 0.396 ± 0.054 | 12 |
| STRAT-H | 1 | 32.6 ± 0.42 | 0.239 ± 0.009 | 60 |
| | 6 | 32.1 ± 0.44 | 0.233 ± 0.010 | 60 |
| NOSTRAT-H | 3 | 31.1 ± 0.45 | 0.232 ± 0.013 | 60 |
| | 5 | 31.1 ± 0.43 | 0.224 ± 0.010 | 60 |

### 3.3.7 Mussel growth rates

At the start of the experiment mussels with a shell length between 20 and 25 mm were selected (mean ± s.e.: 23 ± 0.35, n=30). The ash-free dry weight of the mussels was 0.146 ± 0.006 g. The instantaneous daily growth rates, based on ADW, varied between 0.006 and 0.014. Mussel growth rates were highest in the treatment with low mussel biomass, and at the end of the experiment individual weights in treatment STRAT-L were significantly higher than in the other two treatments (ANOVA followed by LSD-test, $p<0.001$; Table 3.5). Shell lengths were significantly different between all treatments (ANOVA followed by LSD-test, $p<0.10$), with highest shell length in STRAT-L and lowest values in NOSTRAT-H.

## 3.4 DISCUSSION

In this experiment, the response of the pelagic system to benthic grazing under different hydrodynamic conditions was studied. Earlier experiments have shown that mussel grazing can efficiently control phytoplankton biomass irrespective of the level of external nutrient loading (Prins et al., 1995a). In this experiment, nutrient loadings were similar in all treatments, and it was to be expected that the different hydrodynamic conditions would affect the relationship between phytoplankton biomass and mussel grazing. In the continuously mixed mesocosms, grazing control by the mussels would be effective during the entire experiment and consequently phytoplankton biomass would remain relatively low. In the systems with periodic stratification, the upper mixed layer would be uncoupled from the benthos during the period of stratification. This release of the pelagic system from benthic grazing could be expected to result in elevated phytoplankton biomass, provided that other factors (lights, nutrients) were not limiting. The effect of differences in mussel biomass on phytoplankton development could be expected to be most apparent during the periods that the water column was well-mixed and differences in grazing pressure would be affecting the entire water column. In addition, the effects of stratification in itself will be better light conditions for the phytoplankton in the upper mixed layer, but potentially less favourable nutrient conditions as the upper mixed layer is uncoupled from regeneration processes in the benthic system.

### 3.4.1 Mussel activity

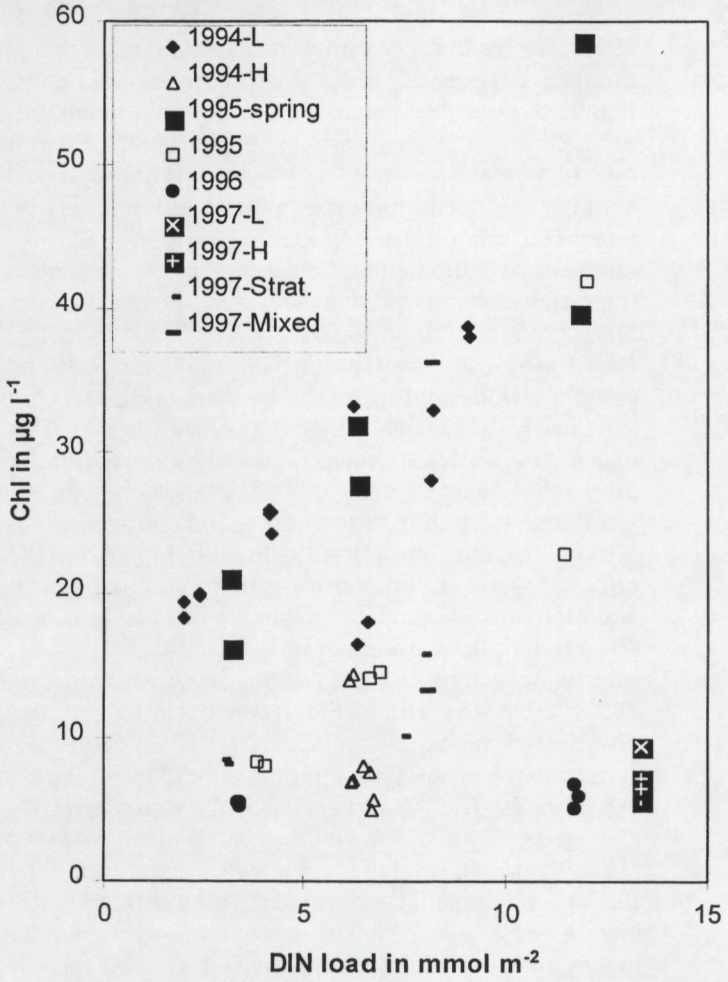
The difference in mussel biomass between the low and high mussel biomass treatments was a factor 5 at the start of the experiment. Due to differences in growth rate this was reduced to slightly more than a factor 3 at the end of the experiment. However, mussel biomass in the high mussel biomass treatments increased from 3 to >4.5 g ADW $m^{-3}$ (Table 3.5), which is high enough for an efficient control of

phytoplankton biomass (Heip et al., 1995; Dame, 1996). In the low mussel biomass treatment, biomass increased from 0.6 to 1.2 g ADW $m^{-3}$, which should allow for some control of phytoplankton biomass at the end of the experiment.
The differences in mussel biomass were only partly reflected in measured filtration rates (Figure 3.17). Most apparent was the decline in filtration rates in the mesocosms after the induction of stratification, while filtration rates stayed more or less constant in the well-mixed system. This reduction in the filtration rates was probably due to reduced oxygen concentrations. During the five days after the induced collapse of the stratification there seemed to be no recovery of filtration activity in STRAT-H, whereas filtration rates increased in STRAT-L. Overall, mussel filtration was higher in the treatments with high mussel biomass than in STRAT-L. There was considerable variation over time, and due to higher growth rates and higher individual clearance rates in STRAT-L, the difference in filtration rates between low and high mussel biomass was much smaller (approximately a factor 2.5) than the initial difference in biomass.

### 3.4.2 Plankton response

Chlorophyll-a concentrations were high in the water that was incubated in the mesocosms. Values dropped very rapidly during the first days of the experiment, and then a bloom occurred after three weeks. At this point, differences between treatments were only minor, and the average chlorophyll-a concentrations over the period before stratification were not significantly different between treatments. During stratification, small blooms occurred in the upper mixed layer of the stratified mesocosms, with maximum levels up to 30 µg $l^{-1}$. Concentrations in the mixed mesocosms remained much lower, but variation within treatments in the stratified mesocosms was very high in comparison to variation between treatments. Overall, differences in phytoplankton biomass and production between stratified and mixed mesocosms, and between the low and high mussel biomass treatments were fairly low and not statistically meaningful. Although in mesocosms with a low mussel grazing pressure the average microzooplankton biomass values were higher than in the mesocosms with a higher mussel grazing pressure, these differences in average biomass were not significant.
No significant differences in microzooplankton biomass were found between the Bottom and Top samples. Also during the 1996 expriment, microzooplankton biomass values for Bottom and Top samples were comparable (Escaravage et al., 1997). Similarly, for the mesozooplankton (calanoid copepods) biomass no significant differences between Bottom and Top samples were found. From literature data an uneven vertical distribution of zooplankton is known. Observations during stratification at the Oyster Grounds indicated that microzooplankton (mainly ciliates) were much more abundant in surface water than in samples from the thermocline and bottom waters (TRIPOS, 1997). There are also data showing an uneven distribution during stratification of ciliates and heterotrophic dinoflagellates (Sleigh et al., 1996) and of invertebrate larvae (Krause & Trahms, 1982). At the Oyster Ground area it was found that the distribution of calanoid copepods most resembled the distribution of chlorophyll, suggesting that the copepods tend to concentrate at the highest food density (Fransz et al., 1984). Thus, the distribution of micro- and mesozooplankton appears to be not only related to the vertical stratification of the water column, but also to the abundance of

**Figure 3.18**
Average chlorophyll-a concentrations in relation to DIN loading in mesocosm experiments carried out in 1994-1997. Experiments in 1994 were 4-week experiments at various times between March and September; "1994-L" are experiments with low mussel biomass (< 2 g ADW $m^{-3}$); "1994-H" are experiments with high mussel biomass (>2 g ADW $m^{-3}$). "1995-spring" indicates average values of the 1995 experiment for the same period as in the present study. "1995" indicates average values for the entire 1995 experiment (March-September). "1996" indicates average values for the 1996 stratification experiment (mussel biomass >2 g ADW $m^{-3}$). "1997-L" indicates average values for the mesocosms with low mussel biomass in the present experiment "1997-H" indicates average values for the mesocosms with high mussel biomass.



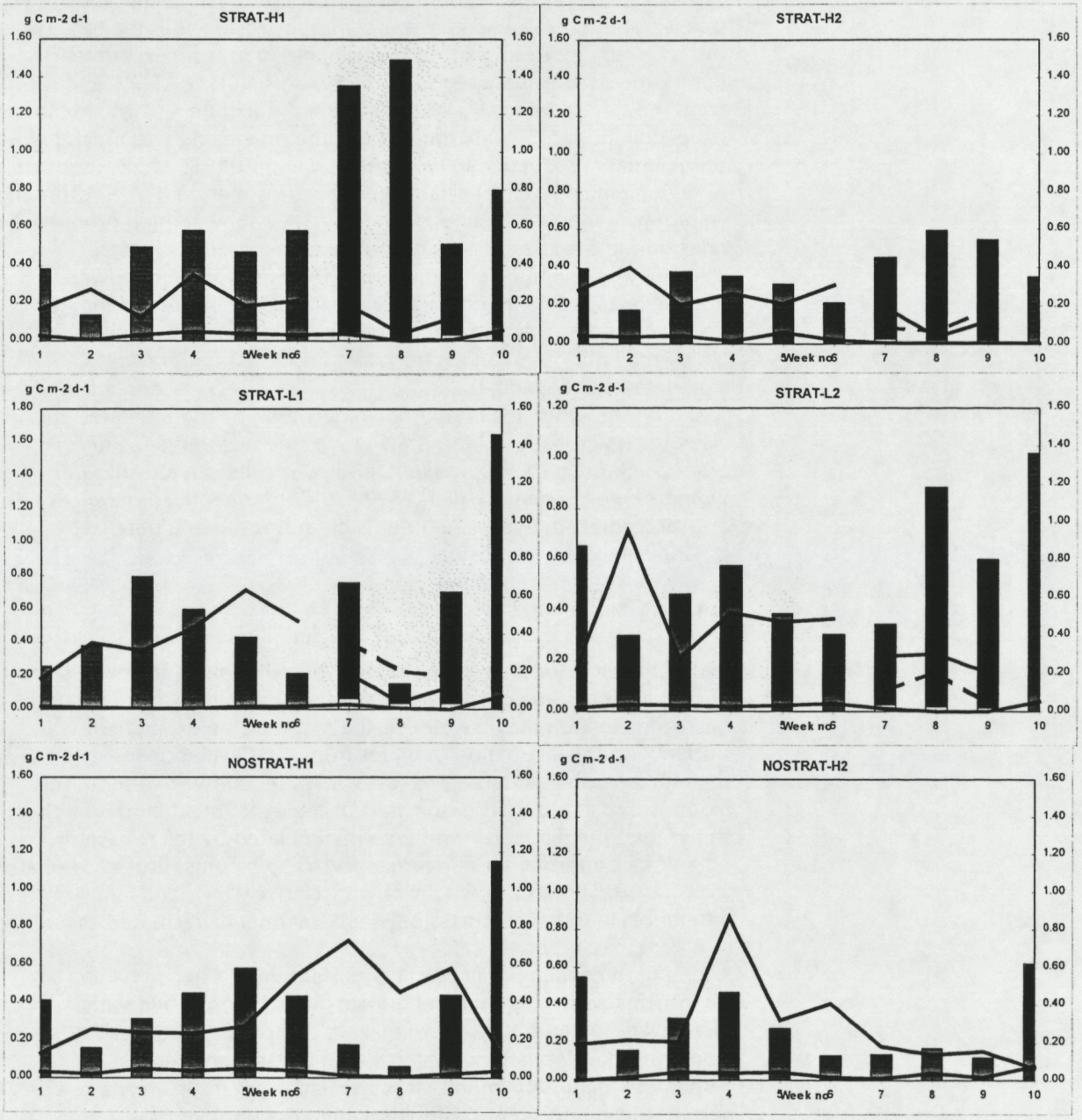

phytoplankton in the different water layers of the stratified water column. Although there were differences between chlorophyll-a values in the Bottom and Top compartments of the stratified mesocosms in this study (Table 3.2), these differences were not mirrored in vertical zooplankton biomass distribution, probably because of the small vertical distance.

### 3.4.2.1 Lack of treatment effects

The question remains why there was no clear response of the phytoplankton to the experimental treatments. Nutrient limitation was only apparent for silicate for some days in the experiment, but was absent during most of the period. The differences between treatments in mussel grazing rates did not result in clear differences in phytoplankton development, suggesting that bivalve grazing was not

acting as a very dominant limiting factor either. Regarding the entire experimental period, there was little variation in chlorophyll-a values, with the exception of the chlorophyll-a values during the second stratification period. This suggests more or less an equilibrium between phytoplankton production and loss terms during a great part of the experimental period.
A comparison of the yield of phytoplankton biomass and of primary production in this experiment with the results of other mesocosm experiments (Peeters et al., 1995; Escaravage et al., 1997; Smaal et al., 1997), shows that both phytoplankton biomass and phytoplankton production were very low in the present experiment (Figure 3.18). The figures show a clear linear response of phytoplankton biomass and production in the short term experiments of 1994 in those cases where mussel biomass was low. With higher mussel biomass, bivalve grazing controls phytoplankton development and reduces phytoplankton biomass. During the spring bloom period in 1995 (for comparative purposes here defined as April-June), levels were more or less similar to the observations in 1994. Lower levels were observed when the entire 1995 experiment is considered, which is due to lower productivity in early spring and late summer. The results for 1996 show very low values for both phytoplankton biomass and production; this was at least partly due to relatively high mussel biomass. The present experiment shows anomalously low phytoplankton biomass and production when compared to these earlier results. This is true for both (temporarily) stratified mesocosms and continuously well-mixed mesocosms, indicating that the low phytoplankton yield was not an effect of reduced turbulence. As there was no difference between low and high mussel biomass treatments, bivalve grazing control did not seem to be the factor either.
Interestingly, turnover rates of the phytoplankton were very high in 1997 (Table 3.3). The P/B ratios were even higher than values observed in the experiments in 1994, when values were high as a consequence of the typical bloom conditions in these short term experiments. The filtration rates by the mussels were relatively low (on average only 5% of the volume $day^{-1}$), meaning that phytoplankton mortality due to bivalve grazing was insignificant when compared to the high phytoplankton turnover. This indicated that there must have been a major loss term causing phytoplankton biomass to remain as low as observed. This loss term overshadowed the effects of mussel grazing.
Zooplankton grazing is a likely explanation for the rather constant phytoplankton loss term. In a number of mesocosms microzooplankton developed slower than usually observed during previous mesocosm experiments. Nevertheless, microzooplankton biomass was not low and similar to more productive waters (see Table 4.3 in Smaal et al., 1997). During the first three weeks of the experiment a large biomass of cirriped larvae was present in all mesocosms (Figure 3.14), and food competition between the cirripeds and microzooplankton may have caused the delayed development of microzooplankton. During the second half of the experiment microzooplankton biomass in most of the mesocosms was somewhat higher than during the first weeks of the experiment, together with an increased copepod biomass. This increase in microzooplankton and copepod biomass compensated for the disappearance of cirriped larvae, and may have resulted in a more or less constant grazing pressure during the entire experimental period.

**Figure 3.19**
A comparison of production and loss rates of the phytoplankton. The vertical bars show the weekly average phytoplankton primary production. The full line shows the estimated microzooplankton grazing in the entire water column or in the top layer during stratification, the broken line line shows the estimated microzooplankton grazing in the bottom layer during stratification. The dotted line shows the mussel filtration rate. See text for explanation of estimates.




A quantitative estimate of microzooplankton grazing rates on the phytoplankton was made from the microscopic estimates of microzooplankton biomass. It was assumed that microzooplankton daily ingestion was equal to their body carbon content (Paffenhöfer, 1998). Mussel consumption rates were calculated from measured clearance rates and water column concentrations of organic carbon. The results showed that microzooplankton consumption was much higher than grazing by the mussels, and in most instances microzooplankton grazing more or less equalled the rates of primary production (Figure 3.19). A closer comparison showed a rather good agreement between periods with production exceeding grazing and

observed peaks in chlorophyll-a levels, and between periods with relatively high microzooplankton grazing and low chlorophyll-a levels: In STRAT-H1 primary production was much higher than grazing in the stratification period (week 7-9, days 146-168) and this resulted in a peak in chlorophyll-a up to 25 $\mu g\ l^{-1}$ in the top layer (Fig. 3.19). In STRAT-H2 the difference between primary production and grazing was smaller, and this resulted in lower chlorophyll-a levels in the top layer (up to 10 $\mu g\ l^{-1}$). Results of microzooplankton grazing experiments during the stratification period in mesocosms NOSTRAT-H1 and -H2 confirmed these trends in phytoplankton production and grazing losses (Appendix I). In STRAT-L1 microzooplankton grazing was much higher than primary production in weeks 6-9 (day 139-168) as a consequence of high biomass levels of ciliates and *Oxyrrhis marina* (Fig. 3-13b), and chlorophyll-a levels remained low. STRAT-L2 showed high production rates during the last 4 weeks of the experiment and increased chlorophyll-a levels. Finally, both NOSTRAT mesocosms showed grazing rates that were equal to primary production rates during the entire experiment, and low chlorophyll-a levels as well. Again, this concurred with observations from microzooplankton grazing experiments (Appendix I).
Based on the estimates of phytoplankton growth and loss rates, it is likely that microzooplankton grazing was the major loss factor for phytoplankton in this experiment, and controlled phytoplankton biomass development to a large extent. Consequently, differences in mussel biomass between treatments did not result in differences in phytoplankton standing stock.

### 3.4.3 Final remarks

In the present experiment, microzooplankton grazing was a major mortality factor for phytoplankton and overshadowed the effects of mussel grazing. One of the causes for the high activity of the microzooplankton may have been the fact that the experiment was started with the incubation of water from the Oosterschelde estuary, at the moment when a dense *Phaeocystis* sp. bloom was present. This bloom collapsed soon after the start of the experiment, and it has been shown that the microbial food web is stimulated by the release of organic carbon from declining *Phaeocystis* sp blooms (Brussaard et al., 1996). In addition, mussel activity was relatively low, and initial differences in mussel biomass between treatments decreased as a result of differences in growth rates.
A long term experiment is planned in 1998. In this experiment, mesocosms with a high mussel biomass will be contrasted with mesocosms without mussels, to make a comparison between planktonic development in systems with a dominant benthic suspension feeder community and the development in a system with pelagic consumers only. To enhance the development of mesozooplankton and prevent excessive microzooplankton growth, the mesocosms will be seeded with mesozoplankton. The experiment will last during the entire growing season (April-August), and stratification periods will be induced during spring and summer.

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# 4 Effects of water column structure and nutrient loading on plankton development

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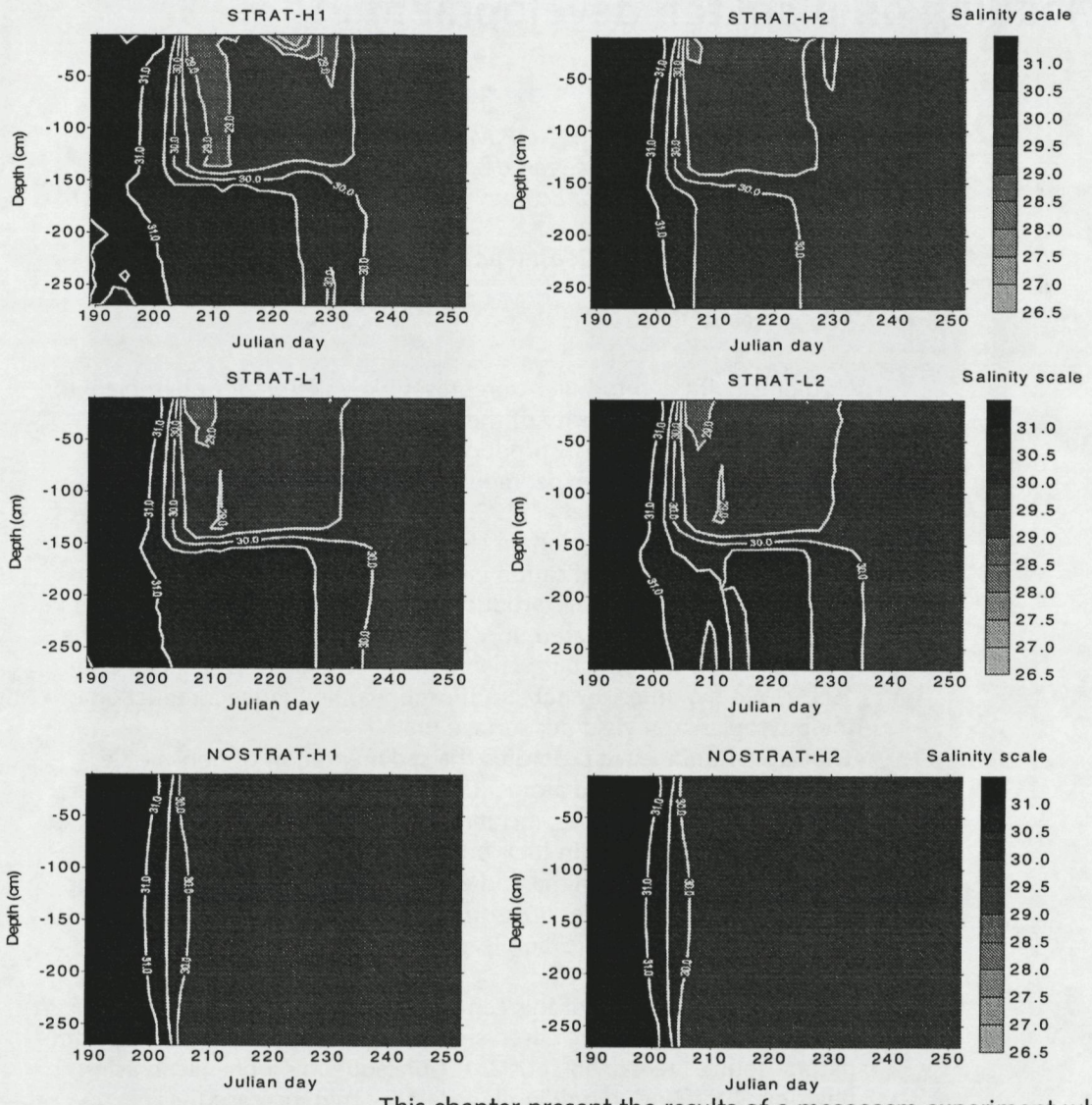
## 4.1 INTRODUCTION

Nutrients, light and turbulence represent key factors for phytoplankton development. The intensity of the response of phytoplankton to nutrient loading (biomass, production increase with N or P-load increase) in mesocosm experiments was closely coupled to the light availability (Escaravage *et al.*,1995a; Escaravage *et al.*, 1996). Lack of turbulence could, under certain circumstances, favour the establishment of a stratification (saline and/or thermal). In stratified areas, most of the primary production and related nutrient uptake take place in the well illuminated upper layer whereas nutrients below the pycnocline are not directly available for phytoplankton (Peeters *et al.*, 1995). We hypothesize that stratification could induce a reduction of the phytoplankton yield per surface unit.
The lack of turbulence promotes the sedimentation of non-motile phytoplankton (mainly diatoms) (Peeters & Peperzak, 1990) whereas actively swimming species, including toxic dinoflagellates (Peperzak *et al.*, 1995), could maintain their position in the upper and well illuminated layer by daytime and eventually migrate overnight to the nutrient rich bottom layer (Olsson & Granéli, 1991). An additional hypothesis is that stratification leads to a competitive advantage of flagellates over diatoms.
During the second half of this century, anthropogenic loadings of N and P to the sea have increased significantly whereas Si- loading from natural sources remained constant. Consequently, non-silicon using phytoplankton (including dinoflagellates) could increase their concentration and bloom duration whereas the development of silicon using phytoplankton (diatoms) was limited by Si-availability (Van Bennekom *et al.*, 1975; Officer & Ryther, 1980; Radach & Berg, 1986; Smayda, 1990; Escaravage *et al.*, 1995a). The sustained high anthropogenic nitrogen loading to the North Sea in the 1990's has led to an increase of the N/P ratio in the coastal waters that may favour the production of noxious substances by potentially harmful algal species (Peperzak, 1994). It was suggested that the co-occurrence of stratification and eutrophication could favour the development of potentially toxic dinoflagellate blooms (Margalef, 1978; Taylor & Pollingher, 1987). A third hypothesis in this study was that increased N-loadings may favour the appearance of potentially toxic dinoflagellate blooms in stratified areas.
A mesocosm experiment was set up to test the hypotheses formulated above:
1.- Stratification induces a reduction of the phytoplankton yield.
2.- Stratification favours flagellates over diatoms.
3.- Increased N-loadings favour the appearance of potentially toxic dinoflagellate blooms in stratified areas.

**Figure 4.1**
Vertical distribution of salinity in the six mesocosms.



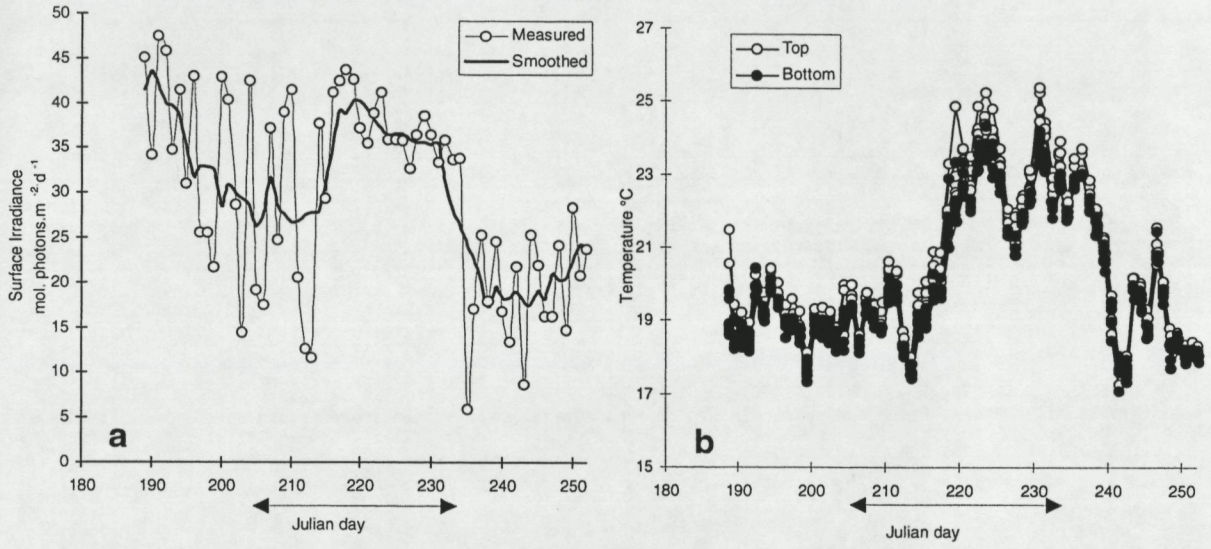

This chapter present the results of a mesocosm experiment where three treatments were applied (replicated) to six mesocosms, combining two nitrogen loadings (High/Low) with two mixing regimes (Stratified/Mixed). The dinoflagellate *Prorocentrum micans*, that is often found in considerable concentrations during blooms of the DSP producing dinoflagellate *Dinophysis acuminata* (Peperzak *et al.*, 1996), was added to the mesocosm water and served as a "model species".

## 4.2 MATERIAL AND METHODS

### 4.2.1 Mixing regime

*Day 189:* the six mesocosms were filled with Oosterschelde water and continuous flushing with nutrients-enriched sea water was started.
*Day 204:* saline stratification was established in the STRAT-mesocosms whereas the NOSTRAT-mesocosms were kept well mixed. Top compartment was flushed with low salinity seawater (ca 27 ‰) whereas the Bottom compartment was flushed with high salinity seawater (*ca* 37 ‰).
*Day 234:* A high mixing rate was restored in STRAT-mesocosms until the end of experiment (day 252).

**Figure 4.2**
a.-Daily surface irradiance (PAR, mol photons.m$^{-2}$.d$^{-1}$) measured under the optical diffuser on top of the mesocosms. Daily measurements were smoothed (weighed moving average, 15 days period) to underline the main trends.b.-Daily average temperature measured in the continuous measuring unit connected to the mesocosms. Stratification period is indicated below the X-axis (↔).




Further details about the experimental set-up and the standard analytical procedures are extensively described in Chapter 2.

## 4.3 RESULTS

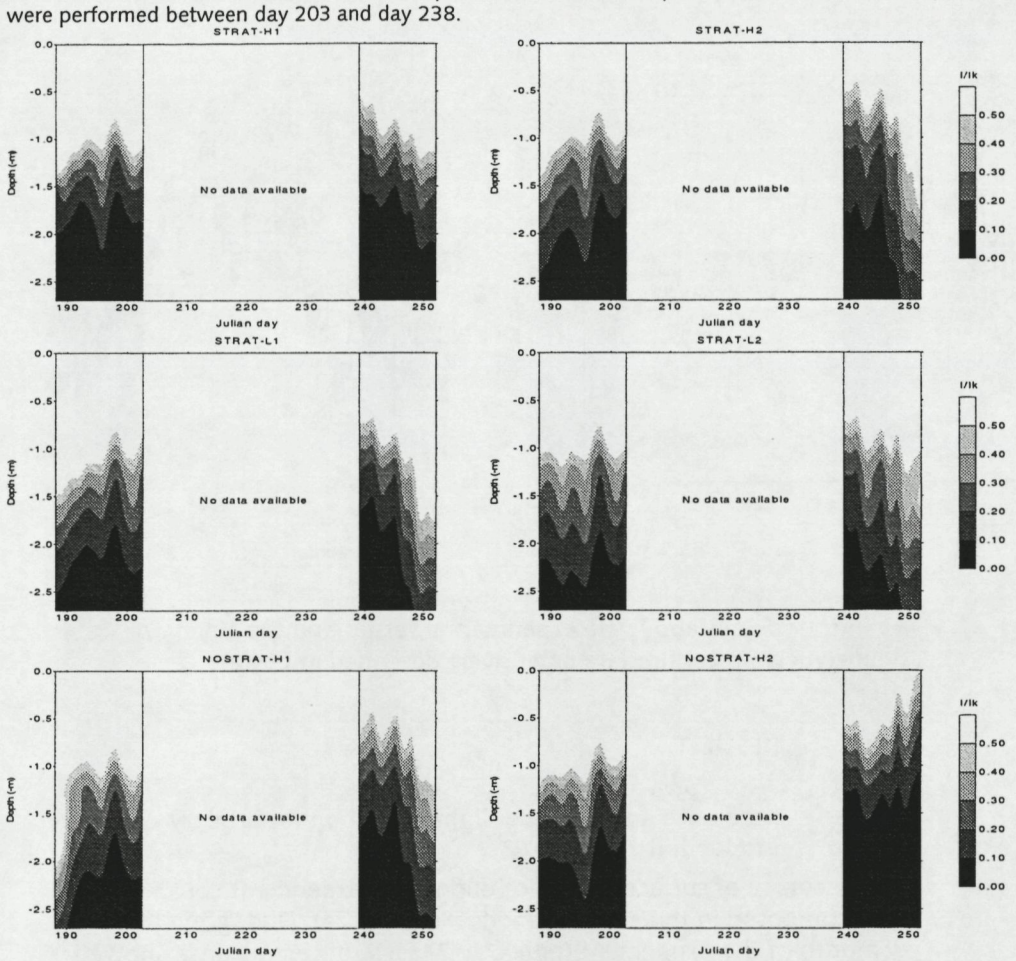
### 4.3.1 Irradiance and potential light limitation for primary production

The course of surface irradiance during this experiment could be decomposed in three main periods (Figure 4.2a). During the first month of the experiment (until Day 215), surface irradiance showed large fluctuations but followed a pronounced declining trend. The following two weeks (until Day 234), daily variations were smaller and surface irradiance was relatively high. A marked drop in surface irradiance occurred on Day 235 and initiated a period of variable and relatively low irradiance.
Variations in surface irradiance were not clearly found back in the course of the index of light limitation (Figure 4.3). Each mesocosm showed a specific light climate mainly dependent on its $K_d$ and on the photosynthetic characteristics of its phytoplankton. All mesocosms had in common that severe light limitation ($I/I_k<0.1$) was restricted to the Bottom compartment whereas the Top compartment showed no or hardly any light limitation.

### 4.3.2 Temperature

The mesocosm water temperature reproduced the trends followed by the surface irradiance (Figure 4.2b). Temperatures were relatively low during the first month of experiment and increased after day 215. The highest temperature was reached during the second week of stratification (before day 225). A sharp decrease before day 230 was followed by a new increase and relatively high temperatures for about 10 days. After day 240, temperature decreased again.

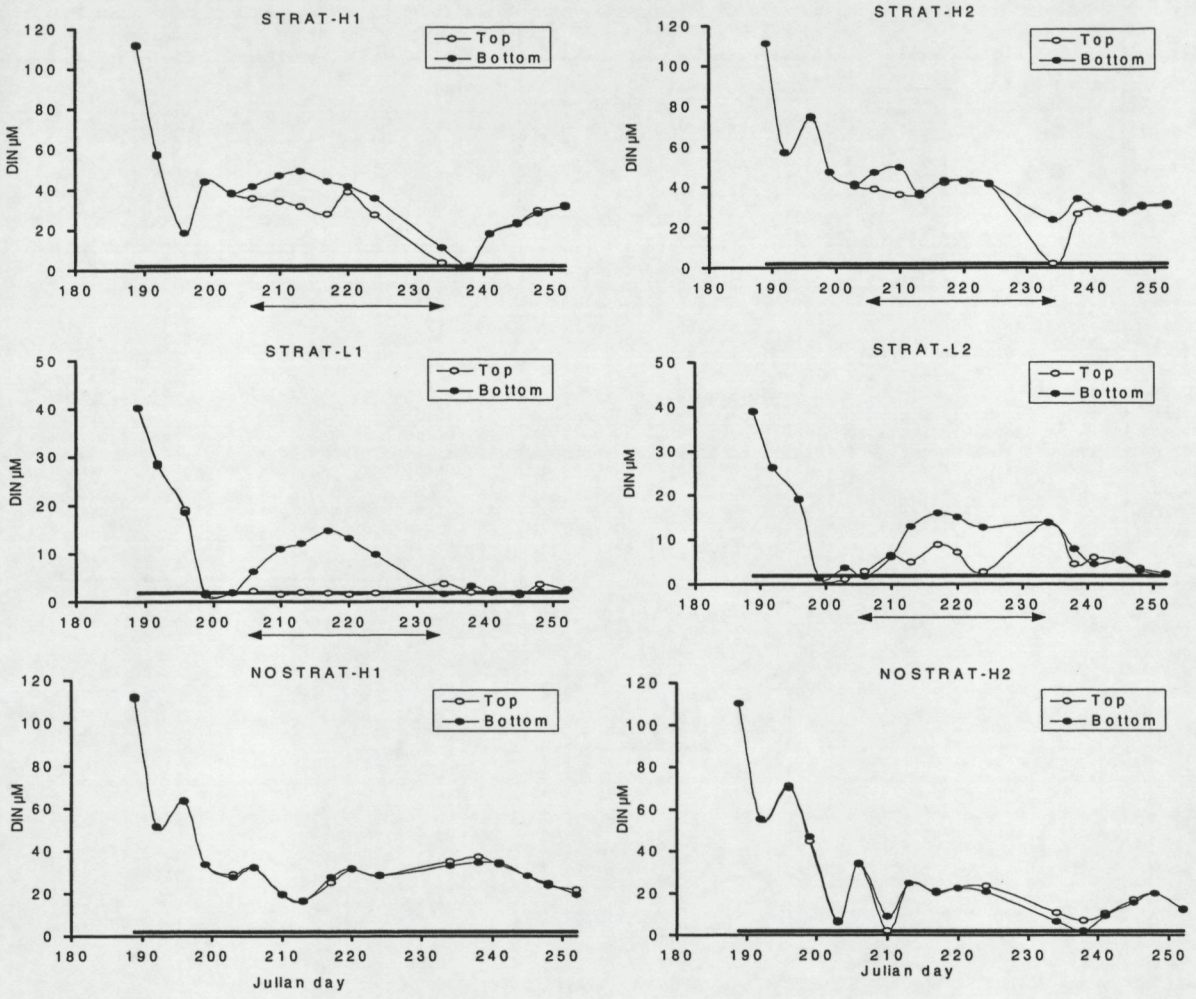
**Figure 4.3**
Vertical distribution of the light limitation index ($I/I_k$) over the mesocosm water column during the present experiment. I: mean daily irradiance, $I_k$: photosynthetic characteristic irradiance ($I_k = P_{max}/a$, $P_{max}$ highest point and a slope of the P/I curves obtained by the $^{14}C$ incubations). When $I/I_k$ is between 0.1 and 0.5, light limitation is considered to be moderate; when $I/I_k$<0.1, light limitation is considered to be severe (See Chapter 2.2.6 for further explanation). Due to technical problems, no $^{14}C$ incubations were performed between day 203 and day 238.




### 4.3.3 Dissolved nutrient concentrations and potential limitation for primary production

The initial inorganic nutrient concentrations were set to the steady state values that were an equilibrium between the continuous nutrient addition and the water renewal rate of the mesocosms. As a result, initial DIN concentrations were lower in mesocosms with the low nitrogen additions (STRAT-L1 and STRAT-L2). DIN, DIP and Silicate concentrations showed a sharp decrease in all mesocosms during the first ten days of the experiment (*ca* until day 200). After this date, concentrations evolved differently in the various treatments.

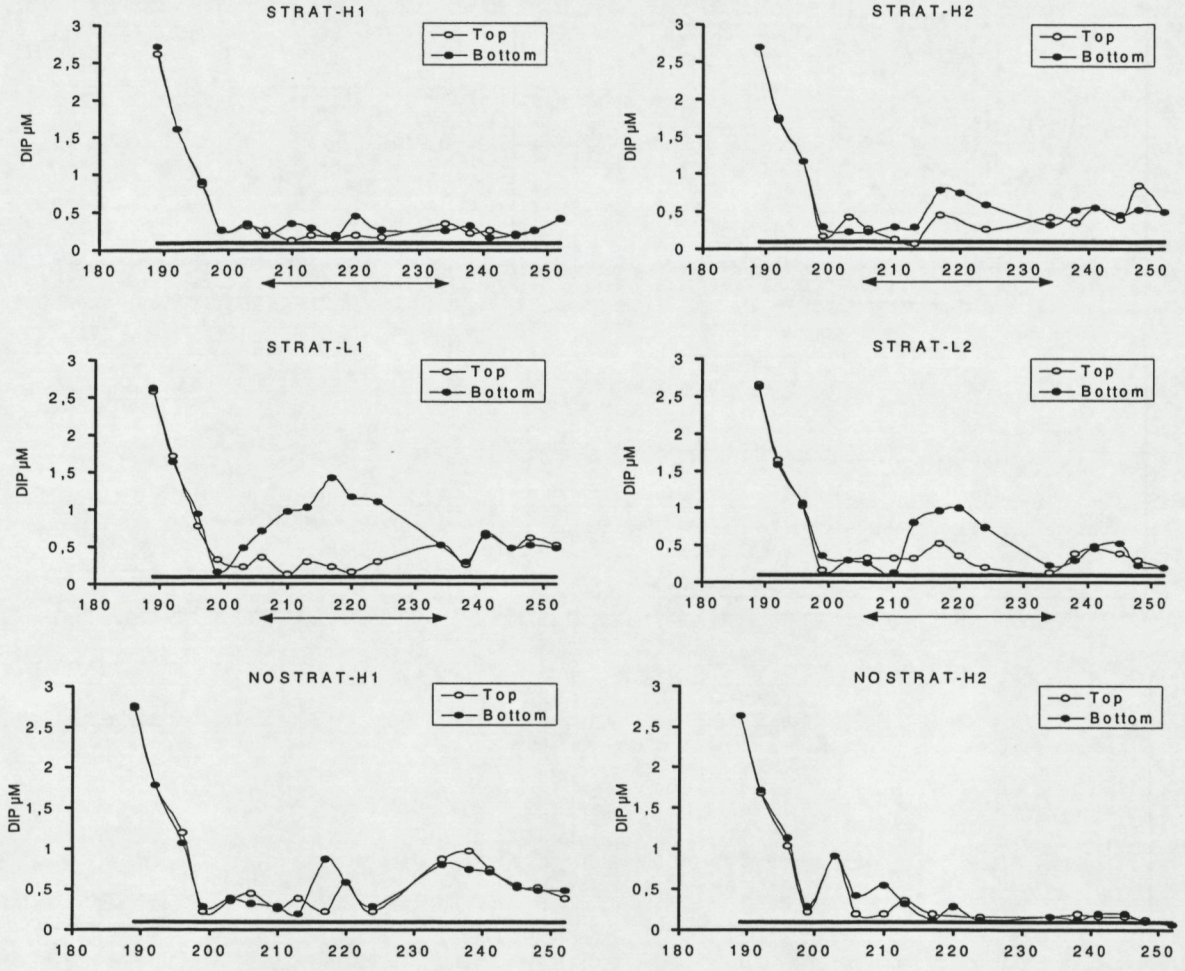
**Figure 4.4**
DIN concentrations in the six mesocosms in the upper layer (Top) and in the lower layer (Bottom). When concentrations decrease below 2 µM (level line plotted on graphs), DIN becomes potentially limiting for the primary production (Ks value from literature sources, see Chapter 2). Stratification period is indicated below the X-axis (↔)




### 4.3.3.1 DIN concentrations

DIN concentrations in the Top and Bottom compartments of the non-stratified mesocosms (NOSTRAT) did not significantly differ and were mostly far above 2 µM (with exception of day 210 and 238 in NOSTRAT-H2) (Figure 4.4). After day 200, DIN concentrations were generally lower in NOSTRAT-H2 than in NOSTRAT-H1.
As a consequence of the treatment used, DIN concentrations were higher in mesocosms STRAT-H than in mesocosms STRAT-L. During the stratification period, the DIN concentrations were generally lower in the Top compartments than in the Bottom compartments of the STRAT mesocosms. This difference between both compartments was more pronounced in the mesocosms with low nitrogen loading. In the latter mesocosms, DIN concentrations remained lower (below or near 2µM) in STRAT-L1 than in STRAT-L2. In the mesocosms with high nitrogen loading, nitrogen never became potentially limiting for primary production during the stratification period. After stratification was ended, DIN concentrations in the Top and Bottom compartments of the STRAT mesocosms reached similar levels again and remained higher in mesocosms with a high nitrogen loading.

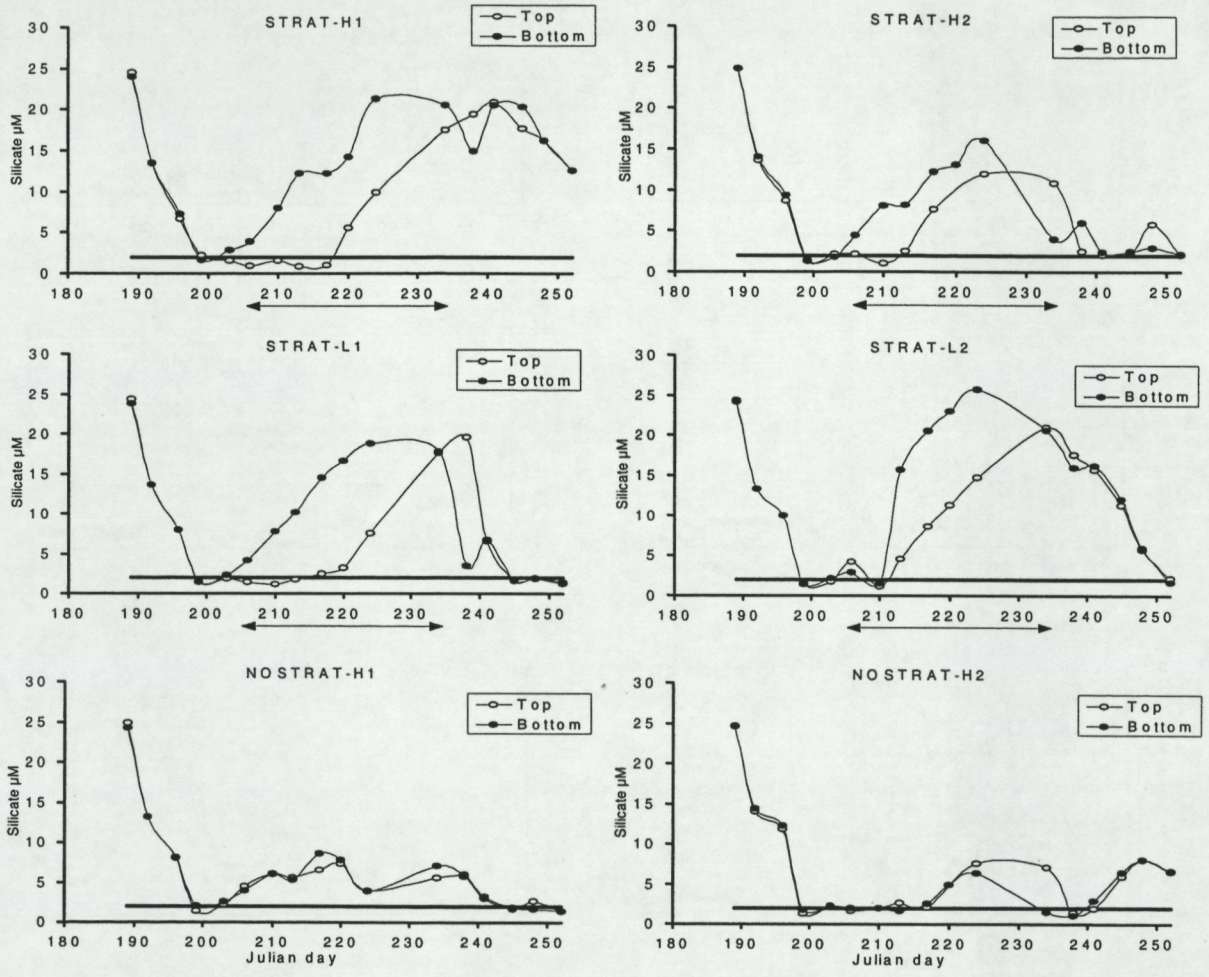
**Figure 4.5**
DIP concentrations in the six mesocosms in the upper layer (Top) and in the lower layer (Bottom). When concentrations decrease below 0.1 µM (level line plotted on graphs), DIP becomes potentially limiting for the primary production (Ks value from literature sources, see Chapter 2). Stratification period is indicated below the X-axis (↔).




### 4.3.3.2 DIP concentrations

No systematic differences in DIP concentrations were observed between the Top and the Bottom compartments of the non-stratified mesocosms (Figure 4.5). As for DIN concentrations, higher DIP concentrations were measured in mesocosm NOSTRAT-H1 than in mesocosm NOSTRAT-H2. DIP concentrations in NOSTRAT-H2 remained just above the threshold for potential phosphorus limitation throughout the second half of experiment.
During the stratification period, DIP concentrations were lower in the Bottom compartments of mesocosms STRAT-H than in mesocosms STRAT-L. DIP concentrations in Top compartments did not differ much among the stratified mesocosms and were mostly higher than 0.1 µM. The differences in DIP concentrations between the Top and Bottom compartments were higher in mesocosms STRAT-L than in mesocosms STRAT-H. DIP concentrations were also higher in the Bottom compartment of mesocosm STRAT-L1 than in mesocosm STRAT-L2.

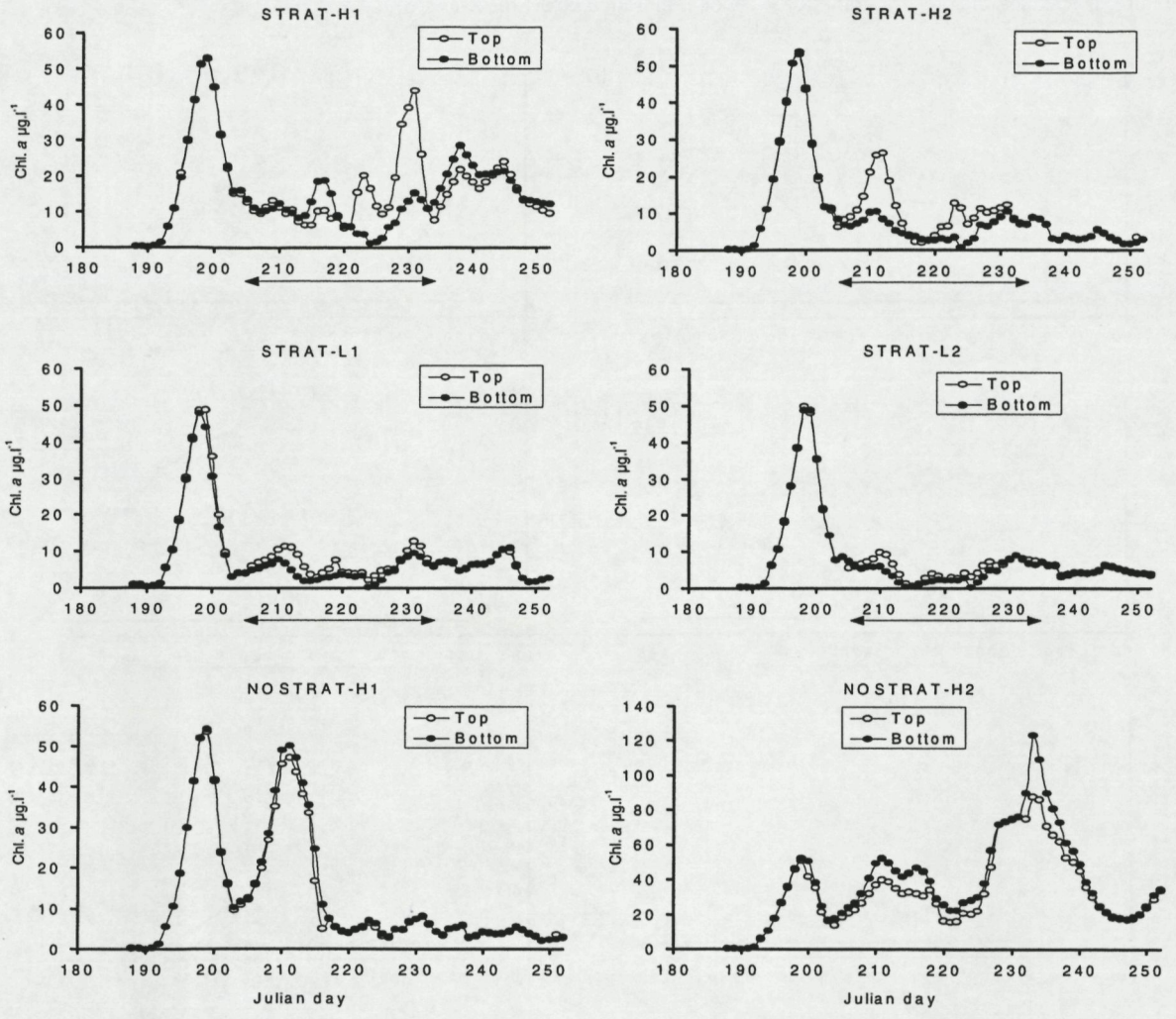
**Figure 4.6**
Silicate concentrations in the six mesocosms in the upper layer (Top) and in the lower layer (Bottom). When concentrations decrease below 2 µ(level line plotted on graphs), DIP becomes potentially limiting for the primary production (Ks value from literature sources, see Chapter 2). Stratification period is indicated below the X-axis (↔).




### 4.3.3.3 Silicate concentrations

During the stratification period, Silicate concentrations reached higher values in the stratified than in the non-stratified mesocosms (Figure 4.6). The course and levels of silicate concentrations were similar in the four stratified mesocosms. After stratification had been installed, Silicate concentrations remained low (near or below 2 µM) in the Top compartments of all stratified mesocosms. This period of low Silicate concentrations in the Top compartment was more or less extended in the various mesocosms: until days 210, 213, 217 and 220 respectively in mesocosms STRAT-L2, STRAT-H2, STRAT-H1 and STRAT-L1. Silicate concentrations in the Bottom compartment began to increase in most of the stratified mesocosms after stratification was installed. After the end of stratification, Silicate concentrations in Top and Bottom decreased towards potentially limiting concentrations, except in mesocosm STRAT-H1 where silicate concentrations remained at levels comparable to those of the first week of experiment. Lower silicate concentrations (potentially limiting) were observed in NOSTRAT-H2 than in NOSTRAT-H1 between days 203 and 217 whereas just the opposite was observed between days 245 and the end of the experiment.

**Figure 4.7**
Chlorophyll-*a* concentrations in the Top and the Bottom compartments of the six mesocosms. Note the different scale used for mesocosm NOSTRAT-H2.




### 4.3.4 Phytoplankton biomass and production

For technical reasons, no $^{14}C$ incubations were performed between day 203 and day 238 (the whole stratification period). Therefore, primary production results will not be presented here.

#### 4.3.4.1 Chlorophyll-a concentrations

A similar chlorophyll-*a* peak (about 55 $\mu g.l^{-1}$) developed in all mesocosms between day 190 and 203 (Figure 4.7).
In the non stratified mesocosms, a second chlorophyll-*a* peak immediately followed this initial biomass development and culminated around 50 $\mu g.l^{-1}$ on day 210 in both mesocosms. No such a peak was observed in the stratified mesocosms where the average chlorophyll *a* concentrations was 15 $\mu g.l^{-1}$ on day 210. Afterwards, chlorophyll-*a* concentrations decreased in NOSTRAT-H1 and remained below 10 $\mu g.l^{-1}$ between day 219 and the end of the experiment. After day 214, chlorophyll-*a* concentrations in NOSTRAT-H2 differed radically from what occurred in NOSTRAT-H1. While the second chlorophyll-*a* concentrations peak declined in NOSTRAT-H1, chlorophyll-*a* concentrations increased again and remained high (above 60 $\mu g.l^{-1}$) in NOSTRAT-H2 between days 230 and 240. Another peculiarity in

mesocosm NOSTRAT-H2 were the systematically lower chlorophyll-*a* concentrations measured in the Top compartment compared to the Bottom compartment, whereas these values never differed that much in NOSTRAT-H1. Lower chlorophyll-*a* concentrations in the Top than in the Bottom compartment were also observed in mesocosm STRAT-H1 around days 215 and 240.

In the stratified mesocosms, the end of the first chlorophyll-*a* peak coincided with the establishment of the stratification. While chlorophyll-*a* concentrations below 10 µg $l^{-1}$ occurred in the STRAT-L mesocosms, they showed peaks between 25 and 40 µg.$l^{-1}$ in the STRAT-H mesocosms. After stratification was ended, the chlorophyll-*a* concentrations in the Top and Bottom compartments remained below 10 µg $l^{-1}$ until the end of experiment in all stratified mesocosms except STRAT-H1. In this latter mesocosm the chlorophyll-*a* concentrations increased again during the last mixed period and showed a peak between 20 and 30 µg $l^{-1}$ in both compartments.

No significant differences were found between the mesocosms during the first two weeks of the experiment (until day 203; ANOVA,$P>0.05$). During this period, nutrients remained above the theoretical limiting threshold for primary production (See Figures 4.4 to 4.6). As a result, phytoplankton was not controlled by the nutrient treatment and could grow identically in all mesocosms. Effects of the nutrient treatment on phytoplankton biomass were expected after this initial period (from day 204 on), when nutrient concentrations became potentially limiting for phytoplankton development. However, there was no significant difference between the chlorophyll-*a* concentrations measured in the Top compartments of treatments L and H (nested ANOVA $F_{ratio}=2.28$, $p=0.21$).

The effect of stratification on chlorophyll-*a* concentrations was studied by comparing concentrations measured in the Top and the Bottom compartments of the stratified mesocosms during the stratification period (days 204 to 234). Significant differences (one way ANOVA) were found in mesocosms STRAT-H1 ($p<0.05$), STRAT-H2 ($p<0.01$) and STRAT-L1 ($p<0.01$) but not in STRAT-L2 ($p>0.05$).

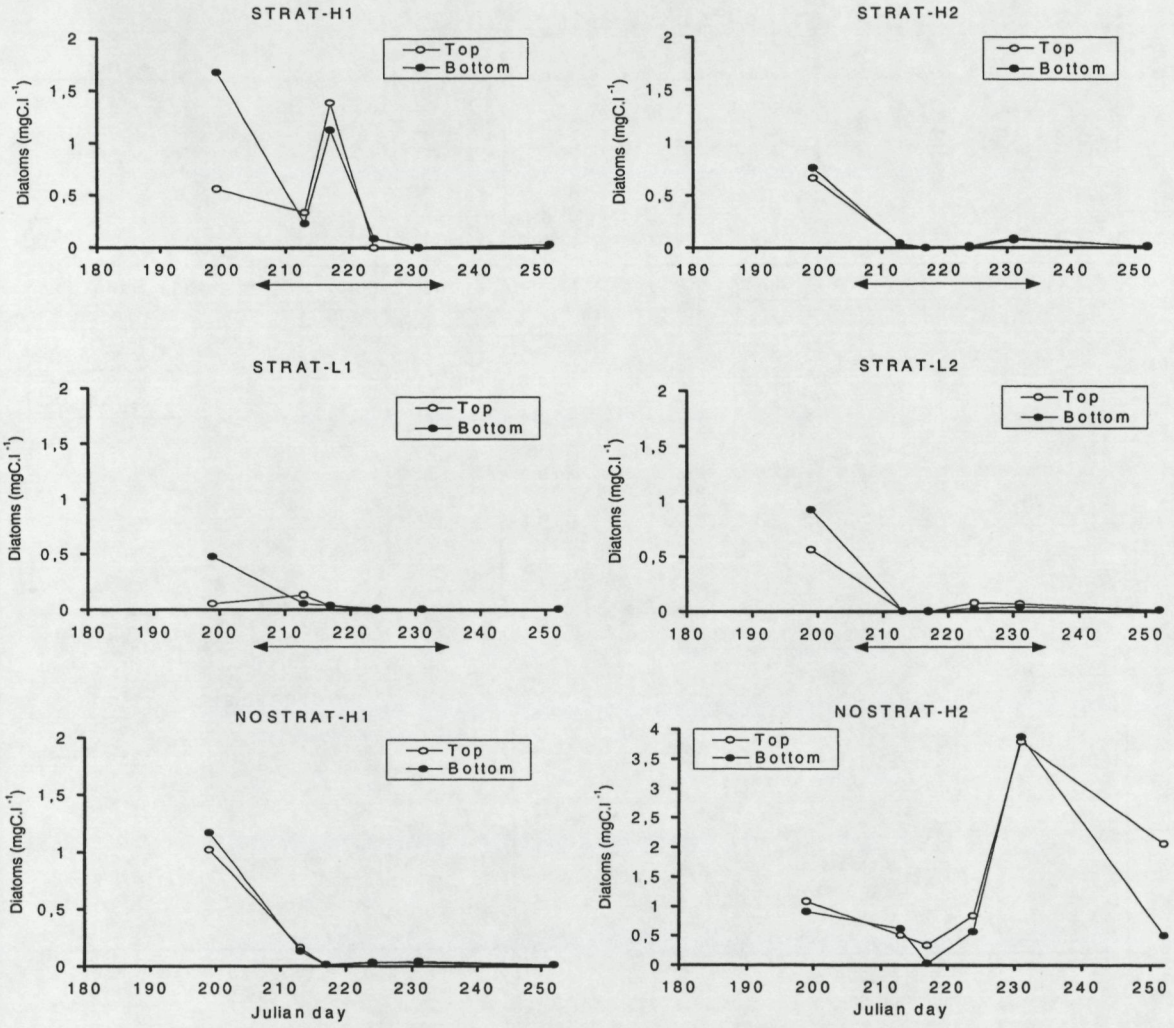
Due to the high heterogeneity between replicates, no significant differences were found between the chlorophyll-*a* concentrations measured in the STRAT and NOSTRAT treatments (nested ANOVA, $p>0.05$).

### 4.3.5 Phytoplankton species composition

Taxonomic entities encountered during the present experiment are listed below. Identification at species level was not always possible. In such case, phytoplankton cells were classified according to their size.

*Asterionella glacialis*
*Biddulphia aurita*
*Cerataulina bergonii*
*Skeletonema costatum*
*Chaetoceros* sp.
*Chaetoceros socialis*
*Chatonella* sp.
*Chrysochromulina* sp.
*Cryptophyceae* sp.
*Cyanophyceae*
*Eucampia zoodiacus*
*Eutreptiella* sp.
*Leptocylindrus danicus*
*Leptocylindrus minimus*
*Lycmophora* sp.
*Nanoflagellates*
*Nitzschia* sp.
*Nitzschia closterium*
*Nitzschia delicatissima*
*Nitzschia longissima*
*Nitzschia seriata*
*Pennate diatoms*
*Phaeocystis* sp.
*Picoflagellates <2µm*
*Plagiogramma brockmanni*
*Prorocentrum micans*
*Pyramimonas* sp.
*Rhizosolenia delicatula*
*Rhizosolenia fragilissima*
*Rhizosolenia pungens*
*Rhizosolenia setigera*
*Rhizosolenia shrubsolii*
*Skeletonema costatum*
*Striatella* sp.
*Tetraselmis marina*
*Thalassionema nitzschioides*
*Thalassiosira* sp.
*Thalassiosira decipiens*
*Thalassiosira fallax*
*Thalassiosira levanderi*
*Thalassiosira nordenskioldii*
*Thalassiosira rotula*

**Figure 4.8**
Total diatom biomass (mgC.l$^{-1}$) measured in the Top and Bottom compartment of the six mesocosms. Stratification period is indicated below the X-axis (↔). Note the different Y-scale used for NOSTRAT-H2.

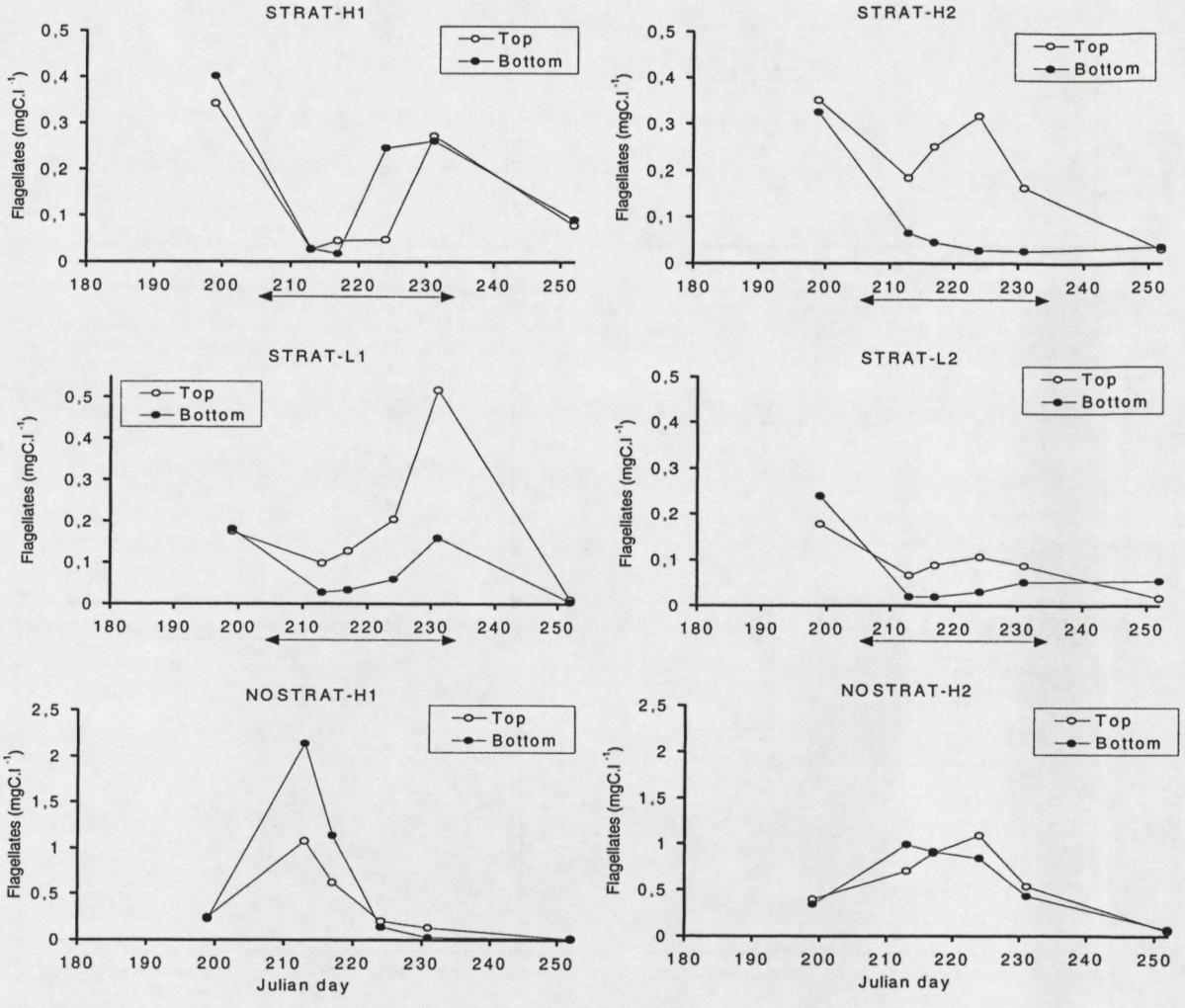



#### 4.3.5.1 Main components of the phytoplankton biomass

Two different functional groups were distinguished among the phytoplankters: flagellates and diatoms (respectively non-silicon and silicon using phytoplankton). Flagellates consisted mainly of three sub-groups: autotrophic nanoflagellates (2-20 µm), Cryptophyceae and *Phaeocystis* sp.. The dinoflagellate *Prorocentrum micans*, artificially introduced in the mesocosms, occasionally also represented a significant part of the non-diatom biomass.

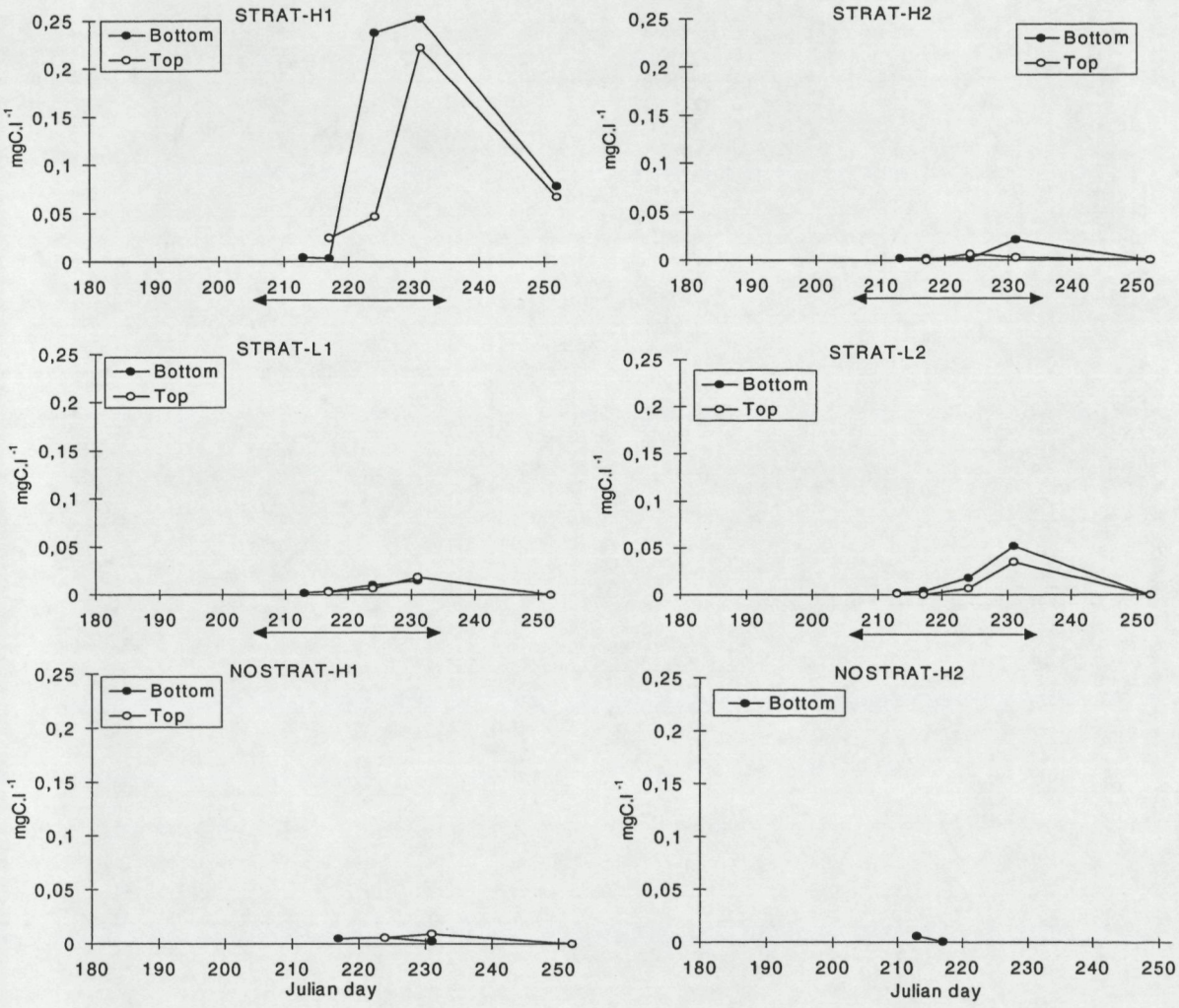
The first phytoplankton development, culminating around day 200 in all mesocosms, corresponded with relatively high diatom biomass, between 0.5 and 1.0 mg C l$^{-1}$ (Figure 4.8). Diatom biomass decreased between days 200 and 220 in all mesocosms except mesocosm STRAT-H1, where a narrow peak was observed on day 217. After day 220 diatom biomass remained low in all mesocosms except mesocosm NOSTRAT-H2, where exceptionally high biomass was reached between days 230 and 240. There was no significant relationship between diatom biomass measured in the Top compartments and nutrient treatment (nested ANOVA $F_{ratio}$=1.90, p=0.24).

**Figure 4.9**
Total Flagellate (non diatom) biomass measured in the Top and Bottom compartments of the six mesocosms. Stratification period is indicated below the X-axis (↔). Note the different Y-scale used for NOSTRAT-H1 and NOSTRAT-H2.



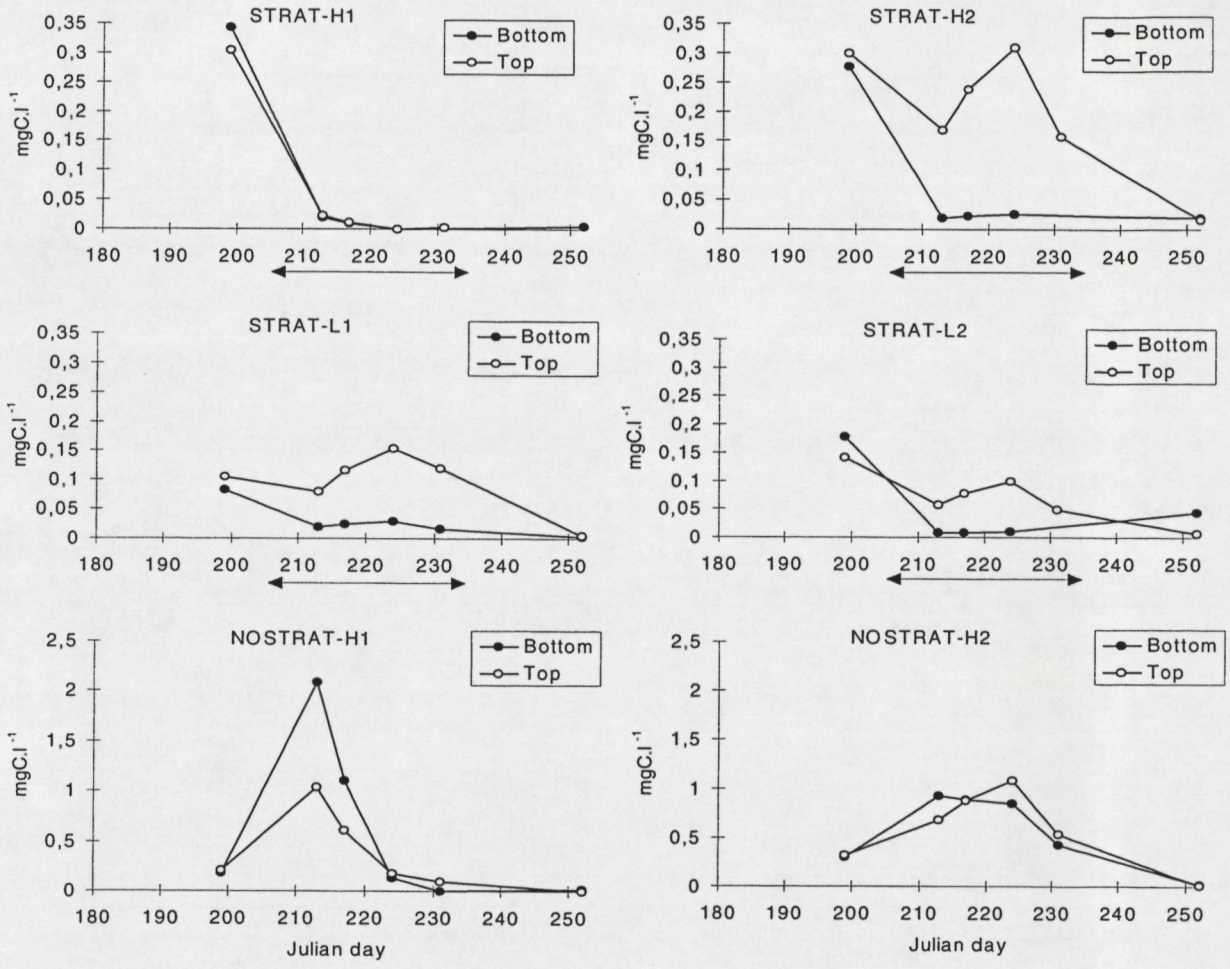

No significant difference was found between the diatom concentrations measured in the Top and the Bottom compartments of the stratified mesocosms during the stratified period (one way ANOVA, $p>0.05$). There was no relationship between the diatom biomass measured during the stratified period and the mixing regimes STRAT and NOSTRAT either (nested ANOVA, $p>0.05$).
In most mesocosms, flagellate biomass represented about one half of phytoplankton biomass during the first phytoplankton development around day 200 (Figure 4.9). Maximum flagellate biomass appeared in all mesocosms except STRAT-L2 between day 210 and day 230. Flagellate biomass was generally higher in the Top compartment of the stratified mesocosms than in the Bottom compartment. Differences were significant in mesocosms STRAT-H2 ($p<0.01$) and STRAT-L2 ($p<0.01$) but not in mesocosms STRAT-H1 and STRAT-L1 ($p>0.05$). Differences in flagellate biomass between the Top and Bottom compartments were also measured in the NOSTRAT mesocosms but were not constant. There was no significant relationship between flagellate biomass measured in the Top compartments and nutrient treatment (nested ANOVA $p>0.05$). Flagellate biomass was

**Figure 4.10**
Biomass of *Prorocentrum micans* measured in the Top and Bottom compartments of the six mesocosms (note the different scale used for STRAT-H1). Stratification period is indicated below the X-axis (↔)



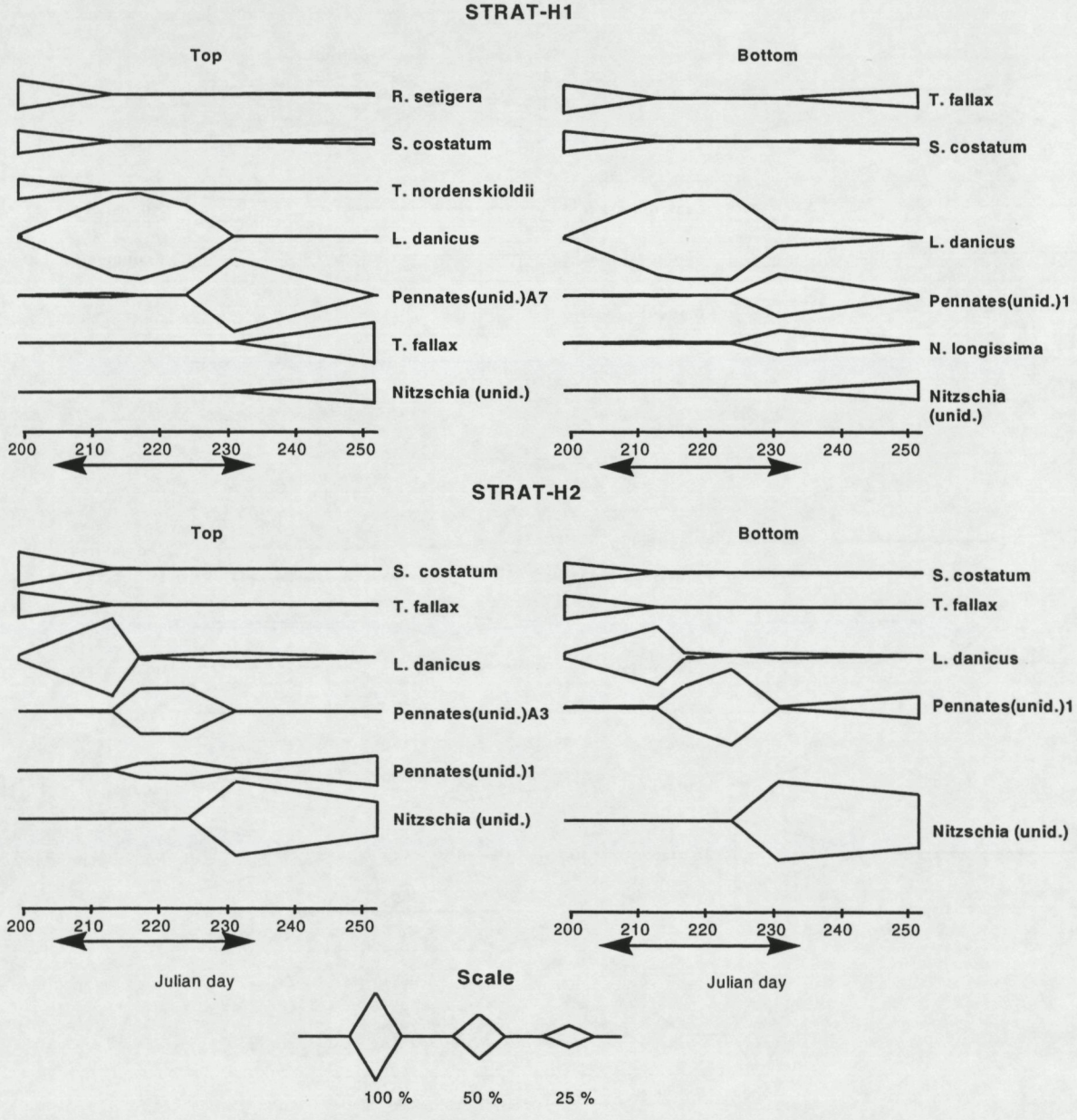

significantly higher in the NOSTRAT mesocosms than in the STRAT mesocosms (nested ANOVA, $p<0.01$).
In most mesocosms, flagellate biomass consisted mainly of *Prorocentrum micans* (Figure 4.10) and *Phaeocystis* sp. (Figure 4.11) except in STRAT-L1 where the large biomass development during the stratification period was due to the raphidophycean flagellate *Chattonella* sp. The highest *P. micans* biomass was found during stratified periods. The biomass that was reached in STRAT-H1 was one order of magnitude higher than in the other mesocosms (Figure 4.11). *P.micans* biomass in the Top compartments was either equal to or lower than the biomass in the Bottom compartments of the stratified mesocosms. No significant relationship was found between *P. micans* biomass measured in the Top compartments and the nutrient treatments (nested ANOVA $p>0.05$). No significant difference was found between the flagellate biomass measured in the Top and Bottom compartments of the stratified mesocosms during the stratified period (one way ANOVA, $p>0.05$). Although higher biomass of *P. micans* was found in the stratified mesocosms, no significant difference between the *P. micans* biomass in the mixing regimes STRAT and NOSTRAT was observed (nested ANOVA, $p>0.05$).

**Figure 4.11**
Biomass of *Phaeocystis sp.* Measured in the Top and Bottom compartments of the six mesocosms (note the different scale used for mesocosms NOSTRAT-H1 and NOSTRAT-H2). Stratification period is indicated below the X-axis (↔). Note the different Y-scale used for NOSTRAT-H1 and NOSTRAT-H2.




*Phaeocystis* sp. biomass represented most of the flagellate biomass in mesocosms STRAT-H2, STRAT-L2, NOSTRAT-H1 and NOSTRAT-H2 (compare Figures 4.9 and 4.11). An important *Phaeocystis* sp. biomass development was observed in all mesocosms except in STRAT-H1 between days 210 and 230. Very few colonies could be observed and most of this bloom was due to free cells. There was no significant relationship between the *Phaeocystis* sp. biomass measured in the Top compartments and the nutrient treatments (nested ANOVA, $p>0.05$). Significantly higher biomass of *Phaeocystis* sp. was measured in the Top than in the Bottom compartments of mesocosms STRAT-H2 (one-way ANOVA, $p<0.001$), STRAT-L1 ($p<0.001$) and STRAT-L2 ($p<0.001$) but not in mesocosms STRAT-H1 ($p>0.05$).
The average biomass of *Phaeocystis* sp. was significantly higher in the NOSTRAT than in the STRAT mesocosms (nested ANOVA, $p<0.01$).

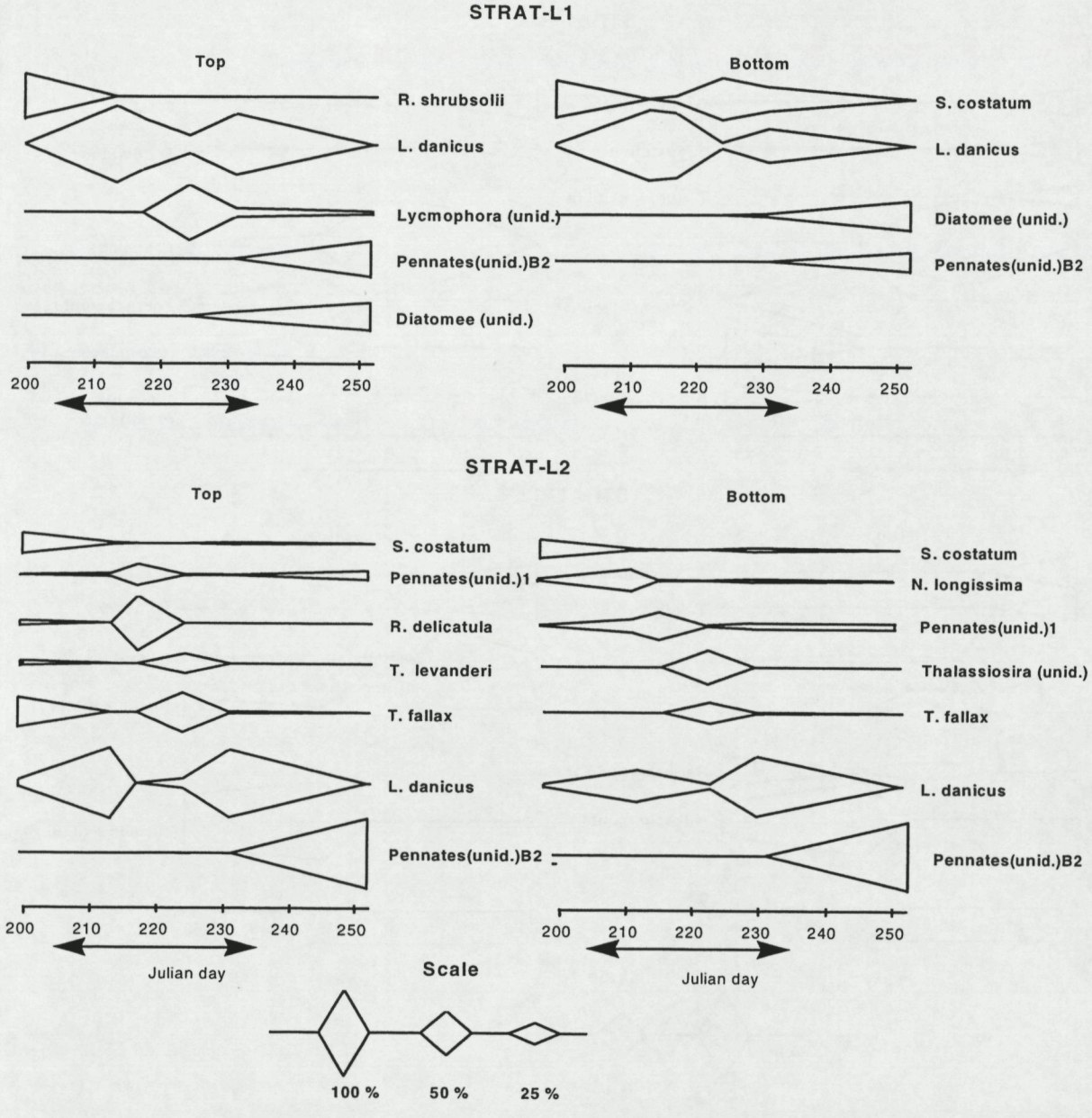
**Figure 4.12**
Kite diagrams of the diatom biomass composition in mesocoms STRAT-H1 and STRAT-H2. The width of the polygons is proportional to the contribution of the corresponding species or group of species to the total diatom biomass (See scale). Stratification period is indicated below the X-axis (↔).




### 4.3.5.2 Main diatom species succession

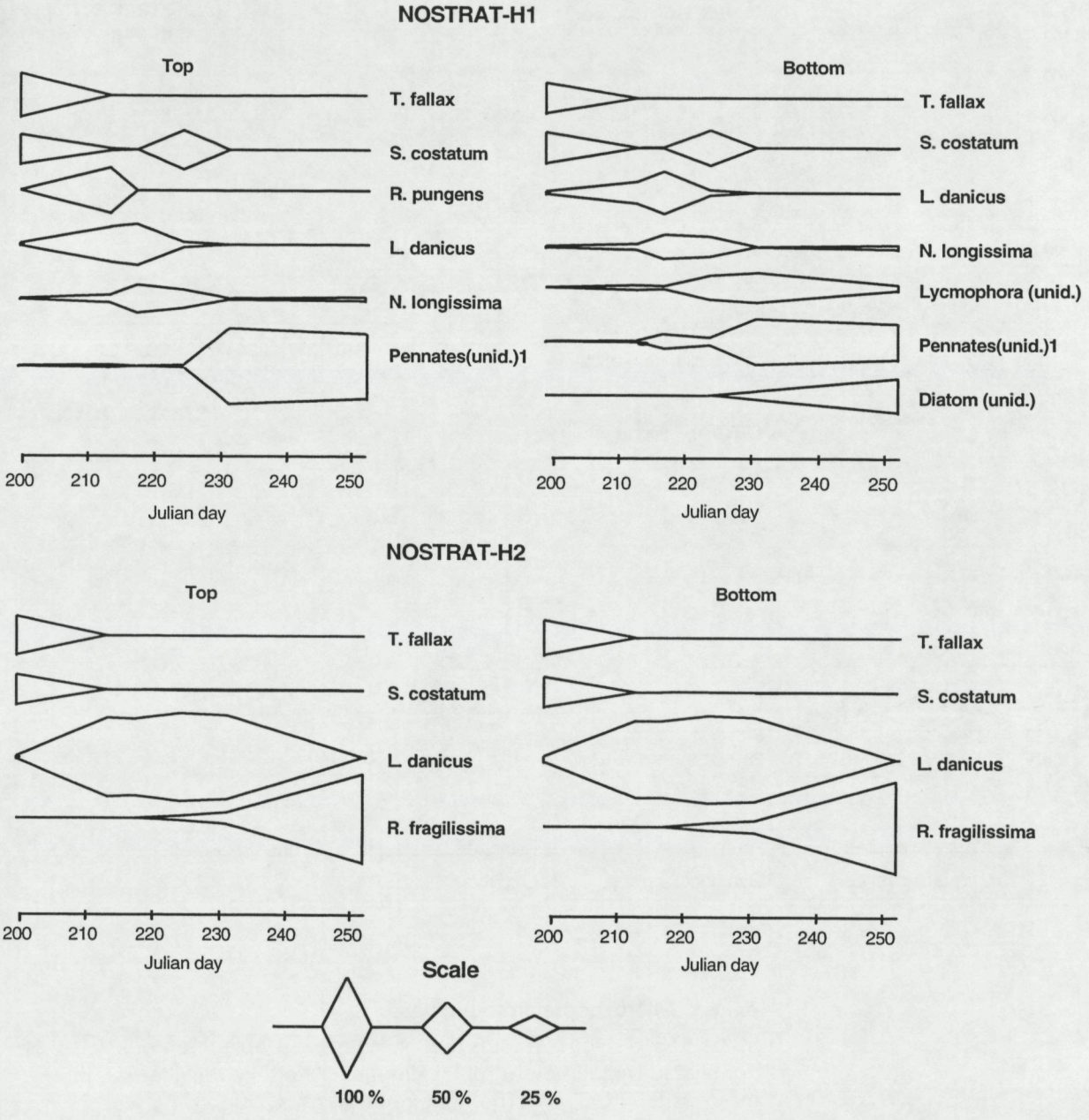
The relative contribution of the different species or groups of species to the diatom biomass is illustrated by the kite-diagrams in Figures 4.12 to 4.14. For a better readability of the graphs, only diatoms species reaching 20 % of the diatom biomass at least once during the experiment are shown.
All STRAT mesocosms showed very similar patterns of species succession within the diatom community. The first diatom development (before the stratification period) was mainly dominated by *S. costatum*, *T. fallax* and *R. shrubsolii*.
No differences were found with respect to the species composition of the diatom community between the top and the bottom compartments of the STRAT-mesocosms.

**Figure 4.13**
Kite diagrams of the diatom biomass composition in mesocoms STRAT-L1 and STRAT-L2. The width of the polygons is proportional to the contribution of the corresponding species or group of species to the total diatom biomass (See scale). Stratification period is indicated below the X-axis (↔).



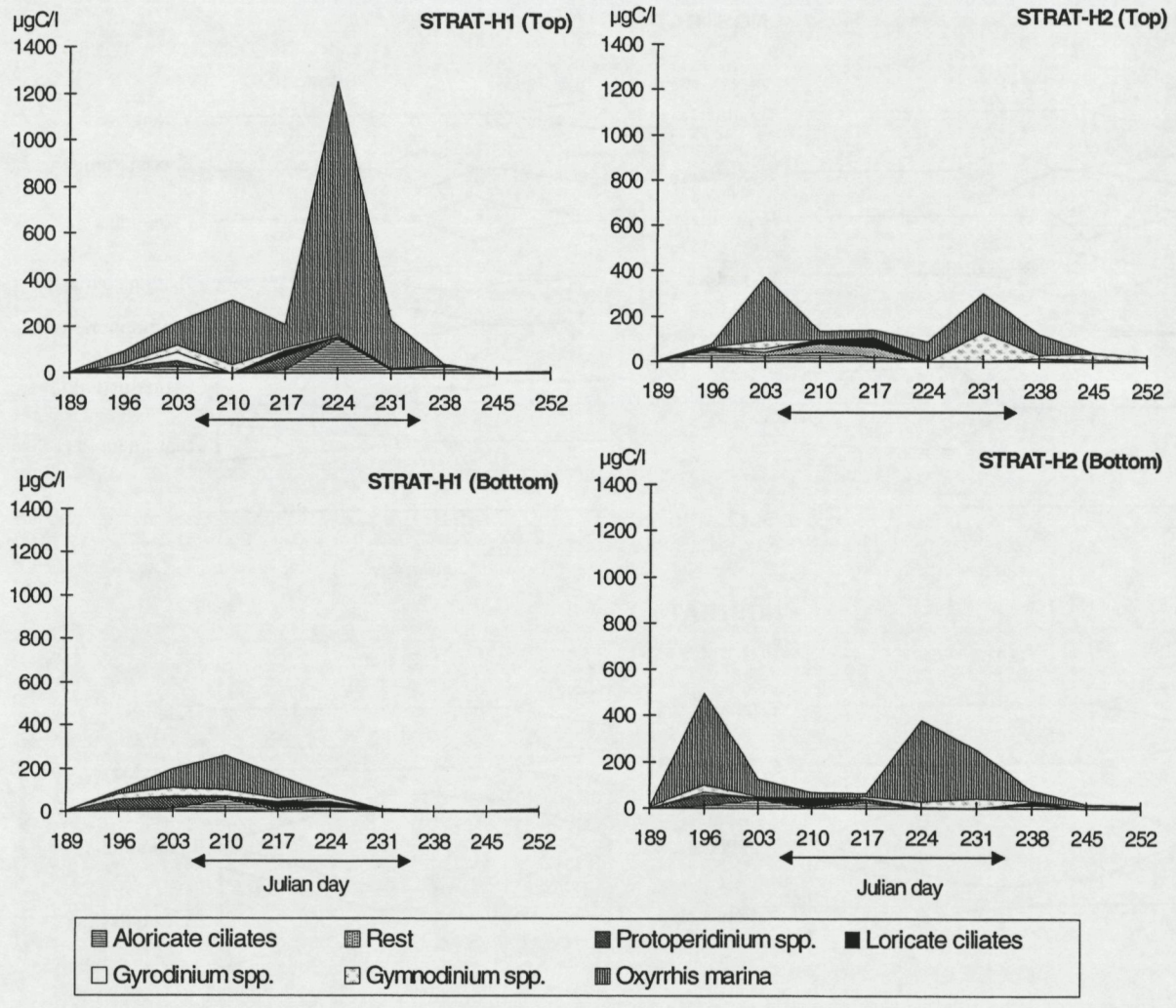

In most stratified mesocosms, *L. danicus* dominated between days 210 and 220. From day 220 onwards, the mesocosms became increasingly dominated by *Nitzschia* sp. and *T. fallax* in STRAT-H mesocosms, and by unidentified pennates in STRAT-L mesocosms
The initial diatom development (until day 210) in the NOSTRAT mesocosms was mainly dominated by *S. costatum* and *T. fallax*, which was similar to the STRAT-mesocosms.

**Figure 4.14**
Kite diagrams of the diatom biomass composition in mesocoms NOSTRAT-H1 and NOSTRAT-H2. The width of the polygons is proportional to the contribution of the corresponding species or group of species to the total diatom biomass (See scale).




The subsequent diatom species succession in NOSTRAT-H1 was similar to what was observed in the stratified mesocosms, with a development of *R. pungens* and *S. costatum* (generating a second major chlorophyll-*a* concentration peak) followed by a biomass dominance of pennates inthe last period of low chlorophyll-*a* concentrations. The diatom succession observed in NOSTRAT-H2 differed greatly from what was observed in the other mesocosms. The initial *S. costatum*, *T. fallax* development was followed by a 3-week bloom of *L. danicus*, subsequently followed by an increasing biomass contribution of *R. fragilissima*.

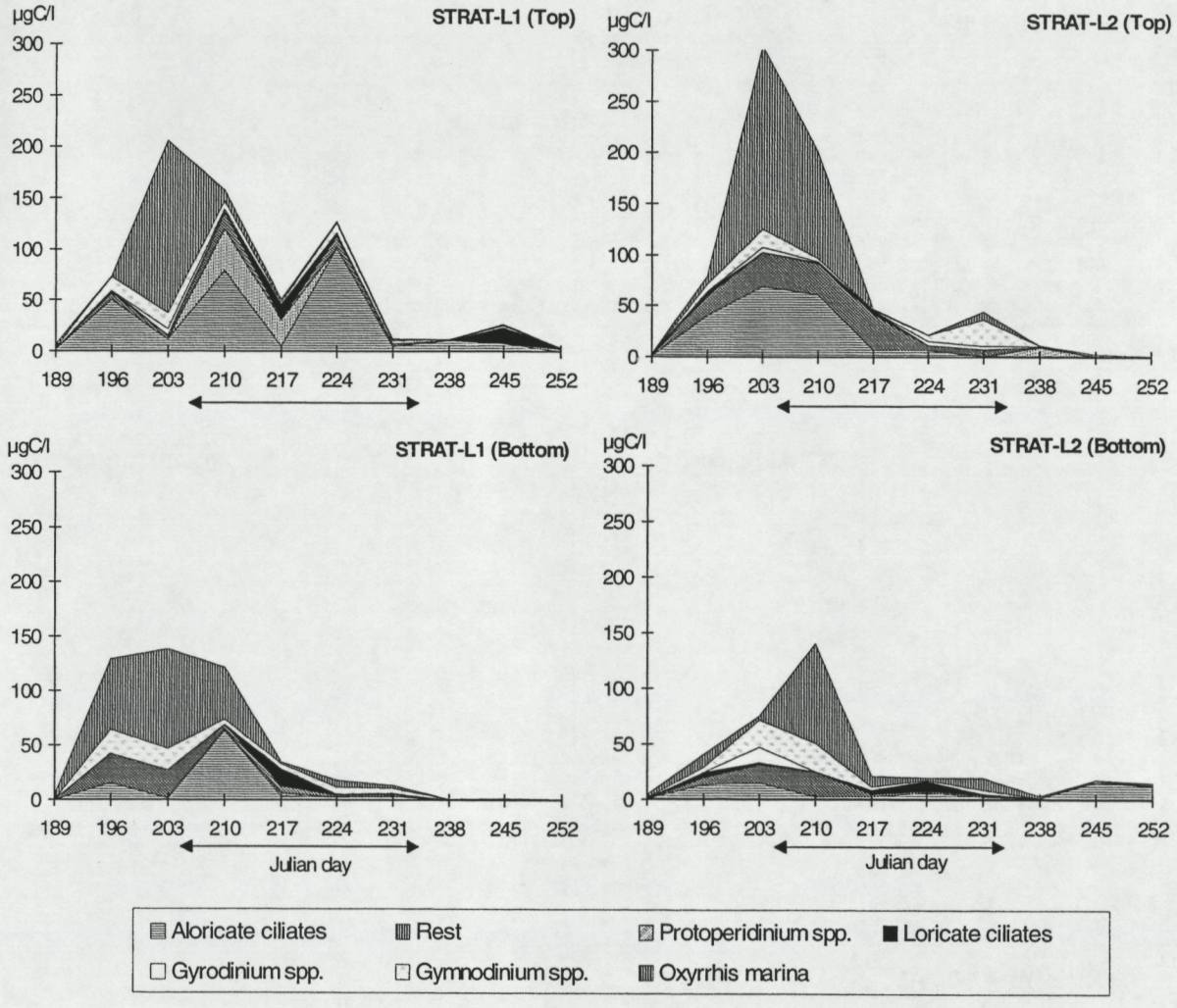
**Figure 4.15**
Microzooplankton biomass (without eggs and cysts) in µgC/l during the 1997-2 experiment in the upper (Top) and lower layers (Bottom) of the STRAT-H1 and STRAT-H2 mesocosms. Stratification period is indicated below the X-axis (↔).




### 4.3.6 Microzooplankton biomass

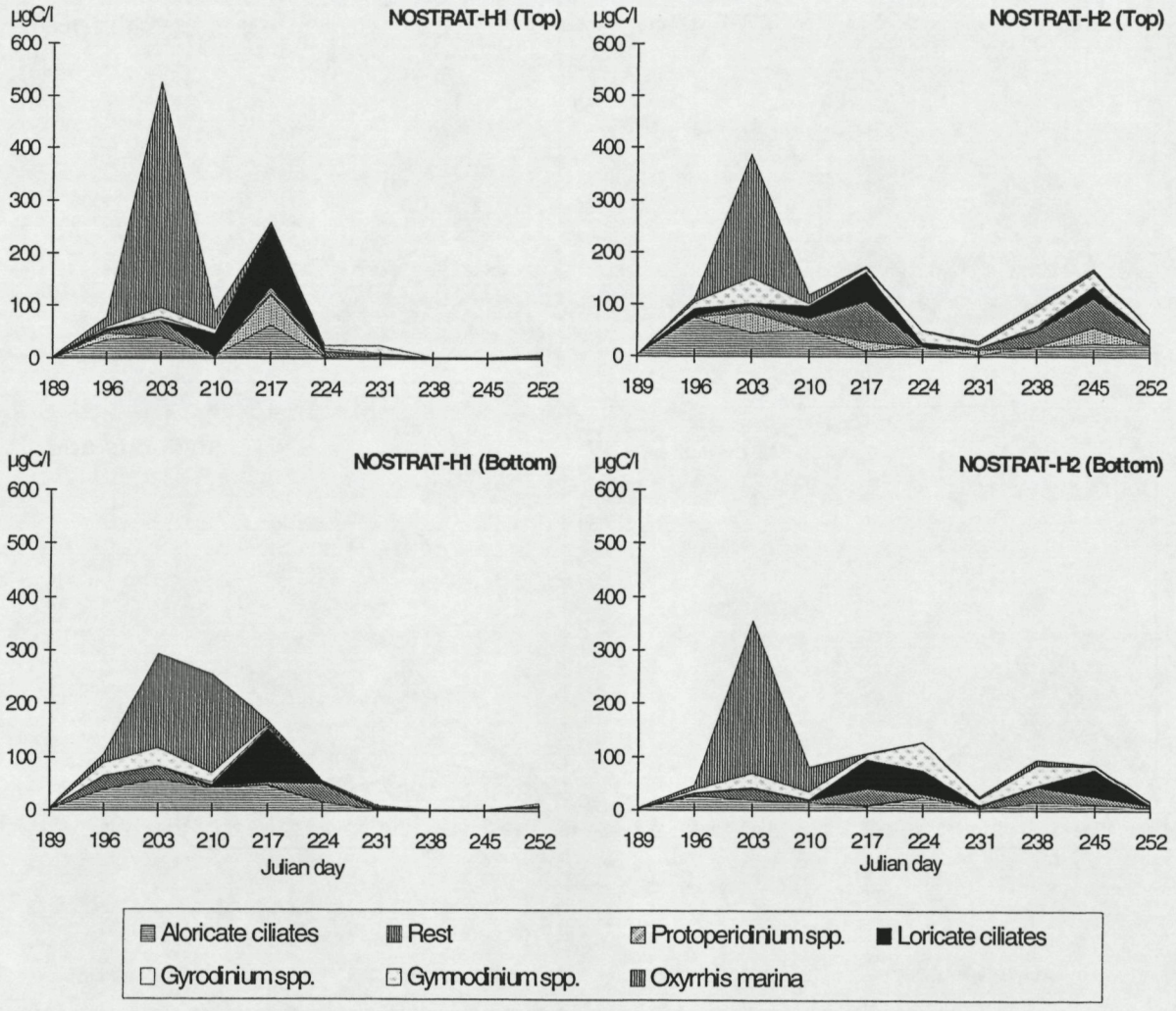
In the STRAT-H mesocosms (Fig. 4.15) microzooplankton biomass was dominated by the heterotrophic dinoflagellate *Oxyrrhis marina*. In mesocosm STRAT-H1 (Top) a huge peak, ca. 1250 µgC $l^{-1}$, was observed at day 224; the contribution of *Oxyrrhis marina* to this peak was almost 1100 µgC $l^{-1}$ (ca. 1.1 million cells.$l^{-1}$). Other peak values in the STRAT-H mesocosms ranged between ca. 250 and 500 µgC $l^{-1}$.

**Figure 4.16**
Microzooplankton biomass (without eggs and cysts) in µgC/l during the 1997-2 experiment in the upper (Top) and lower layers (Bottom) of the STRAT-L1 and STRAT-L2 mesocosms. Stratification period is indicated below the X-axis (↔).




In the STRAT-L mesocosms (Fig. 4.16) maximum microzooplankton biomass values remained lower than in the STRAT-H and NOSTRAT mesocosms, and was between ca. 130 and 300 µgC $l^{-1}$. Especially during the second half of the experiment biomass values had declined to very low levels not exceeding 25 µgC $l^{-1}$. Aloricate ciliates and especially *Oxyrrhis marina* contributed substantially to microzooplankton biomass.
In mesocosms NOSTRAT-H (Fig. 4.17) biomass reached peak values between ca. 300 and 500 µgC $l^{-1}$. Half of the biomass in these mesocosms originated from *Oxyrrhis marina*. Table 4.1 gives the microzooplankton biomass, averaged over the entire experimental period, for the Top and Bottom compartments of the mesocosms, and also the averaged biomass in the whole water column during the experimental period. The highest average biomass values were observed in the STRAT-H mesocosms and the lowest in the STRAT-L mesocosms.

**Figure 4.17**
Microzooplankton biomass (without eggs and cysts) in µgC/l during the 1997-2 experiment in the upper (Top) and lower layers (Bottom) of the NOSTRAT-H1 and NOSTRAT-H2 mesocosms.

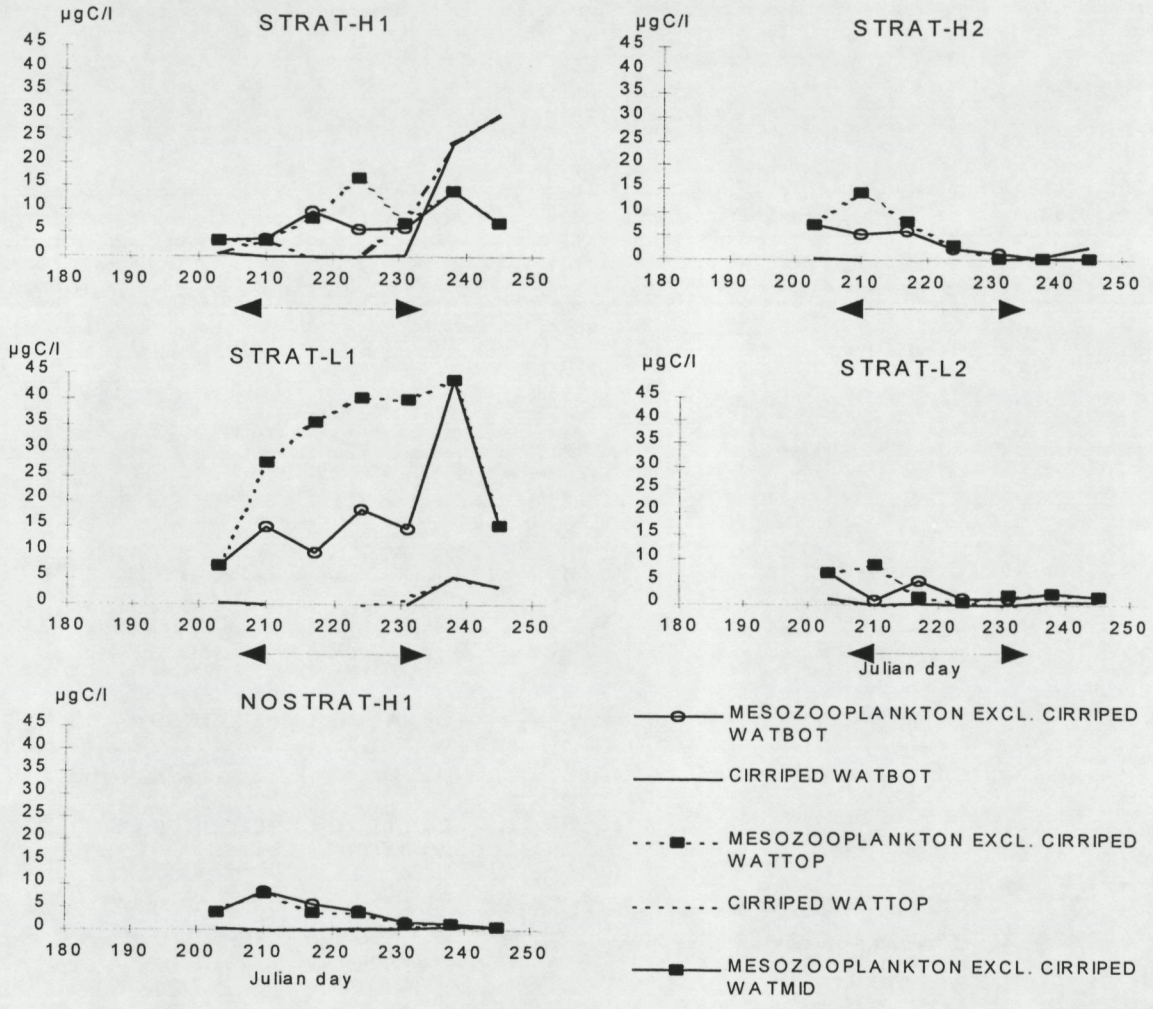



A paired t-test showed no significant difference between Bottom and Top biomass, neither for the entire experiment nor for the stratification period. Average biomass values (averaged over the whole experimental period and the whole water column) were log-transformed for a comparison between treatmentsby ANOVA, followed by a Bonferroni post-hoc test. Biomass in STRAT-H was significantly higher than STRAT-L ($p=0.003$) and NOSTRAT-H ($p=0.042$). NOSTRAT-H was significantly higher than STRAT-L ($p=0.013$).

**Table 4.1**
Microzooplankton biomass (averaged over the entire experimental period) in the Bottom and Top compartments of the 6 mesocosms and in the whole water column (in italics).

| Treatment | Mesocosm | Microzooplankton biomass (µgC $l^{-1}$) | | |
|---|---|---|---|---|
| | | Top | Bottom | Entire water column |
| STRAT-H | 1 | 237.9 | 84.3 | *161.1* |
| | 2 | 130.0 | 149.6 | *139.8* |
| STRAT-L | 1 | 68.0 | 46.7 | *57.4* |
| | 2 | 71.7 | 36.1 | *53.9* |
| NOSTRAT-H | 1 | 101.9 | 90.9 | *96.4* |
| | 2 | 119.2 | 94.5 | *106.9* |

**Figure 4.18**
Total mesozooplankton biomass(without eggs and cysts) during the 1997-2 experiment in upper (WATTOP) and lower layers (WATBOT) of all mesocosm except NOSTRAT-H2 discarded from the zooplankton study (see text for explanation). Stratification period is indicated below the X-axis (↔).

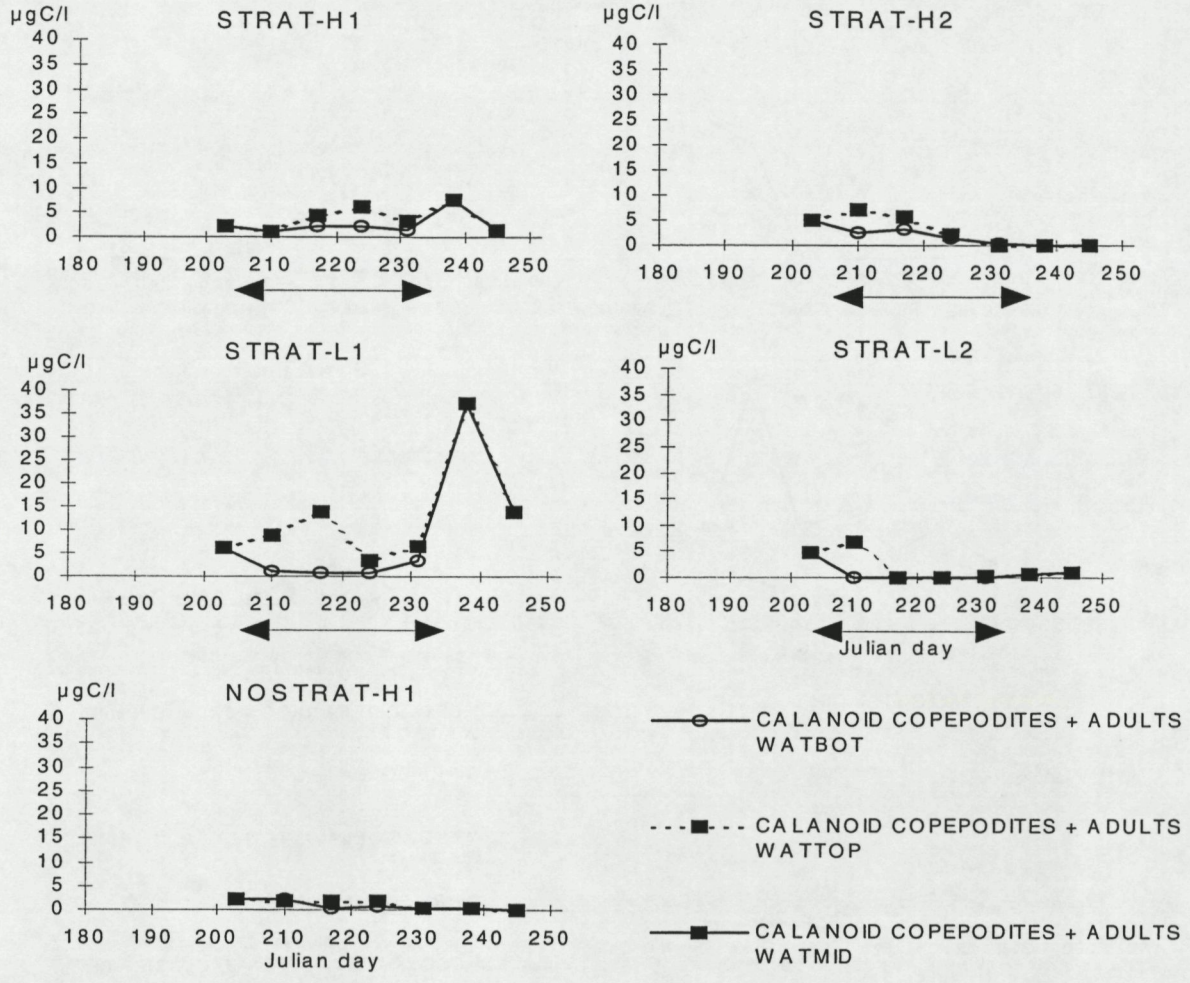



### 4.3.7 Mesozooplankton biomass

Mesozooplankton development in the mesocosms is shown in Figures 4.18-4.21. Only mesozooplankton samples, taken during and after the stratified period, were analysed. Samples from mesocosm NOSTRAT-H2 were not analysed because chlorophyll-a development differed extremely from what was measured in the other mesocosms (see Figure 4.7).

The highest biomass values were recorded in mesocosm STRAT-L1 and biomass in the Top samples was ca. twice as high as in the Bottom samples (Figure 4.18). At the end of the experiment cirriped larvae developed in mesocosm STRAT-H1. Obviously large differences existed between replicate mesocosms.

**Figure 4.19**
Biomass of calanoid copepodites + adults during the 1997-2 experiment in upper (WATTOP) and lower layers (WATBOT) of all mesocosm except NOSTRAT-H2 discarded from the zooplankton study (see text for explanation). Stratification period is indicated below the X-axis (↔).




Biomass values of calanoid copepodites and adults in the Top samples were higher than in the Bottom samples, but were still very low with the exception of a peak value found at day 238 (Figure 4.19). Biomass values of calanoid nauplii (Figure 4.20) reached values similar to the biomass of copepodites and adults, which may point to a developing copepod population. In general, biomass values of nauplii were highest in the Top samples.

**Figure 4.20**
Biomass of calanoid nauplii during the 1997-2 experiment in upper (WATTOP) and lower layers (WATBOT) of all mesocosm except NOSTRAT-H2 discarded from the zooplankton study (see text for explanation). Stratification period is indicated below the X-axis (↔).

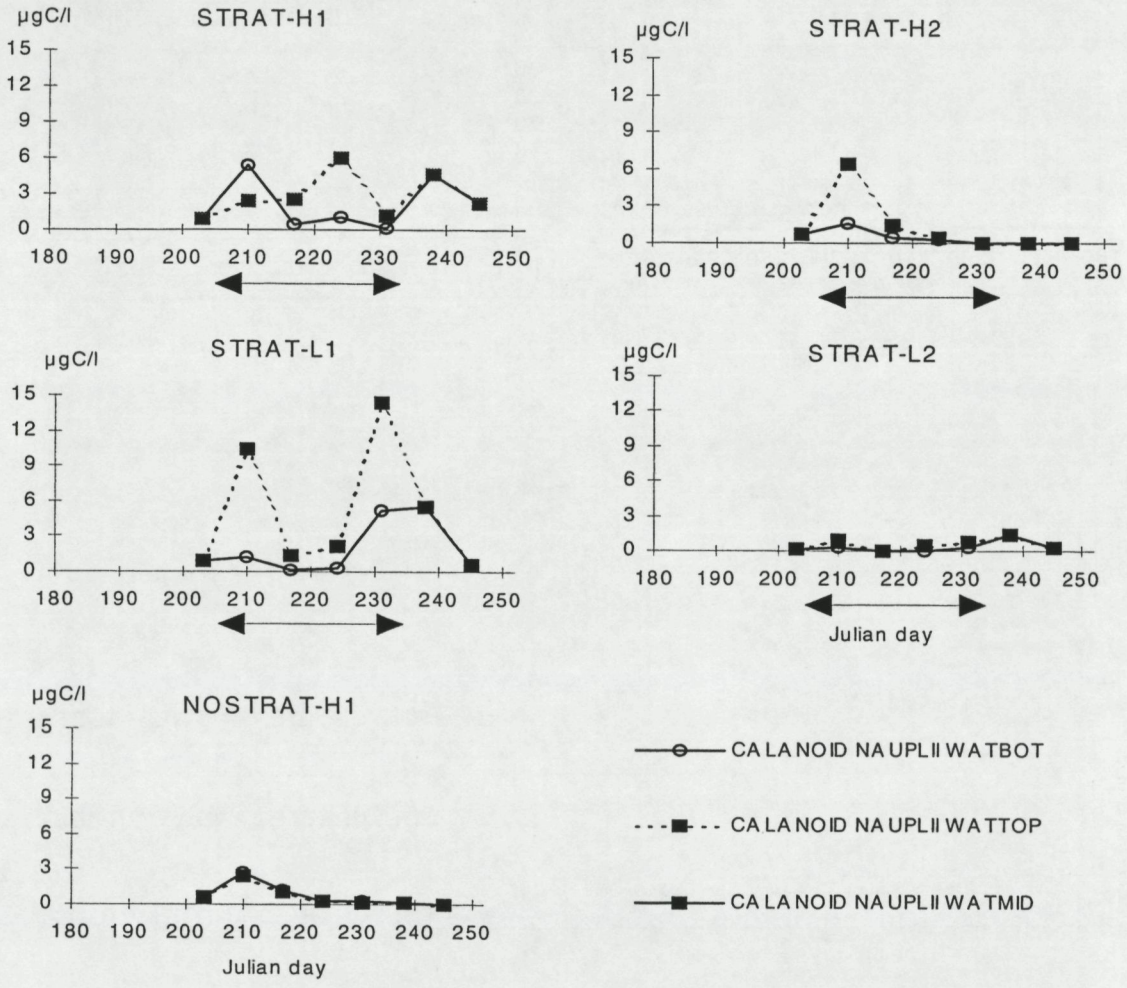



Figure 4.21 shows a strong development of polychaete larvae and nematodes in mesocosm STRAT-L1 during the stratified period. On the other hand, only low values were found in the replicate mesocosm STRAT-L2.Biomass in the Top samples was greater than in the Bottom samples. Large polychaete larvae contributed most to biomass.

**Figure 4.21**
Biomass of polychaetes and nematodes catched during mesozooplankton sampling during the 1997-2 experiment in upper (WATTOP) and lower layers (WATBOT) of all mesocosm except NOSTRAT-H2 discarded from the zooplankton study (see text for explanation). Stratification period is indicated below the X-axis (↔).

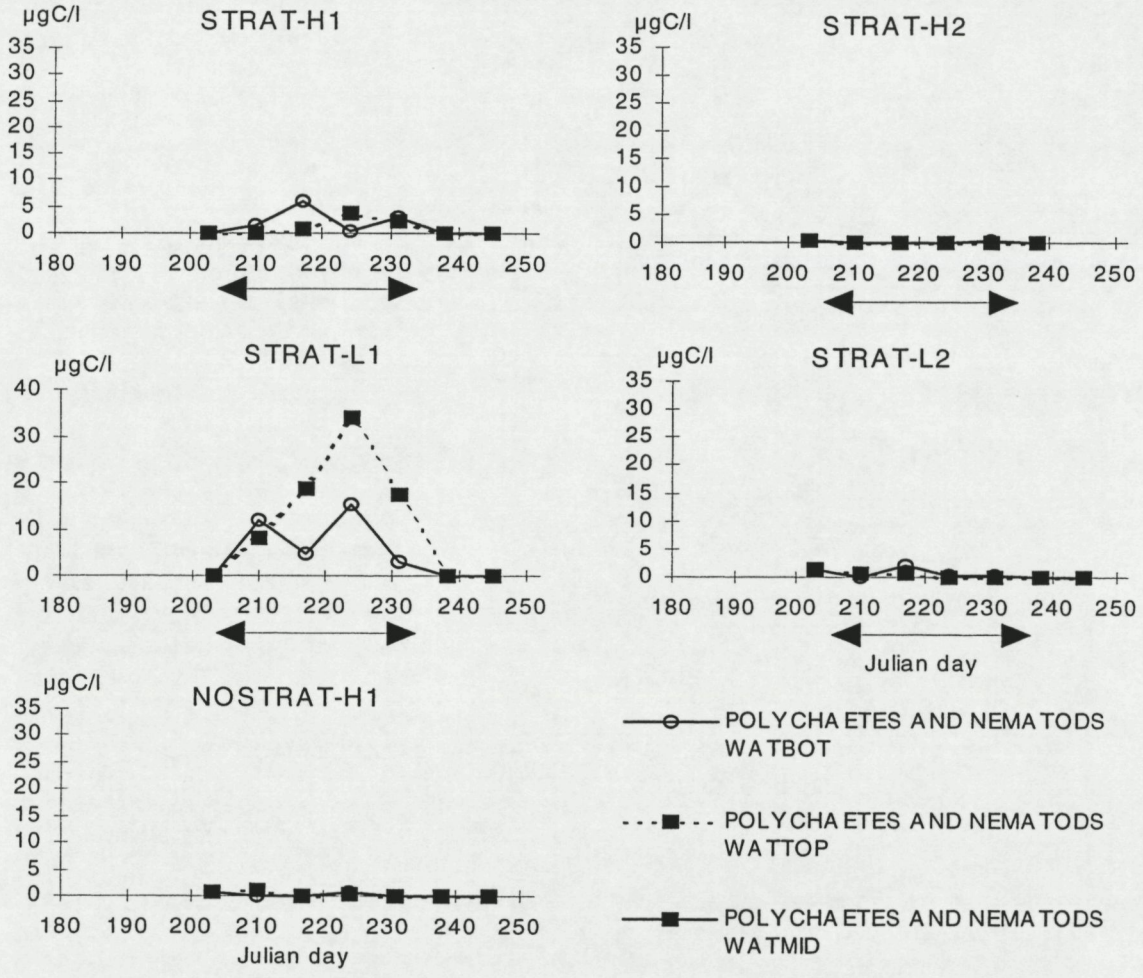



Table 4.2 gives the average calanoid copepod (all stages) biomass, for the Top and Bottom compartments and also the average biomass for the entire water column. A significant difference between the Bottom and Top samples was found in mesocosm STRAT-L1 for the entire experimental period (paired t-test, p=0.05), but not in the other mesocosms. Differences in average biomass between treatments were not significant (ANOVA, p= 0.62).

**Table 4.2**
Calanoid copepod (all stages) biomass (averaged over the entire experimental period) in the Bottom and Top compartments of the 6 mesocosms and in the whole water column (in italics).

| Treatment | Mesocosm | Calanoid copepod biomass (µgC $l^{-1}$) | | |
|---|---|---|---|---|
| | | Top | Bottom | Entire water column |
| STRAT-H | 1 | 6,6 | 4,7 | *5,7* |
| | 2 | 4,2 | 2,2 | *3,2* |
| STRAT-L | 1 | 17,9 | 11,0 | *14,5* |
| | 2 | 2,5 | 1,3 | *1,9* |
| NOSTRAT-H | 1 | 1,9 | 1,7 | *1,8* |
| | 2 | - | - | - |

**Table 4.3**
Mussel shell length and ash-free dry weights at the start and the end of the experiment

| Treatment | Tank # | Shell length in mm (mean ± s.e.) | ADW in g (mean ± s.e.) | n |
|---|---|---|---|---|
| start | | 25.6 ± 0.18 | 0.160 ± 0.006 | 22 |
| STRAT-H1 | 1 | 36.0 ± 1.00 | 0.328 ± 0.023 | 2 |
| STRAT-H2 | 4 | 35.3 ± 0.59 | 0.402 ± 0.008 | 15 |
| STRAT-L1 | 2 | 31.7 ± 0.72 | 0.315 ± 0.019 | 15 |
| STRAT-L2 | 5 | 32.3 ± 0.69 | 0.269 ± 0.011 | 15 |
| NOSTRAT-H1 | 3 | 34.9 ± 0.91 | 0.320 ± 0.020 | 14 |
| NOSTRAT-H2 | 6 | - | - | 0 |

### 4.3.8 Mussel growth rates

At the start of the experiment mussels with a shell length between 25 and 27 mm were selected. The average ash-free dry weight of the mussels was 0.160 g (Table 4.3). A high mortality occurred in mesocosms STRAT-H1 and NOSTRAT-H2. In the other mesocosms the instantaneous daily growth rates, based on ADW, varied between 0.008 and 0.015 $day^{-1}$. The high mortality in STRAT-H1 was probably caused by low oxygen concentrations during the stratification period. However, it is not clear why there was no or hardly any mortality in other stratified systems where oxygen concentrations reached similar low levels. All mussels in mesocosm NOSTRAT-H2 died, which was related to the massive phytoplankton bloom in that mesocosm, leading to low oxygen levels; also, high $H_2S$ concentrations were observed at the end of the experiment.
Mussel ADW in mesocosm STRAT-H2 was significantly higher than the mussel weights in all other mesocosms except mesocosm STRAT-H1 (ANOVA, $p<0.010$). Differences between treatments in ADW at the end of the experiment were not significant. There was no correlation between mussel growth and phytoplankton biomass.

## 4.4 DISCUSSION

### 4.4.1 Abiotic conditions

*Stratification.*-Due to the continuous addition of both demineralized water and hypersaline solution, stratification could be kept steady for about 30 days without need for further major intervention. A gradient of about 1 ‰ over 20 cm appeared to guarantee the steadiness of the stratification (See STRAT-H1, STRAT-L1 and STRAT-L2 on Figure 4.1). In mesocosm STRAT-L2, the initial salinity gradient of about 0.5 ‰ over 20 cm appeared not steep enough for the establishment of a steady stratification (see Figure 4.1). These observations agreed with results of the 1996 pilot experiment where an initial gradient of about 1 ‰ over 30 cm was found to be a prerequisite for a durable stratification (Escaravage *et al.*, 1998).
*Irradiance.*-The last 20 days of stratification (days 215-234) coincided with stable sunny conditions and were preceded and followed by periods of less favourable weather conditions with low or highly variable irradiance (Figure 4.2). This pattern resembled field conditions during thermohaline stratification in the central and northern North Sea, when stable and sunny conditions allow the establishment of a pycnocline (Peeters *et al.*, 1995). As a result of the high intrinsic light attenuation of the mesocosm (small diameter/height ratio, black colour), the light climate in the two compartments were very contrasting. Light was never a limiting factor for primary production in the Top compartment whereas phytoplankton in the Bottom compartment had to face moderate to severe light limitation throughout the stratification period (Figure 4.3). In such conditions, the presence of stratification corresponds to a segmentation of the mesocosm in an auto- and a heterotrophic sub-system.
*Temperature.*-Temperature of the mesocosm water was clearly affected by solar radiation (Figure 4.2). The warming up of the Top compartment contributed to a strengthening of the saline stratification
*Dissolved Inorganic nutrients.*-For all nutrients, the first 10 days of the experiment corresponded to the consumption of the initial nutrient stock by phytoplankton. During this period all nutrients were far above the limiting threshold for primary producers and phytoplankton could evolve similarly in all mesocosms, as it was free of any nutrient limitation. The different nutrient treatments applied to the mesocosms became effective only after the depletion of this initial stock (after day 200, see Figures 4.4, 4.5, 4.6). Accumulation of nutrients within the Bottom compartment was a common feature in the stratified mesocosms, whereas concentrations showed no vertical gradients in the non-stratified mesocosms. Increases in silicate concentrations, occurring in the Top compartments of the stratified mesocosms, indicated a low demand for this nutrient by diatoms. In most cases, inorganic nitrogen and phosphorus concentrations remained relatively steady in the Top compartment, indicating a balance between the continuous addition and uptake by the phytoplankton.

### 4.4.2 Phytoplankton species composition

#### 4.4.2.1 Diatoms

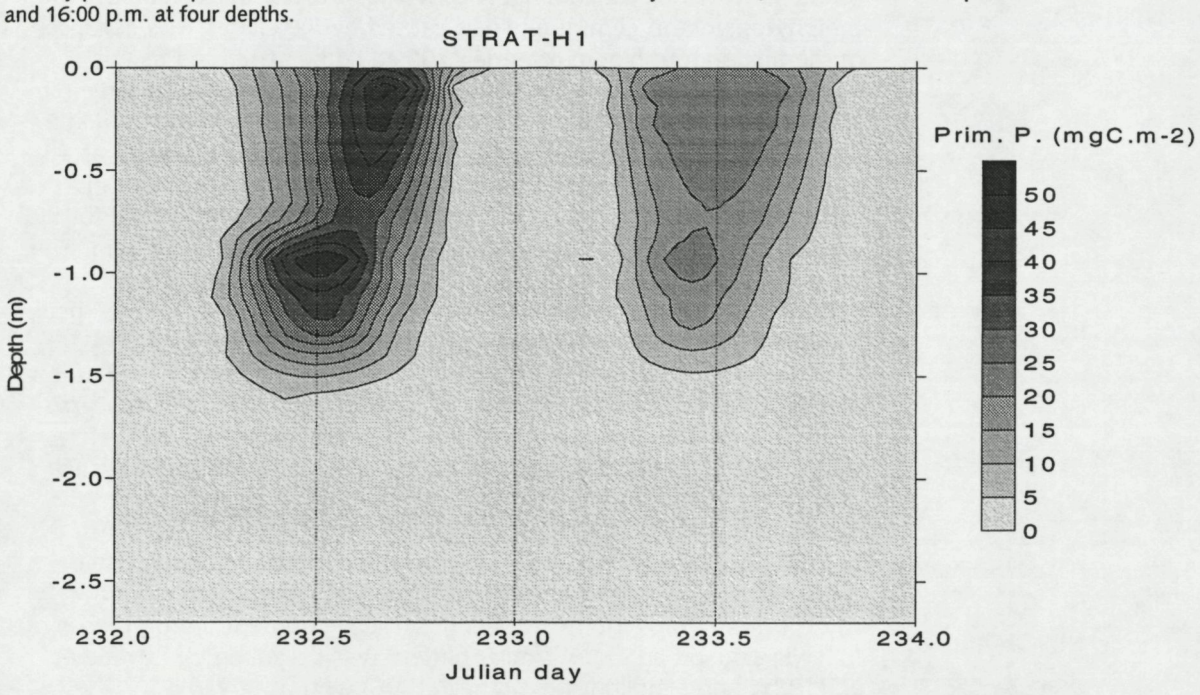
Following the second hypothesis formulated in the introduction, diatoms were expected to be favoured by high mixing regimes when compared to stratified conditions. With exception of NOSTRAT-H2, diatoms did not play a significant role in this experiment, as they

mainly reached bloom concentrations during the initial period when nutrients were not yet limiting. Afterwards, most of the dynamics in the phytoplankton community was caused by flagellates. The collapse of the first diatom bloom after day 200 could be attributed to the depletion of the initial nutrient stock. A similar feature was observed in a summer experiment in 1994 (Escaravage *et al.*,1995b), where diatom biomass increased intensively at the beginning of the incubation as a response to the high nutrient concentrations in the mesocosm. This initial growth went on until one of the nutrients became limiting for primary production, and was followed by a *Phaeocystis* sp. bloom. The main difference between the experiment in 1994 and the present experiment was that in the former experiment the *Phaeocystis* sp. peak was followed by a second massive (ca 50 $\mu g.l^{-1}$) diatom bloom (*L.danicus*). This second diatom bloom appeared to develop well at low nutrient (N and Si) concentrations, and could outcompete *Phaeocystis*, which is a relatively bad competitor for nutrient uptake (Hegarty & Villareal, 1998). With exception of NOSTRAT-H2, there was not such a second peak of diatom biomass development during the present experiment.
In the STRAT mesocosms, the 3-week long stratification could have contributed to the removal through sinking of the diatoms from the water column, preventing any significant development during the following mixed period. A similar pattern was described by Estrada *et al.* (1987) and Schöllhorn & Granéli (1996) who observed a replacement of diatoms by flagellates in unmixed mesocosms. The sustained low phytoplankton concentrations in NOSTRAT-H1 after the collapse of the *Phaeocystis* sp. bloom after day 220 is more difficult to interpret, since neither nutrients nor light were strongly limiting for primary production at that time. Potential grazers (micro-and mesozooplankton) were also at relatively low concentrations after day 224 (Figures 4.17 and 4.18).
Due to the relative bad development of diatoms in mesocosm NOSTRAT-H1 when compared with *Phaeocystis sp.*, the hypothesis (2) formulated in the introduction that stratification may favor flagellates to the detriment of diatoms.

#### 4.4.2.2 Prorocentrum micans

The addition of *Prorocentrum micans* was mainly meant to test the third hypothesis formulated in the introduction: Increased N-loadings may favour the appearance of potentially toxic phytoplankton blooms in stratified areas.
*Prorocentrum micans* became dominant in terms of biomass in mesocosm STRAT-H1, whereas its development was relatively poor in the other mesocosms. *Prorocentrum micans* concentrations did not systematically differ between the Top and the Bottom compartment in mesocosm STRAT-H1. The main reason for this apparent lack of response to stratification may be the ability for diurnal vertical migration. Edler & Olsson (1985) and Olsson & Granéli (1991) described strong diurnal vertical migration of *Prorocentrum micans* with ascent starting before sunrise and descent before sunset. The latter pattern was also observed during an intensive measurements survey conducted in STRAT-H1 on days 233-234. At 7:00 am the mesocosm water was clear and we could easily see the mixer at 80 cm depth, whereas water became brownish with time and at 16:00 the mixer was not visible anymore. At 19:00 the water became clearer. Further evidence for the diurnal vertical migration of *Prorocentrum micans* comes from primary production measurements done in STRAT-H1 at 10:00 a.m. and 16:00 p.m. during this survey (Figure 4.22). The

**Figure 4.22**
Primary production profiles measured in mesocosm STRAT-H1 on days 232-233, measurements were performed at 10:00 a.m. and 16:00 p.m. at four depths.

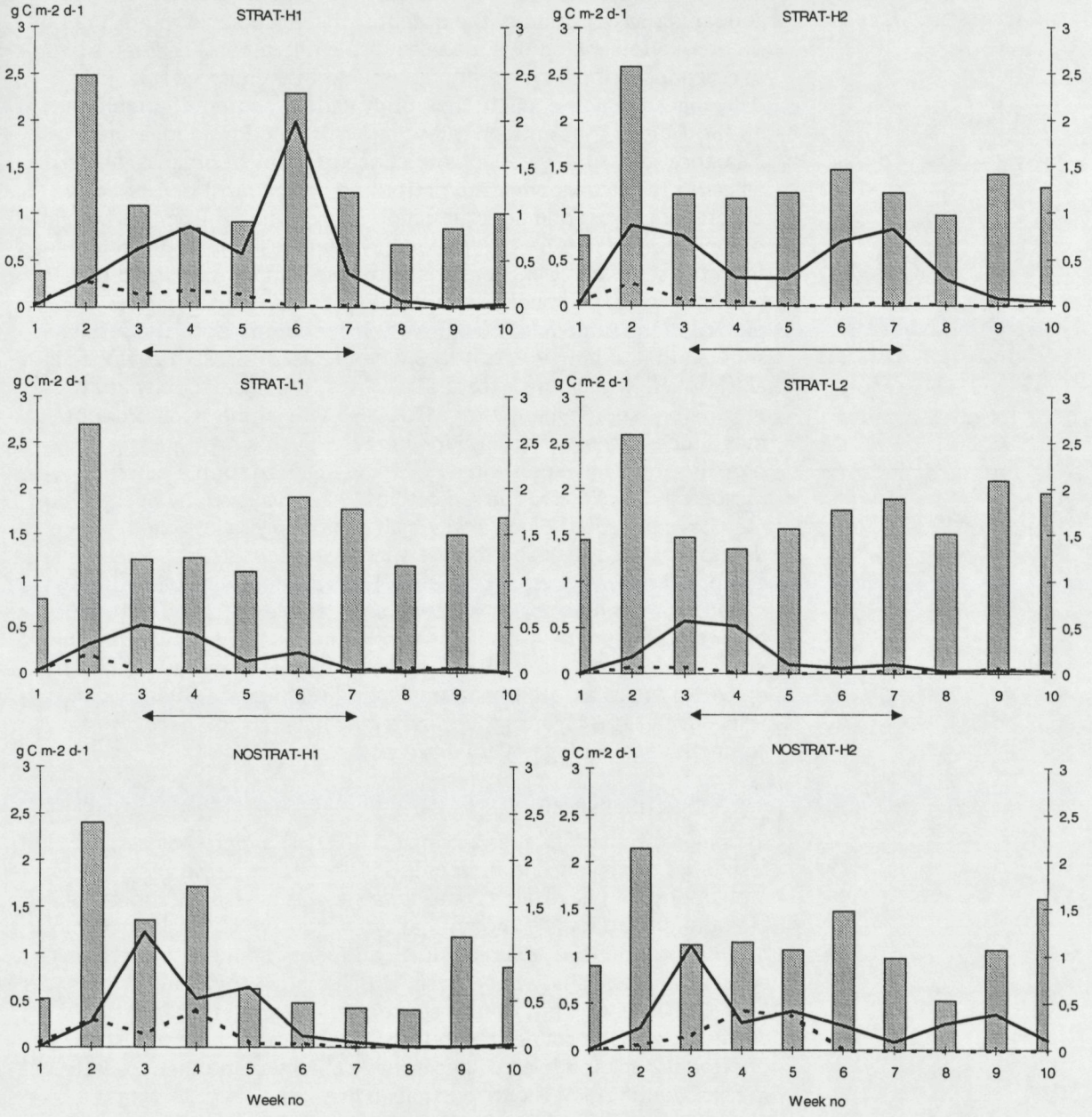



migration of *Prorocentrum micans* was clearly illustrated by the upwards displacement of the production maximum during daytime. The standard sampling for the phytoplankton species counts in the present experiment was performed around 7:00 am, and in these samples *Prorocentrum micans* appeared to be regularly distributed over the water column. Samples collected around 16:00 a.m. would certainly have led to different results.
The observation that the highest *Prorocentrum micans* were measured in a stratified mesocosm with a high nitrogen loading rate converges with the hypothesis (3) formulated in the introduction that increased N-loadings may favour the appearance of potentially toxic phytoplankton blooms in stratified areas. The fact that it was *Phaeocystis* sp. and not *Prorocentrum micans* which dominated in the duplicate STRAT-H2 will be discussed in the following section.

#### 4.4.2.3 Phaeocystis sp.

When STRAT-H1 is excepted, *Phaeocystis* sp. appeared to develop successfully in the stratified mesocosms with significantly higher biomass in the Top than in the Bottom compartment. Since most of the *Phaeocystis* cells . The significantly higher *Phaeocystis* sp. biomass found in the NOSTRAT mesocosms compared to the STRAT mesocosms may have resulted from the mixing, as this prevented the formation of a local biomass maximum with related local nutrient depletion.
In all stratified mesocosms *Phaeocystis* sp. represented the main part of the flagellate biomass, except in STRAT-H1 where *Prorocentrum micans* was the dominant species. *Oxyrrhis marina* formed a major fraction of microzooplankton biomass in both the stratified and in the mixed mesocosms, and in Bottom as well as in Top samples. In addition, microzooplankton biomass was high in comparison with mesozooplankton biomass. Thus, the microzooplankton may have had a serious grazing impact on the phytoplankton. Protozoa, such as ciliates and heterotrophic dinoflagellates are the primary consumers of

**Figure 4.23**
A comparison of the production and loss rates of the phytoplankton. The vertical bars show the weekly average phytoplankton water column gross primary production estimated from the oxygen method applied to the continuous oxygen measurements. The full line shows the estimated microzooplankton grazing in the entire water column. The dotted line shows the mussel filtration rate. See text for explanation of estimates. Stratification period is indicated below the X-axis (↔).




*Phaeocystis* single cells in natural populations from the North Sea (Weisse & Scheffel-Möser, 1990). From laboratory experiments Hansen et al. (1993) concluded that the ciliate *Strombidinopsis acuminatum* and the heterotrophic dinoflagellate *Oxyrrhis marina* ingested Phaeocystis single cells at much higher rates than did the copepod *Temora longicornis*. Further, Admiraal & Venekamp (1986) reported grazing of tintinnids on *Phaeocystis* single cells and also on single cells grasped from the surface of bladder-like colonies. Finally, *Oxyrrhis marina* can be considered an effective grazer on the smaller phytoplankton size fraction (see references in Wetsteyn & Vink-Lievaart, 1997). In all mesocosms *Phaeocystis* was present as single cells and thus may have been subject to protozoan grazing. The increasing biomass of *Oxyrrhis marina*, culminating in the huge peak in mesocosm STRAT-H1, probably is responsible for the fast decrease of

*Phaeocystis* biomass in this mesocosm. This collapse of the Phaeocystis sp. bloom may have given the dinoflagellate *Prorocentrum micans* the opportunity to reach the high observed biomass after day 220. The much higher biomass of *Phaeocystis* in the other mesocosms coincided with lower microzooplankton biomass than in STRAT-H1.
A quantitative estimate of the potential microzooplankton grazing rate on the phytoplankton was made from the microscopic estimates of microzooplankton biomass. It was assumed that microzooplankton daily ingestion was equal to their body carbon content (Paffenhöfer, 1998). Mussel consumption rates were calculated from measured clearance rates and water column concentrations of organic carbon. Since no $^{14}C$ incubations were performed during most part of this experiment, gross primary production was estimated from the oxygen diel cycles. The (C/O2) production quotient was empirically determined from the series of $^{14}C$ incubations performed during the first three weeks of the experiment. Primary production and grazing rates were plotted in the same figure (Figure 4.23) for comparison. The results showed that, during weeks 4 to 6 when *Phaeocystis* sp. developed in all stratified mesocosms except STRAT-H1, the microzooplankton grazing pressure in mesocosm STRAT-H1 was of the same order of magnitude as the primary production rate. Such a high grazing pressure could have prevented *Phaeocystis* sp. to form a bloom in mesocosm STRAT-H1. The removal of *Phaeocystis* sp. by overgrazing may have benefited *P. micans*, which is probably a less eatable item due to its armed shell and relatively large size (*ca*. 30 µm) when compared to *Phaeocystis* (*ca* 8 µm). The question remained why *P. micans* did not reach biomass levels comparable to STRAT-H1 in the other stratified mesocosms. Laboratory cultures have shown that the growth rate by *P. micans* was significantly lower than by *Phaeocystis*: up to 1 $d^{-1}$ (Costas, 1990) and up to 2 $d^{-1}$ (Peperzak, 1993) respectively. This higher growth rate may have given *Phaeocystis* a competitive advantage over *P. micans*.

#### 4.4.2.4 Other species

In one of the stratified mesocosms (STRAT-L1) a high biomass, resulting from a concentration of $2.6*10^6$ cells.$l^{-1}$, of the raphidophycean flagellate *Chattonella* sp. was measured. From Dutch biomonitoring data it is known that two *Chattonella* species occur in our marine waters: *C. marina* and *C. antiqua*. The highest observed concentration of *Chattonella* sp. in Dutch marine (offshore) waters was ca. $1.0*10^6$ cells.$l^{-1}$, but usually concentrations are less then $4.0*10^4$ cells.$l^{-1}$. It must be stated that in Lugol-preserved samples the shape of *Chattonella* sp. and related species have changed and that accurate species identification is only possible in live samples. *C. marina* is a potentially neurotoxic species (Tseng et al., 1993) and *C. antiqua* is known as a red-tide organism in the Seto Inland Sea (Japan), killing farmed fish (see references in Nakamura & Umemori, 1990).

### 4.4.3 Phytoplankton biomass

The absence of nutrient limitation during the first 10 days of the experiment was clearly illustrated by the similarity in the initial chlorophyll-*a* increases observed in the six mesocosms.
After stratification had been installed, the differential nutrient uptake due to phytoplankton production was clearly illustrated by the N- and P-concentration increases observed in the bottom compartment of the stratified mesocosms. No such increase was measured in the NOSTRAT mesoscosms where the development of the second phytoplankton

bloom (around day 210) was probably responsible for the uptake of most of the nitrogen and phosphorus added to the mesocosms. This observation converges with the first hypothesis formulated in the introduction that stratification could induce a reduction of the phytoplankton yield. However, due to heterogeneity between the NOSTRAT mesocosms no significant difference was found between the chlorophyll-a concentrations in the STRAT and in the NOSTRAT mesocosms.

The effect of stratification on the vertical distribution of chlorophyll-*a* concentrations was most clear in the STRAT-H mesocosms with significant differences in phytoplankton biomass development between the Top and the Bottom compartments. The differences were smaller in the STRAT-L mesocosms but still significant in STRAT-L1.

Although maximum chlorophyll-*a* concentrations in the Top compartments of the stratified mesocosms were highest in the mesocosms with high nitrogen loading (Figure 4.7), no significant relationship could be found between phytoplankton biomass and nutrient treatment. This was mainly due to the relatively short duration of the peaks and to the large differences observed between replicates within each treatment. A good agreement was observed between the phytoplankton biomass measured in mesocosms STRAT-L1 and STRAT-L2 (Figure 4.7), with average chlorophyll-*a* concentrations of 8.5 and 8.2 $\mu g\ l^{-1}$ respectively. More variation was observed within treatments STRAT-H and NOSTRAT-H, with average chlorophyll-*a* concentrations equalling 15.9 and 10.1 $\mu g\ l^{-1}$ in STRAT-H, and 13.4 and 36.2 $\mu g\ l^{-1}$ in NOSTRAT-H.

The main discrepancies between replicates appeared after day 225 in the NOSTRAT-H treatment and after day 235 in the STRAT-H treatment. In both cases, it was observed that the mesocosms with the highest chlorophyll-*a* concentrations (STRAT-H1 and NOSTRAT-H2) had higher concentrations in the Bottom than in the Top compartment (Figure 4.7). The higher chlorophyll-*a* concentrations observed in STRAT-H1 and NOSTRAT-H2 than in their replicates may have resulted from a lower grazing pressure exerted by zooplankton and/or mussels. However, the comparison between gross primary production ($O_2$ method) and potential loss rates of the phytoplankton in Figure 4.23 did not indicate significantly lower grazing rates in STRAT-H1 and NOSTRAT-H2 during the period when high chlorophyll-*a* concentrations were measured. Another potential cause for the high chlorophyll-*a* concentrations in STRAT-H1 and NOSTRAT-H2 could have been a malfunctioning of the automatic valves used for the continuous measurements, resulting in an input of nutrient-rich filtered seawater into the mesocosms. It was observed that barnacles had settled in parts of the pipes and valves regulating the flow of water from the mesocosms to the measuring unit, which could have affected valve closure. However, an analysis of the inorganic nutrient concentrations and mesocosm nutrient balances gave no indication of nutrient enrichment exceeding the experimental dosage of nutrients. When NOSTRAT-H2 is not considered and observation is restricted to the stratification period, the effect of stratification on phytoplankton biomass development could be studied by comparing mesocosm NOSTRAT-H1 with mesocosms STRAT-H. The main effect of stratification appeared to be the extension of the phytoplankton growth period, allowing the occurrence of summer blooms developing on nutrients diffusing from the Bottom layer, after depletion of nutrients in the Top compartment. This pattern agrees well with field and model observations (Holligan *et al.*, 1984; Stigebrandt & Djurfeldt, 1996).

### 4.4.4 Zooplankton development

After the start of the experiment, the increase in chlorophyll-a was immediately followed by an increase in microzooplankton biomass. There were significant differences between treatments and the highest microzooplankton biomass values occurred in the mesocosms with the highest nutrient loads. In mesocosm NOSTRAT-H2 more microzooplankton biomass might haven been expected, based on the observed high chlorophyll-a concentrations. No significant differences between Bottom and Top samples were found during the mixed and stratified periods, showing a lack of relation between the vertical chlorophyll-a distribution and the vertical distribution of microzooplankton.
Mesozooplankton biomass in the mesocosms did not reach high values. In mesocosm STRAT-L1 the highest biomass was found, mainly formed by some large polychaete larvae in the samples. In the other mesocosms calanoid copepods, in particular, contributed to mesozooplankton biomass, but there were no differences in copepod biomass between treatments.
In only one mesocosm (STRAT-L1) a significant difference was found between the Bottom and Top samples, for the entire experiment. The number of observations during the stratification period was too small to give significant differences. Thus, generally no uneven vertical distribution of microzooplankton or mesozooplankton was found, neither under mixed nor under stratified conditions. From literature it is well-known that stratified conditions almost always will lead to an uneven distribution of zooplankton in the water column (see references in Chapter 3), forced by an uneven distribution of chlorophyll containing food particles. Figure 4.7 shows that there were no large differences in phytoplankton biomass between Bottom and Top (with the exception of some smaller peaks in the STRAT-H mesocosms). Consequently an uneven distribution of zooplankton was not to be expected, as was the case also in the experiment described in Chapter 3.

## 4.5 CONCLUSIONS

-The mesocosms are suitable for the study of processes involved with stratification events.
-Stratification decreased the nutrient availability for phytoplankton and induced lower peaks of phytoplankton biomass when compared to well mixed mesocosms (Hypothesis 1).
-Contrary to expectations (Hypothesis 2), highest flagellate biomass was observed in the mixed mesocosms. This high biomass was mainly *Phaeocystis* sp., that grows well under nutrient-replete conditions.
-In the stratified mesocosms, microzooplankton grazing was an important factor determining phytoplankton species composition. When grazing controlled *Phaeocystis* sp., *Prorocentrum micans* became dominant in the stratified mesocosm with high nitrogen loading (Hypothesis 3). With reduced grazing control, *Phaeocystis* sp. outcompeted *Prorocentrum micans.*
-There was no clear effect of nitrogen loading on flagellate abundance in the stratified mesocosms, due to a short bloom of *Chattonella* sp. in one of the mesocosms with low nitrogen loading.
-Maximum chlorophyll-a levels were highest in the Top layer of the stratified mesocosms with high nitrogen loading, although differences between high and low nitrogen treatments were not statistically significant due to the short duration of the bloom.
-Microzooplankton biomass was highest in the mesocosms with high nitrogen loading. Also, microzooplankton biomass in the stratified

mesocosms with high nitrogen loading was higher than in the mixed mesocosms with similar nutrient loading. Mesozooplankton biomass showed no differences between treatments

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# Appendix I : The impact of zooplankton grazing on algal growth dynamics

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contribution EUPRO

1997

## SUMMARY

The main purpose of the EUPRO-project was to establish quantitative relationships between haline stratification and various nitrogen/phosphorus (N/P) load ratios on one hand, and the algal species composition and productivity on the other hand, by means of mesocosm experiments. Here, the NIOZ contribution to EUPRO, a study on the impact of zooplankton grazing on algal species selection and growth dynamics, is presented. After a pilot study in 1996, the measurements on phytoplankton were performed during Experiment 1 (also see Chapter 3) from 27$^{th}$ May until 12$^{th}$ June 1997 (from Julian day 147 to 163). Measurements were carried out in the completely mixed mesocosms NOSTRAT-H, and the haline stratified mesocosms STRAT-H.

The specific growth and grazing rates were determined according to the Landry and Hasset (1982) dilution method. The different dilution series were analysed for chlorophyll-a and concentration of autofluorescent particles (determined by flow-cytometry).

Algal growth rates varied between -0.024 and 0.022 $h^{-1}$. Combining all the data, it was found that the average specific growth rate was 0.006±0.009 $h^{-1}$ (n=16) according to the Chl-a method, whereas the flow-cytometric detection (FC) indicated a value of 0.010±0.007 $h^{-1}$ (n=15). On the average, the grazing rates were somewhat higher, i.e. 0.013±0.005 $h^{-1}$ (n=16) (chl-a detection) and 0.014±0.006 $h^{-1}$ (n=14) according to the FC method.

In mesocosms NOSTRAT-H1 and -H2 similar patterns were observed where at relatively constant grazing rates, the dynamics in algal biomass were effected by variation in their specific growth rates. A dense bloom developed in the upper layer of STRAT-H1 in the presence of grazing activity. In STRAT-H2 also the highest chl-a was observed in the upper water layer.

Combination of all data indicated that the turn-over of the pelagic foodweb, indicated by the specific algal growth rates and microzooplankton grazing rates, decreased with eutrophication. In other words, nutrient recycling became less efficient with increasing eutrophication. The presented data can be used for the improvement of mathematical models which evaluate the impact of eutrophication on coastal ecosystems.


## INTRODUCTION

EUPRO (Peeters et al. 1995), "De invloed van Eutrofiëring op de PROductiviteit van mariene ecosystemen", is a Dutch mesocosm research programme subsidised by BEON. Three institutes, the RIKZ, NIOO-CEMO, and NIOZ, investigated the impact of nutrient load and zoobenthos grazing on the algal species composition in marine mesocosms. The main purpose of the project was to establish quantitative relationships between haline stratification and various nitrogen/phosphorus (N/P) load ratios on one hand, and the algal species composition and productivity on the other hand. Here, the NIOZ contribution to EUPRO, a study on the impact of zooplankton grazing on algal species selection and growth dynamics in the mesocosms, is presented. EUPRO is embedded in BEON as part of the national eutrophication research, being initiated as a response to enhanced eutrophication of the Dutch coastal waters (Anonymous 1991; Klein & van Buuren 1992; Laane 1992; Peeters et al. 1993) and the potential increase of nuisance blooms and harmful algal species (Zevenboom et al. 1991; Peperzak 1994).

In oligotrophic waters, the planktonic food web is mainly driven by pico- and nano-phytoplankton productivity. The dynamics of these algae are largely controlled by their (microzooplankton) predators. During the recent decade, evidence has been accumulating that indicates that also in eutrophicated systems with a dominance of larger algal species, microzooplankton is at least as active as in oligotrophic waters. Very often, their grazing rates balance the growth rates of the pico- and nanophytoplankton (Verity 1986; Sherr et al. 1989; Paranpaje 1990; Frost 1991; Landry et al. 1992; Riegman et al. 1993). Since larger phytoplankton, such as diatoms, dinoflagellates, and colony forming algae lie outside the microzooplankton prey spectrum (Strom & Welschmeyer 1991), a logical consequence of size differential grazing control is that nutrient input in nutrient controlled areas will lead to blooms of larger algae (Kumar et al. 1991; Riegman et al. 1993; Riegman & Kuipers 1994). These changes in species composition may facilitate shifts in fluxes and the productivity of the pelagic foodweb. Additionally, eutrophication may favour K-selection on algal species, resulting in the dominance of poorly growing and therefor poorly productive, and possibly even toxic, algae (Smayda 1990; Riegman and Kuipers 1994; Riegman 1995).
In 1996, a pilot study on the impact of stratification of mesocosms was performed within the EUPRO project at Middelburg (Escaravage et al., 1997). Attempts were made to introduce haline stratification in 3 $m^3$ MERL-type mesocosms located at the RIKZ field station at Jacobahaven. The residence time of the mesocosms was 30 days and six mesocosms were exposed to various nutrient loads and benthos compartments. The results of the microzooplankton grazing measurements during the pilot study in 1996 are presented elsewhere (Riegman & Snoek 1996). Algal biomass was in the oligotrophic range (0.5-3 $\mu g\ l^{-1}$) for all investigated mesocosms. Grazing and algal growth rates were found to be highly variable and on the average 0.3 $d^{-1}$ in the most eutrophic mesocosm (M4), and 0.44 $d^{-1}$ in the most oligotrophic mesocosm (M5). This indication, that the mesocosms with the lowest algal biomass showed a higher algal turn-over than the more eutrophic mesocosms, was not very sound due to strong fluctuations in algal biomasses, as well as turn-over rates.
In 1997 the NIOZ made a contribution by measuring the microzooplankton grazing on phytoplankton during Experiment 1 (also see Chapter 3) from 27$^{th}$ May until 12$^{th}$ June 1997 (from Julian day 147 to 163). Measurements were carried out in the completely mixed mesocosms NOSTRAT-H, and the haline stratified mesocosms STRAT-H. In this final report, the results are presented and evaluated under the consideration of "today's state of the art" in marine food web research studies.

## MATERIAL AND METHODS

The technical details about the mesocosms and the experimental design are given in Chapters 2 and 3. In principle, attempts were made to stratify mesocosms STRAT-H, whereas mesocosms NOSTRAT-H remained completely well mixed. Within the period 27th May until 12th June 1997 (Julian days 147-163), a 25 l sample was collected from one of the mesocosms every working day. 50% of this sample was filtered over Whatman GF/F. The filtrate was used to make the dilution series (Landry & Hasset 1982). The dilutions were made in duplicate. The concentration at t=0 was 20, 40, 60, 80, and 100% of the original algal density at the time of sampling. 1 l polycarbonate bottles were used to incubate the dilutions on a rotating wheel (5 rpm) for 24 h at ambient temperature and irradiance levels. From each

dilution, 200 ml samples were taken at t=0 and t=24h. These samples were filtered over Whatman GF/F. Additionally, 10 ml sub-samples were taken for flow-cytometric analysis of auto-fluorescent particles (algae). After the bottles were filled completely, they were sealed with household wrapping foil (plastic) and closed with polycarbonate screw caps in such a way that no air bubbles were present during the incubation at 15 °C, with a L:D rhythm of 16-8 h and at irradiance levels of 60 $\mu E\ m^{-2}\ s^{-1}$.
Chlorophyll-a was determined after addition of 10 ml 90% acetone (in demi) to the GF/F filters and extraction during at least 24 h at -35 °C in the dark. The extract was measured on a Hitachi F-2000 fluorimeter before and after addition of two drops 10N HCl. Spinach chl-a (Sigma) was used as a standard.
For each dilution the net algal growth rate was calculated and the linear regression between the different dilutions yielded the values for the specific growth and grazing rates. The subsamples for the flow-cytometer were analysed for the total number of autofluorescent particles, followed by similar mathematical treatment as the chl-a data.

**RESULTS**

Two different types of results can be distinguished. Firstly, the results are presented which involve a comparison between measurements on the dilution series by determining of chlorophyll-a (chl-a) and by the determination of autofluorescent particles by means of the flow-cytometer (FC). Secondly, the development of algal biomass, their growth rates and the grazing rates, will be presented for the different mesocosms.
In principle, detection of chl-a or flow-cytometric measurement of the number of fluorescent particles should yield the same values for grazing and growth rates. As shown in Fig. I.1, there was a reasonable agreement between the results of both methods with respect to the estimated algal growth rates in those cases where these rates were estimated simultaneously. Algal growth rates varied between -0.024 and 0.022 $h^{-1}$. Combining all the data, it was found that the average specific growth rate was 0.006±0.009 $h^{-1}$ (n=16) according to the chl-a method, whereas the flow-cytometric detection (FC) indicated a value of 0.010±0.007 $h^{-1}$ (n=15). On the average, the grazing rates were somewhat higher, i.e. 0.013±0.005 $h^{-1}$ (n=16) (chl-a detection) and 0.014±0.006 $h^{-1}$ (n=14) according to the FC method. In those measurements where grazing was detected simultaneously by both methods, the chl-a method resulted in a 60% lower specific grazing rate than the FC method estimate (Fig. I.2).
Comparing the different mesocosms indicated that the algal species composition was not identical in all mesocosms. Where the fully mixed mesocosm 5 (NOSTRAT-H2) and the stratified mesocosms 1 and 6 (STRAT-H) contained on the average smaller algae with an average chl-a content of 0.28 pg chl-a/cell, the fully mixed mesocosm 3 (NOSTRAT-H1) was dominated by algae with a higher cellular pigment content (0.71 pg chl-a/cell) (Fig. I.3).
In mesocosm STRAT-H1, the chl-a levels increased in the upper layer, and decreased in the lower layer (Fig. I.4). In the upper layer, growth and grazing rates were in balance with each other, and lower than in the deeper layer. Here, at low algal biomass, growth decreased with time, whereas grazing pressure increased. In total, the balance between growth and grazing was negative, resulting in a decrease in chl-a in the deeper layer.

**Figure I.1.**
Evaluation of all estimated growth rates: The correlation between the specific phytoplankton growth rate determined by chl-a and by flow-cytometry.

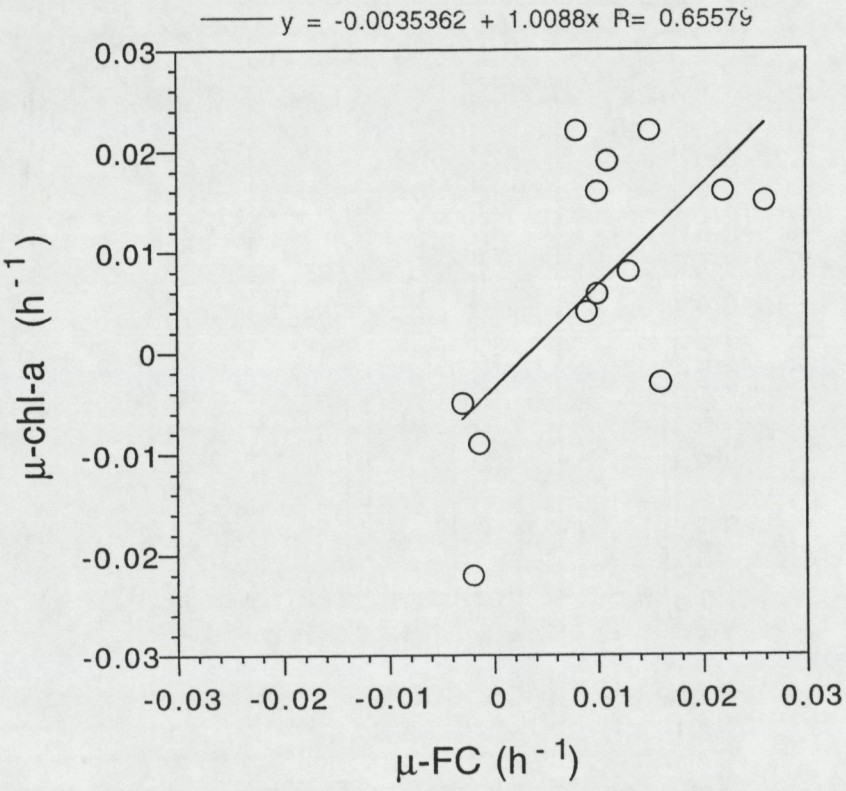



**Figure I.2.**
Evaluation of all estimated grazing rates: The correlation between the biomass specific grazing rate on phytoplankton, determined by chl-a and by flow-cytometry.

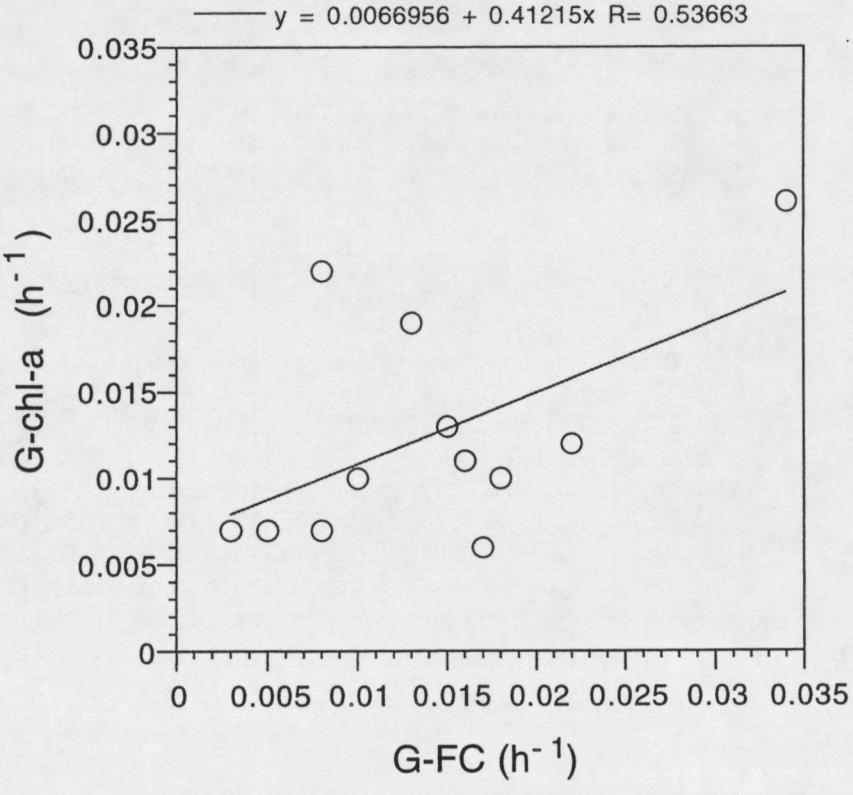


**Figure I.3.**
The average cellular chlorophyll-a content of phytoplankton in the different mesocosms during the period 27th May '97 - 12th June '97.

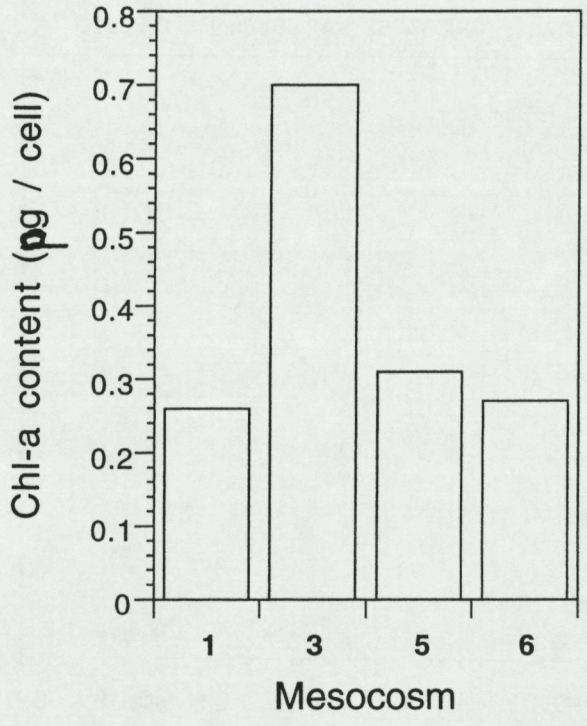



**Figure I.4.**
Variations during the time course (three weeks) of the experiment in algal biomass (chl-a), and specific grazing and growth rates in mesocosm STRAT-H1. Samples were collected from the upper layer (U), the middle layer (M), or the lower layer (L).

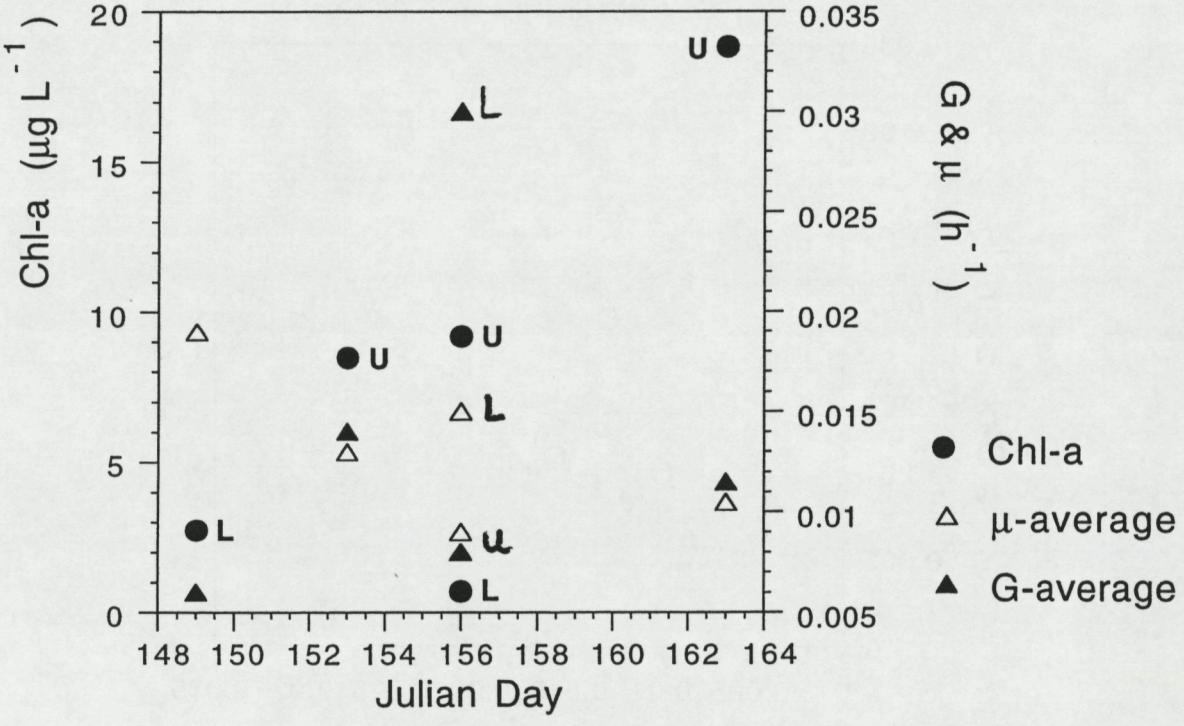


**Figure I.5.**
Variations during the time course (three weeks) of the experiment in algal biomass (chl-a), and specific grazing and growth rates in mesocosm NOSTRAT-H1. Symbols and abbreviations as in Fig. I.4.

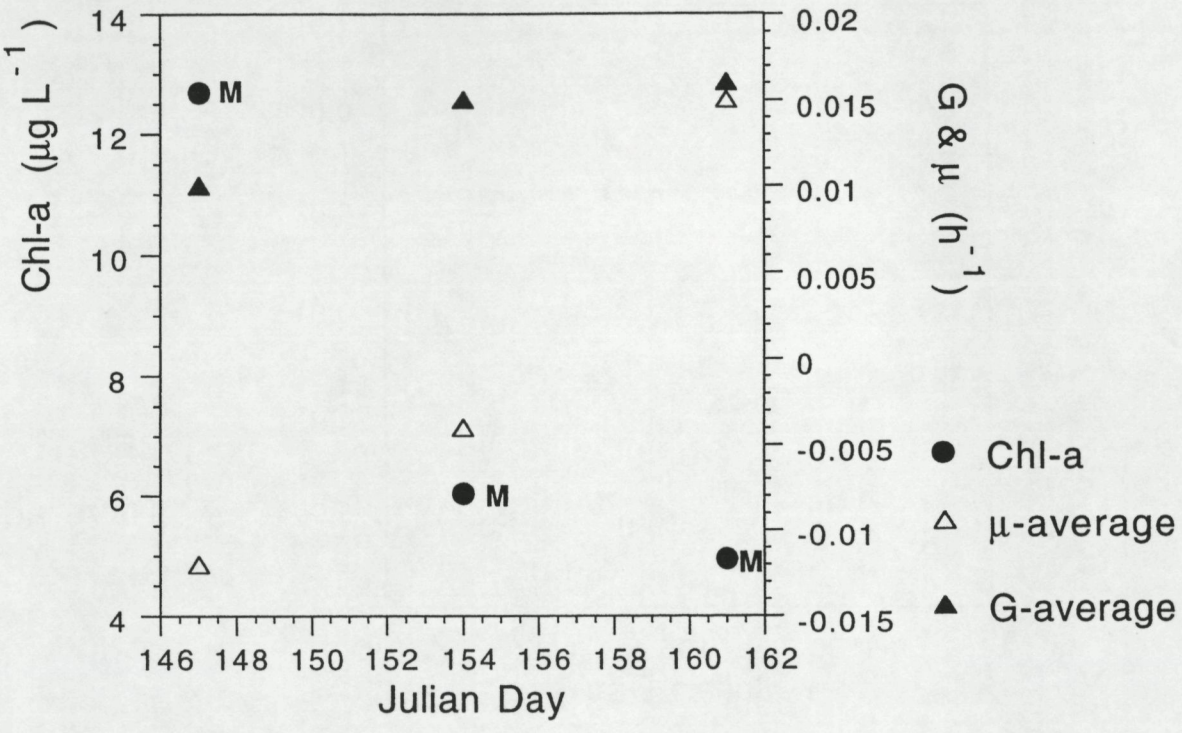



**Figure I.6.**
Variations during the time course (three weeks) of the experiment in algal biomass (chl-a), and specific grazing and growth rates in mesocosm NOSTRAT-H2. Symbols and abbreviations as in Fig. I.4.

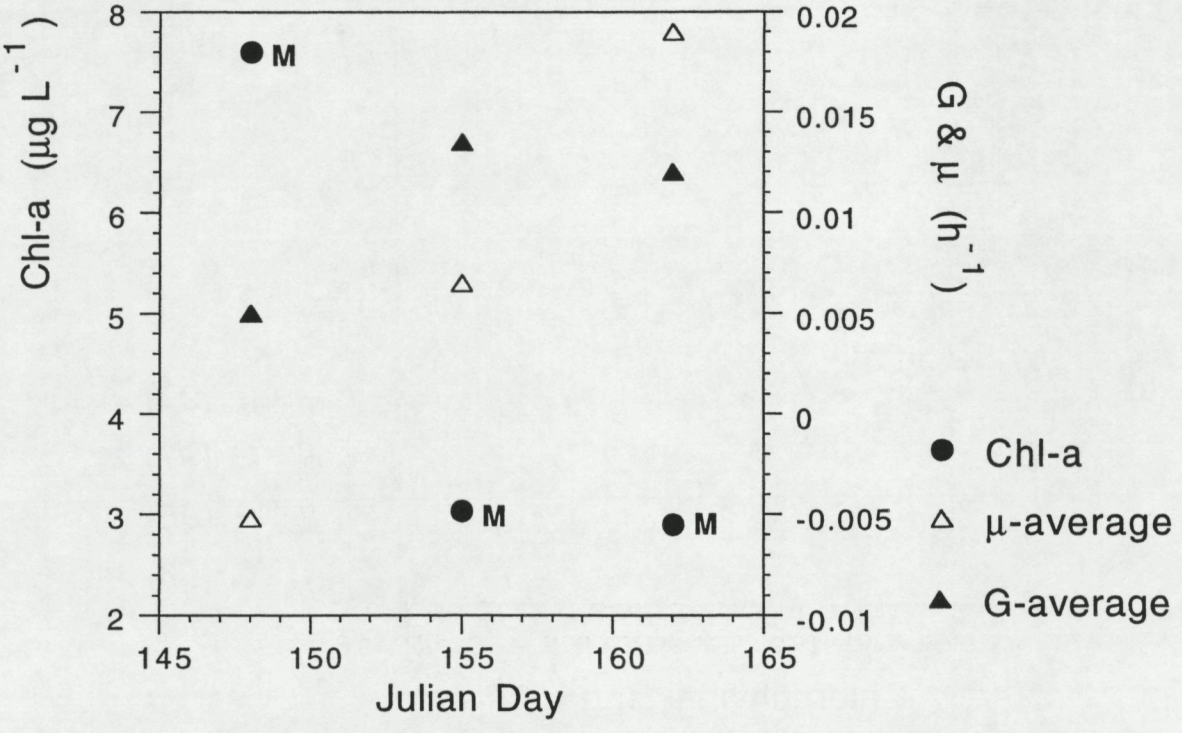


**Figure I.7.**
Variations during the time course (three weeks) of the experiment in algal biomass (chl-a), and specific grazing and growth rates in mesocosm STRAT-H2. Symbols and abbreviations as in Fig. I.4.

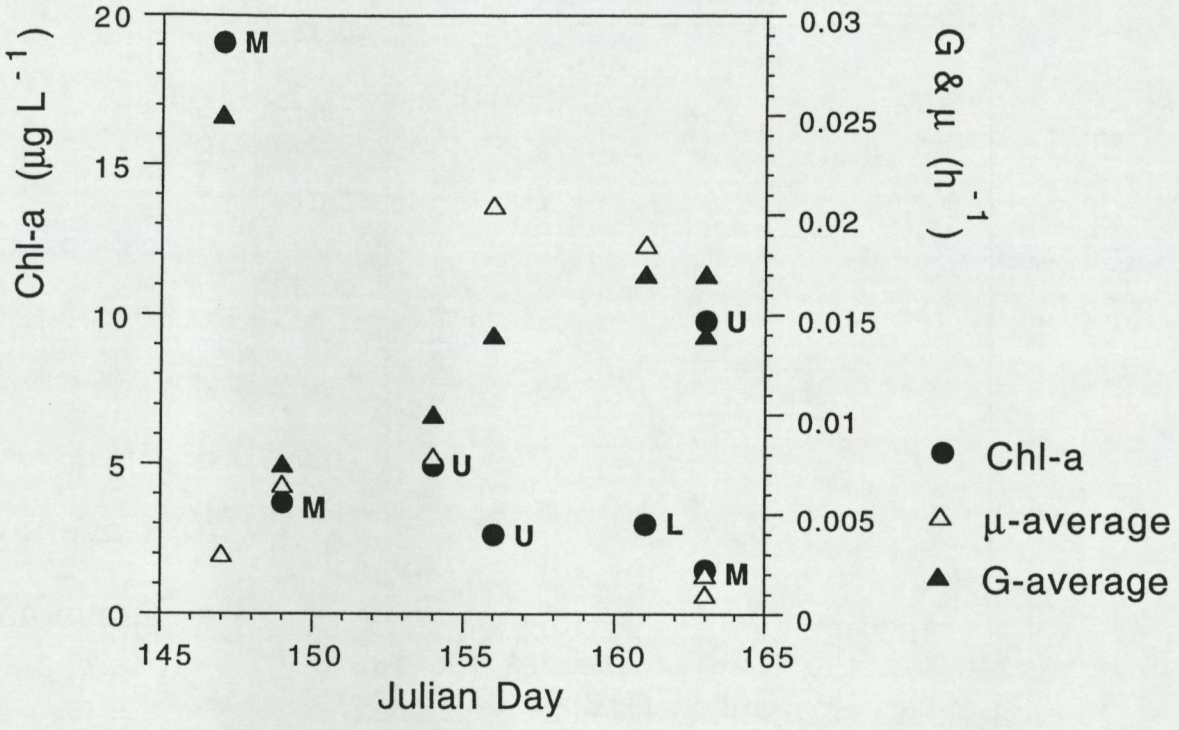
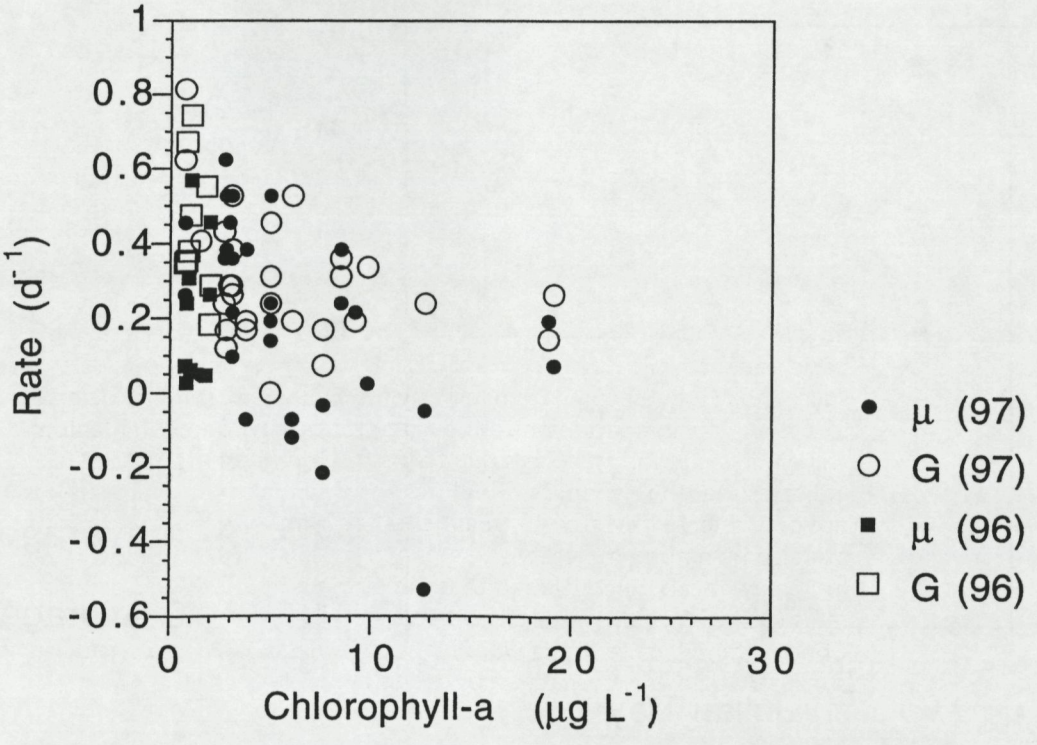



**Figure I.8.**
Compilation of all data on the specific rates of grazing on, and growth rates of phytoplankton as a function of the algal biomass (chl-a) in the four different mesocosms.



In mesocosm NOSTRAT-H1, the algal biomass decreased from 12.7 to 5 µg $l^{-1}$ (Fig. I.5). The grazing pressure on the phytoplankton was constantly high (±0.015 $h^{-1}$), whereas the specific algal growth rate was low at the start of the measurements and increased when algal biomasses started to decrease. At day 161, at the end of the measurement period, growth and grazing were balanced at a much lower algal biomass level than at the start of the experiment.
In mesocosm NOSTRAT-H2, a similar pattern as in NOSTRAT-H1 was observed (Fig. I.6). At a grazing pressure of ±0.01 $h^{-1}$, and a negative growth rate (indicating autolysis of algal cells) at the start of the period, the algal biomass declined from 7.6 to 3 µg $l^{-1}$. The specific growth rate of the algae started to increase and became even higher than the grazing losses at the end of the period.
In the stratified mesocosm STRAT-H2, grazing was in almost all cases higher than the specific growth rate of the algae (Fig. I.7). After the highest growth rate was reached at day 156, it declined whereas the grazing rate showed a small increase.
Combining all data on grazing and growth, indicates that there was some sort of inverse correlation with algal biomass (Fig. I.8). This implies that the turn-over of nutrients in the pelagic foodwebs in the mesocosms was lower when higher algal biomasses were present.
A compilation of all results is given in Table I.1.

## DISCUSSION

The observation that lower growth rates were measured when chl-a was used as biomass than when flow-cytometry was used has also been observed in 1996. The chl-a per cell decreased during the time course of some of the experiments (data not shown). This indicates that during these incubations physiological adaptation occurred where the irradiance values were slightly higher than the *in vivo* levels. In some cases, even negative algal growth rates were observed. This is not necessarily due to active degradation of chl-a since flow-cytometry indicated the same results as the chl-a detection. One, usually overlooked, factor in algal succession in mixed communities is autolysis. It has been shown, that 3-20 % of the phytoplankton can die per day, due to natural mortality (Brussaard et al. 1995, 1996). This mortality is not necessarily due to viral infection, but can also be triggered by nutrient limitation. The dynamics of autolysis mortality is species dependent, and dependent on the type and extent of nutrient stress (Brussaard 1997). Field populations are always exposed to changing environmental conditions. Algae tend to adapt to these variations in environmental conditions, but their magnitude of adaptation is not for each species sufficient enough to compensate for environmentally induced stress (*i.e.* a physiological unbalance in the basic metabolism). Consequently, species which are capable to adapt successfully may become dominant by multiplication. Others, being less successful in adaptation to the given set of environmental factors, will diminish, amongst others due to losses by natural mortality. This indicates that the dynamics of natural populations of phytoplankton is not only affected by growth, sedimentation, viral diseases and grazing, but also by natural mortality (Riegman in press).
In general it can be concluded that the observed growth and grazing rates (0.25-0.35 $d^{-1}$) were lower than in 1996 (0.3-0.44 $d^{-1}$). Concomitantly, chl-a levels were higher than in 1996. This conclusion suggests an inverse correlation between the turn-over of phytoplankton, and its biomass. In Fig. I.8, this inverse relation is clearly recognisable. If this observation is indicative for a general trend

**Table I.1**
Compilation of presented data on phytoplankton biomass, growth and grazing rates during May-June 1997 in the RIKZ mesocosms at Jacobahaven.

| JULIAN DAY | MESOCOSM | CHL-A $\mu g\ L^{-1}$ | CELL CONCEN-TRATION $mL^{-1}$ | SPEC. GROWTH RATE (CHL-A) $d^{-1}$ | SPEC. GROWTH RATE (FC) $d^{-1}$ | SPEC. GRAZING RATE (CHL-A) $d^{-1}$ | SPEC. GRAZING RATE(FC) $d^{-1}$ |
|---|---|---|---|---|---|---|---|
| 147 | NOSTRAT-H1 M | 12.7 | 17880 | -0.53 | -0.05 | 0.24 | 0.24 |
| 154 | NOSTRAT-H1 M | 6.04 | 13900 | -0.12 | -0.07 | 0.53 | 0.19 |
| 161 | NOSTRAT-H1 M | 4.95 | 5170 | 0.53 | 0.19 | 0.47 | 0.31 |
| 147 | STRAT-H2 M | 19.1 | | 0.17 | | 0.26 | |
| 149 | STRAT-H2 M | 3.73 | 7400 | -0.07 | 0.38 | 0.17 | 0.19 |
| 154 | STRAT-H2 U | 4.96 | | 0.14 | 0.24 | 0.24 | |
| 156 | STRAT-H2 U | 2.65 | 12360 | 0.36 | 0.62 | 0.24 | 0.43 |
| 161 | STRAT-H2 L | 3.01 | 13800 | 0.53 | 0.36 | 0.29 | 0.53 |
| 163 | STRAT-H2 M | 1.48 | | 0.05 | | 0.41 | |
| 163 | STRAT-H2 U | 9.8 | 62800 | | 0.02 | | 0.34 |
| 148 | NOSTRAT-H2 M | 7.6 | 14600 | -0.22 | -0.03 | 0.17 | 0.07 |
| 155 | NOSTRAT-H2 M | 3.04 | 17100 | 0.09 | 0.22 | 0.26 | 0.38 |
| 162 | NOSTRAT-H2 M | 2.9 | 13000 | | 0.46 | | 0.29 |
| 149 | STRAT-H1 L | 2.74 | 9537 | 0.38 | 0.53 | 0.17 | 0.12 |
| 153 | STRAT-H1 U | 8.47 | 43700 | 0.38 | 0.24 | 0.31 | 0.36 |
| 156 | STRAT-H1 U | 9.18 | | 0.22 | | 0.19 | |
| 156 | STRAT-H1 L | 0.7 | 2098 | 0.46 | 0.26 | 0.62 | 0.82 |
| 163 | STRAT-H1 U | 18.83 | 86800 | 0.19 | 0.31 | 0.14 | 0.41 |
| Av. | | 6.77 | 22868 | 0.16 | 0.25 | 0.29 | 0.33 |
| s.d. | | 4.25 | 17814 | 0.22 | 0.16 | 0.11 | 0.13 |

in marine ecosystems, it would mean that the turn-over of nutrients slows down when a foodweb becomes more eutrophic. Observations which indicate a similar trend have been reported by Herndl (1991) who studied the microbial foodweb along a trophic gradient at the Atlantic Reef off Belize. The turn-over of bacteria, cyanobacteria, and heterotrophic nanoflagellates decreased with eutrophication. In the hypertrophic Italian Comacchio Lagoons, Sorokin et al. (1996)

observed dense blooms of cyanobacteria populations with hardly any growth or grazing activity. The eutrophication induced reduction in biomass turn-over in pelagic food webs may be the result of a shift in algal species composition towards less edible species. This mechanism has been discussed in detail elsewhere (Riegman et al. 1993; Riegman 1995, in press). Basically, it is assumed that with increasing primary production, the biomass of herbivores will increase, thereby effectively increasing the top-down control of the algal species composition. Algal species which are poorly edible will increase in density whereas the biomass of edible species will be kept under grazing control. Apart from toxicity, and cell shape, there may be a correlation with cell size (Stolte et al. 1994; Stolte & Riegman 1995; Riegman et al. 1996). Eutrophic foodwebs are usually dominated by larger species (Raimbault et al. 1988). The smaller species usually grow faster (Raven 1984), but their predators are also small. The relatively high intrinsic maximum growth rate of small herbivores enables them to control their prey density more easily than larger herbivores can do (Banse 1976).
Another possible explanation for the reduced growth rates at higher algal biomass might be that either light-limitation or nutrient-limitation of the growth rate will become more severe at higher cell densities. Implicitly, this means that the clearance of the watercolumn and/or the nutrient recycling by herbivoric grazing apparently is not enough to compensate for phytoplankton growth demands. So the key question seems whether the predators are inadequate as a functional group, or whether their food source becomes less suitable as a consequence of selective grazing by the very same herbivores. Of course, both mechanisms may be involved at the same time.
Detailed information concerning the algal dynamics in the different mesocosms is presented in Chapter 3. The data on phytoplankton growth rates and microzooplankton grazing presented here were used for a more extensive analysis of the dynamics of the pelagic food web in the different mesocosms. Quantification of these processes will be useful for the validation of mathematical models which have been designed to simulate and evaluate the impact of eutrophication of marine ecosystems.

## ACKNOWLEDGEMENTS

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# Appendix II : Short-term changes in photosynthesis: a comparison between C-fixation and PSII electron flow

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## INTRODUCTION

Photosynthesis can be a very dynamic process. The time scale of the primary process is in the order of µs –ms and the time scale of dark reaction, i.e. the fixation of $CO_2$ is in the order of a sec-min. Hence, it can be expected that large changes can occur in a relatively short time. In order to investigate this, we undertook a detailed sampling program on 14 and 15 May 1997 (Julian days 134-135, Experiment 1), in which mesocosms STRAT-L1 and STRAT-L2 (see Table II.1) were sampled in the top (0.2m depth) and bottom compartment (2.7 m depth) 5 times during the day. Photosynthesis was measured in two ways: 1), using the standard C-fixation method, and 2), by measuring photosystem II electron flow using a PAM-fluorometer.

## METHODS

### C-fixation

Particulate C-fixation was measured by incubating duplicate 50 ml samples (to which 925 kBq $ml^{-1}$ $^{14}C$-$NaHCO_3$ (Amersham) was added) in a rotating incubator on board the R.V. Luctor, immediately after sampling. Dissolved inorganic carbon was determined by potentiometric titration. The rate of C-fixation in the dark bottles was subtracted from the light bottles in order to avoid overestimates of primary production by phytoplankton due to chemosynthetic processes. The photosynthesis/irradiance (P/E-curves) were fitted according to Eilers and Peeters (1988), as described in Chapter 2.

### Chlorophyll fluorescence

Variable chlorophyll (chl) fluorescence originates mainly from light emission antenna of (PSII). Excitation energy obtained by absorption of the light harvesting pigment protein complexes of photosystem II (PSII) cannot only be used for photochemistry, but can also be lost by thermal dissipation (heat) or as fluorescence. These processes compete and influence the energy conversion efficiency or quantum yield (see Krause and Weis, 1991; Kolber and Falkowski, 1993; Dau, 1994 and Horton and Ruban, 1994 for reviews). Linear electron transport will only commence following charge separation. When the cells are fully dark adapted, the primary electron acceptor of PSII, $Q_A$, is in the oxidised state (all reaction centres are "open"), non-photosynthetic energy dissipating mechanism will be minimal, and the quantum yield will be maximal. Closure of PSII reaction centres by capture of irradiance will increase the fluorescence yield and induce energy dissipating processes (non-photochemical (qN) quenching). This will decrease the quantum efficiency. When all energy dissipating mechanisms are saturated, photoinhibition can occur, leading to net degradation of the D1-reaction centre II protein (Ohad et al., 1994). In this case, qN-quenching will slowly relax upon transfer to the dark.

Variable fluorescence was measured with a PAM 101-103 fluorometer (Walz Effeltrich, FRG), which controlled the Schott KL-1500/E light source used for administering the saturating light pulses. The photon flux density (PFD) of the light pulse was more than 10000 µmol $m^{-2}$ $s^{-1}$, i.e. high enough to close all reaction centres. Saturating light pulses were given at 30 sec intervals. We made the pulse length, which varied between 500-700 ms, as short as possible in order to avoid an intrusive effect of the pulses. For actinic light a Schott KL1500 light source was used, in combination with the PAM fibre optics. 8-9 exposures (2 minutes each) were made with PFD's ranging from 0 to 800 µmol $m^{-2}$ $s^{-1}$ in order to construct photosynthesis (i.e. ETR) light curves. The photon irradiance was adjusted by changing the voltage of the Schott Kl1500 lamp. The sensitive ED101US/PM emitter detector unit (Walz) was used.
Quenching coefficients were calculated after van Kooten and Snel (1990).
The maximum energy conversion efficiency or quantum efficiency of PSII charge separation ($\Phi_P°$) is calculated as:

$$\Phi_P° = (Fm - Fo)/Fm = Fv/Fm \quad (1).$$

Fo is the minimum fluorescence, Fm is the maximum fluorescence yield of a minimally 15 min dark adapted sample.

According to Genty et al. (1989) the effective quantum efficiency of charge separation in actinic light is

$$\Phi_P = (Fm' - Fs)/Fm' = \Delta F/Fm' \quad (2).$$

$\Delta F/Fm'$ can be used to calculate the linear rate of photosynthetic electron transport (ETR) as (Genty et al., 1988; Kolber & Falkowski, 1993; Hofstraat et al., 1994):

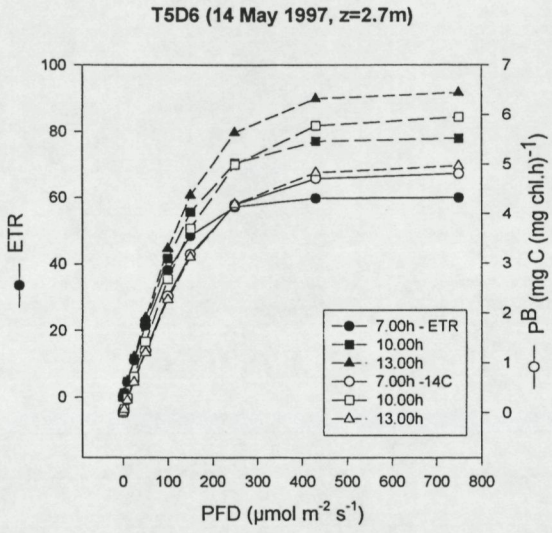
$$ETR = \Delta F/Fm' \times PFD \times \sigma_{PSII} \quad (3),$$

where $\sigma_{PSII}$ is the functional cross section of PSII. The product of PFD and $\sigma_{PSII}$ equals the amount of absorbed light by a PSII unit. ETR is called Je by Hofstraat et al. (1994). As we could not measure $\sigma_{PSII}$, relative ETR was calculated as $\Delta F/Fm' \times PFD$.

## RESULTS AND DISCUSSION

Fig. II.1 shows an example of some light response curves from bottom compartment samples taken from STRAT-L1. As can be seen, there was some change in the shape of the P/E-curve, and the lowest activity was observed in the morning sample. Both the chlorophyll specific C-fixation and the ETR curves showed the same pattern. From the data, the photosynthetic parameters $P^B_{max}$, $ETR_{max}$ and the slopes of the lines ($\alpha^B$ and $\alpha$(ETR) resp.) were calculated using the Eilers and Peeters (1988) fit procedure, in order to investigate whether the observed changes in photosynthetic characteristics were statistically significant (Table II.1). On 14 May, the difference in $P^B_{max}$ between both depths was only significant for STRAT-L1. On both days phytoplankton in STRAT-L2 had higher $P^B_{max}$ as well as $\alpha^B$-values compared to STRAT-L1. $\alpha^B$-values were also different between both depths in both mesocosm on 14 May. However, at the following day, the difference between both depths had disappeared.

**Figure II.1**
Light response curve for C-fixation and PSII electron flow (ETR) in mesocosm STRAT-L1.

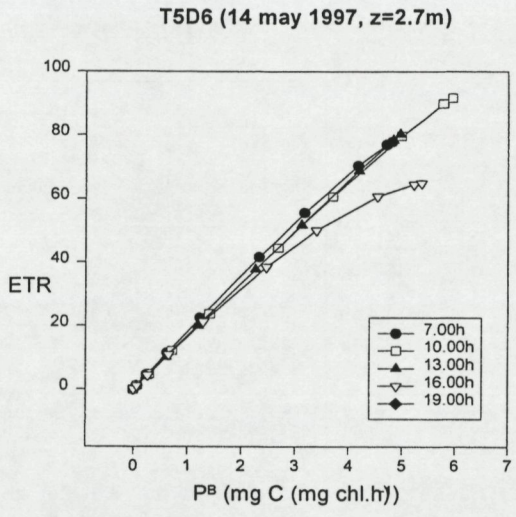



The pattern in PSII electron flow (ETR) showed a different pattern. Maximum rates of ETR of phytoplankton in mesocosm STRAT-L2 differed between both depths on 14 May, but were absent the next day. The opposite was found for mesocosm STRAT-L1. These differences with depths suggest that mixing was not complete. Comparison of both mesocosms showed that $ETR_{max}$ was different

**Table II.1**
Comparison of photosynthetic parameters obtained from C-fixation measurements ($P^B_{max}$ and $\alpha^B$) and PSII electron flow ($ETR_{max}$ and $\alpha$(ETR)) in mesocosms STRAT-L1 and STRAT-L2. The data should be compared pairwise, i.e. L1 at 0.2m depth with L1 at 2.7m depth. *: $p<0.05$; **: $p<0.01$; ns: not significantly different.

| meso | $P^B_{max}$ | | $a^B$ | | $ETR_{max}$ | | a(ETR) | |
|---|---|---|---|---|---|---|---|---|
| **14-05-97** | | | | | | | | |
| L1 0.2m | 6.01 | | 0.0297 | | 65.7 | | 0.380 | |
| L1: 2.7m | 5.26 | * | 0.0267 | * | 75.0 | ns | 0.451 | ** |
| L2: 0.2m | 7.77 | | 0.0345 | | 85.6 | | 0.443 | |
| L2: 2.7m | 6.79 | ns | 0.0305 | ** | 92.5 | * | 0.448 | ns |
| L1 both | 5.63 | | 0.0281 | | 70.3 | | 0.415 | |
| L2 both | 7.28 | ** | 0.0329 | ** | 89.1 | * | 0.446 | ** |
| **15-05-98** | | | | | | | | |
| L1 0.2m | 11.82 | | 0.0595 | | 76.3 | | 0.450 | |
| L1: 2.7m | 11.09 | ns | 0.0538 | ns | 87.5 | * | 0.450 | ns |
| L2: 0.2m | 18.55 | | 0.0860 | | 91.1 | | 0.450 | |
| L2: 2.7m | 17.68 | ns | 0.0794 | ns | 89.9 | ns | 0.450 | ns |
| L1 both | 11.50 | | 0.0570 | | 81.9 | | 0.450 | |
| L2 both | 18.16 | ** | 0.0831 | ** | 90.5 | ns | 0.450 | ns |
| Both days | | | | | | | | |
| L1 | 8.41 | | 0.0418 | | 76.1 | | 0.433 | |
| L2 | 12.44 | ** | 0.0567 | ** | 89.8 | * | 0.448 | ** |

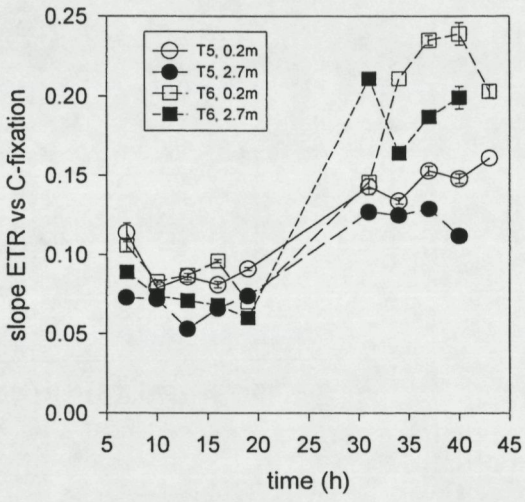
**Figure II.2**
C-fixation plotted as a function of ETR of mesocosm STRAT-L1. Samples from 2.7 m depth.




between both mesocosms on 14 May, but not on 15 May. It is interesting to notice that although the differences in $P^B_{max}$ were very large on 15 May, $ETR_{max}$ was not. Over the 2-day period, all photosynthetic parameters of phytoplankton from mesocosm STRAT-L1 were lower than those of phytoplankton in mesocosm STRAT-L2. This indicates that the relationship between PSII electron flow and chorophyll specific rate of C-fixation is quite complicated.

To investigate the relationship between C-fixation and PSII electron flow (ETR) we plotted the C-fixation rate as a function of ETR (Fig. II.2). As can be seen in this graph, the relationship was quite linear, and the slopes of the lines were similar. However, some non-linearity at high irradiances was observed at a sample from 16.00h. Possible explanations for non-linearity between ETR and $P^B$ are discussed by Flameling and Kromkamp (1998).
The reciprocal of the slope of the lines as plotted in Fig. II.2 can be viewed as the calibration factors needed to convert ETR data in chl-specific rates of C-fixation. These reciprocal slopes are plotted in Fig. II.3. As can be seen, the diurnal change in the slope was rather small, but the differences between the slopes from samples taken at the two different depths were significant, indicating incomplete mixing. However, on 14 May (time 7.00-19.00 h) the slopes obtained from mesocosm STRAT-L1 samples were not significantly higher than those measured from samples coming from mesocosm STRAT-L2. The next day, the differences between both depths were even more pronounced in both mesocosms, and also the difference between the corresponding slopes from both mesocosms were different. It is also clear that the values were higher (i.e. the reciprocal of the slope as shown in Fig. II.2 is smaller). This indicated that the amount of C-fixed per ETR increased on 15 May (31.00-43.00h in Fig. II.3). Hence, the phytoplankton showed a more efficient photosynthesis on 15 May 1997.

**Figure II.3**
Reciprocal of slopes between rates of C-fixation as function of ETR.
T5=STRAT-L1; T6=STRAT-L2




With the data plotted in Fig. II.3 the rates of C-fixation per mg of chlorophyll *a* ($P^B$) at depth z can be calculated as:

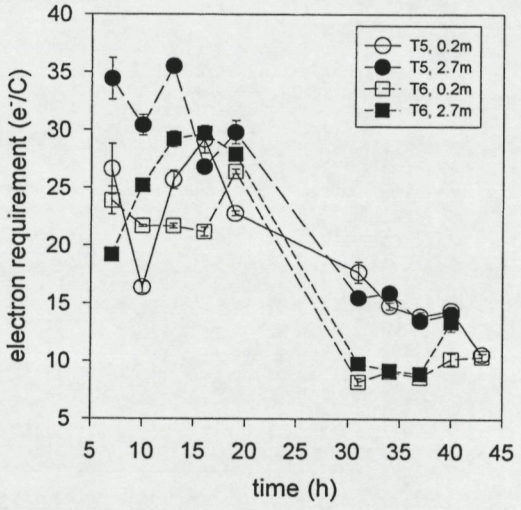
$$P^B_z = c \times PFD_z \times DF/Fm'_z \quad (4)$$

where c stands for the slope as plotted in Fig. II.3, $PFD_z$ for the irradiance at depth z and $DF/Fm'_z$ for the effective PSII quantum efficiency at that depth. This equation would be very useful if there was a constant value for c. However, as is obvious from Fig. II.3, this was not the case. The constant c in Eq. 4 is a composite parameter and is influenced by both the number of PSII units per mg of chlorophyll *a* ($n_{PSII}$), by the functional absorption cross section of PSII ($\sigma_{PSII}$, a measure of the amount of light absorbed by a PSII unit used for photosynthesis), and by the electron requirement for carbon fixation ($\Psi$):

$$P^B_z = \sigma_{PSII} \times PFD_z \times DF/Fm'_z \times n_{PSII} \times \Psi \quad (5)$$

It is likely that during a P/E-curve $n_{PSII}$ will not change. We could not measure $\sigma_{PSII}$ as we did not have the right equipment, but it is known that at high irradiance $\sigma_{PSII}$ can change (Kolber and Falkowski, 1993, Flameling, 1998). $\Psi$, *i.e.* the electron requirement for carbon fixation can be calculated from a plot of the quantum efficiency of PSII against the quantum efficiency of carbon fixation, which can be calculated from the rate of C-fixation at a particular irradiance and the absorption per unit chlorophyll (i.e. the optical absorption cross section $a^*$). Assuming a value for $a^*$ of 0.015 $m^2$ mg $chl^{-1}$, we calculated the quantum efficiency of carbon fixation (moles C fixed per mol quanta absorbed) and used this to calculate the electron requirement $\Psi$. The result is shown in Fig. II.4. As can be seen, assuming an a* of 0.015 $m^2$ mg $chl^{-1}$, phytoplankton photosynthesis was less efficient on the first day of the measurements, when 20-35 electrons were needed to fix a C-molecule. As shown in Fig. II.3, photosynthesis of phytoplankton at the deeper layers was more efficient than phytoplankton sampled near

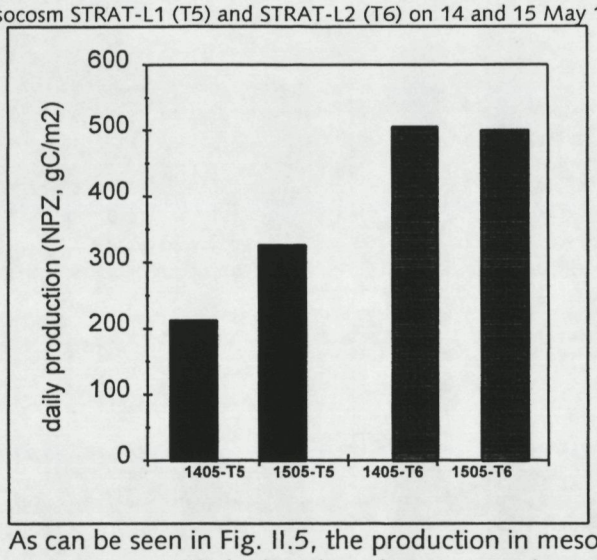
**Figure II.4**
Electron requirement for C-fixation.




the surface layer, but these differences were only observed during the first day (14 May) of the measurement program. It is difficult to explain these large differences between two consecutive days, especially as the bloom was seemingly collapsing. However, two days later the chlorophyll concentrations rose again, and the more efficient photosynthesis measured at the second day of the experiment might have been an early sign of improving conditions leading to a new bloom.

What is clear from these data, is that primary production can most likely change significantly from day to day. If this is the case, sampling frequencies have to be increased, although this will be costly and logistically very difficult. The photosynthesis measurements based on the variable chlorophyll fluorescence technique (i.e. the PAM or pump & probe based methods) are not fully developed yet to be used as an alternative to the C-fixation method, but further research has the potential to develop this technique so that practical application becomes possible. The advantage of variable fluorescence techniques is that it is an optical technique which can, in principal, be used without sample handling and can measure photosynthesis *in situ* and on-line. Although some differences in photosynthetic parameters of samples obtained at 0.2m or 2.7m depth were observed (see Fig. II.1), the differences were not very large, so differences between estimates of daily water column primary production made using those samples were small (not shown). Using all data available, accurate estimates were made for the net photic zone daily primary production in the mesocosms. From these estimates, it became apparent that large differences can occur.

**Figure II.5**
Daily net photic zone production in mesocosm STRAT-L1 (T5) and STRAT-L2 (T6) on 14 and 15 May 1997.




As can be seen in Fig. II.5, the production in mesocosm STRAT-L1 on 15 May was 50% higher than the previous day. Differences in mesocosm STRAT-L2 on the other hand were very small.

**Figure II.6**
Contour plots of the rates of primary production in mesocosm STRAT-L1.

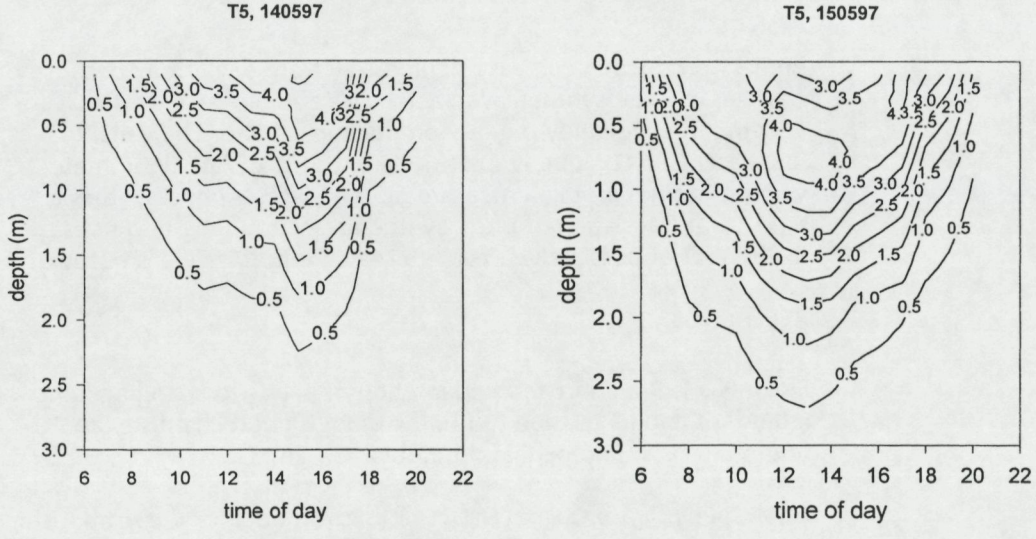
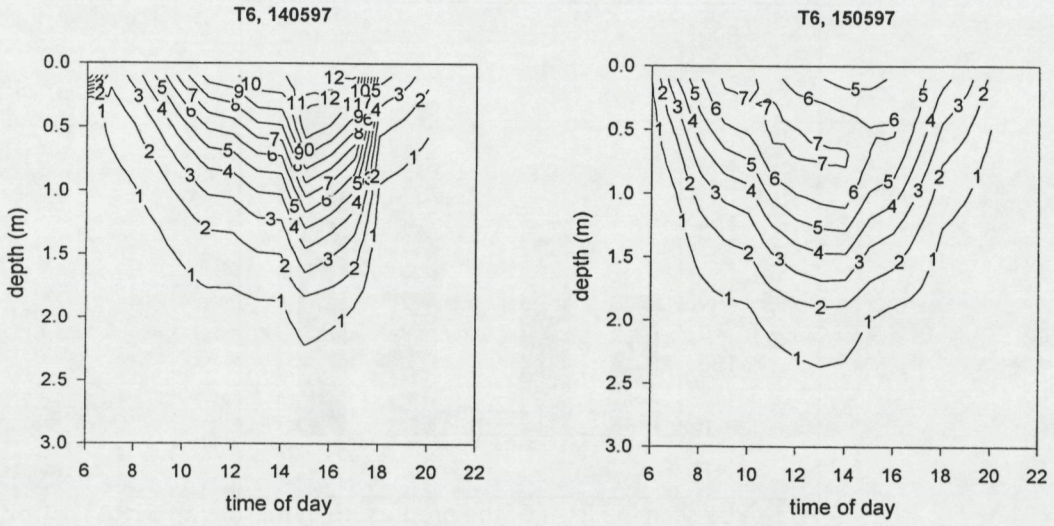



Fig. II.6 shows the production pattern with depth during the day in mesocosm STRAT-L1. As can be seen, the maximum production rates were similar (actually a little higher at 14-05-97), but due to the higher incident irradiance at the next day, there was a higher production at greater depths. Also some photoinhibition could be observed at 15-05-97. As a consequence, the highest production rates were observed between 0.5 and 1.0 m depth.
A different pattern was observed in mesocosm STRAT-L2 (Fig. II.7). The highest rates were observed at 14-05-97, due to a higher biomass. This despite the fact that the incident irradiance as well as the specific rates of photosynthesis were lower (Table II.1). As in mesocosm STRAT-L1, on 15 May production at the surface was reduced due to

**Figure II.7**
Contour plots of the rates of primary production in mesocosm STRAT-L2.




photoinhibition and highest rates at noon were found between 0.5 and 0.75 m depth. Because the incident irradiance was higher, there was a significant higher production rate at greater depths. This compensated the lower production in the surface layer, and was the cause for the fact that the overall production rates for both days were very similar.

## CONCLUSIONS

Primary production can vary significantly from day to day because of changes in photosynthetic characteristics and biomass. This calls for new methods to study primary production on-line. The variable fluorescence technique employed here, using a PAM-fluorometer is very promising, as it can measure rates of (gross) photosynthesis "real-time", but translation of photosynthetic electron flow into rates of C-fixation is not possible yet. More research into this field is necessary.

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# Appendix III: Reeds verschenen BEON-rapporten

| | | | |
|---|---|---|---|
| BEON rapport nr. | 1. | BEON Meerjarenplan 1988-1993. | 1987 |
| BEON rapport nr. | 2. | BEON Jaarwerkplan 1988. | 1988 |
| BEON rapport nr. | 3. | BEON Modellering. | 1988 |
| BEON rapport nr. | 4. | BEON meerjaren Uitvoeringsprogramma 198 8-1993. | 1989 |
| BEON rapport nr. | 5. | BEON Jaarwerkplan 1989. | 1989 |
| BEON rapport nr. | 6. | Findings of the BEON Workshop in preparation for the Third North Sea Conference. | 1989 |
| BEON rapport nr. | 7. | Beleidspresentatie BEON 23 juni 1989 Den Haag. | 1989 |
| BEON rapport nr. | 8. | Effects of Beamtrawl Fishery on the Bottom Fauna in the North Sea. | 1990 |
| BEON rapport nr. | 9. | BEON Jaarwerkplan 1990. | 1990 |
| BEON rapport nr. | 10. | BEON Voortgangsrapport 1988-1989. | 1990 |
| BEON rapport nr. | 11. | Beleidspresentatie BEON 31 mei 1990 Den Haag. | 1990 |
| BEON rapport nr. | 12. | Beleidspresentatie BEON 20 juni 1991 Den Haag. | 1991 |
| BEON rapport nr. | 13. | Effects of Beamtrawl Fishery on the Bottom Fauna in the North Sea. II. The 1990 - studies. | 1990 |
| BEON rapport nr. | 13 A. | BEON Jaarwerkplan 1991. | 1991 |
| BEON rapport nr. | 14. | BEON Jaarwerkplan 1992. | 1992 |
| BEON rapport nr. | 15. | Beleidspresentatie BEON 19 juni 1992 Den Haag. | 1992 |
| BEON rapport nr. | 16. | Effect of Beamtrawl Fishery on the Bottom Fauna in the North Sea. III. The 1991 - studies. | 1992 |
| BEON rapport nr. | 17. | Beleidspresentatie BEON 12 december 1991. | 1992 |
| BEON rapport nr. | 18. | Trace Element Geochemistry at the Sediment Water Interface in the North Sea and the Western Wadden Sea. | 1993 |
| BEON rapport nr. | 19. | Effecten van met benzo(a)pyreen verontreinigd sediment op de Helmkrab (Corystes cassivelaunus). Rapportage Project BEONADD I/II.I | 1993 |
| BEON rapport nr. | 20. | Scavenging seabirds behind fishing vessels in the Northeast Atlantic. (With emphasis on the Southern North Sea). | 1993 |
| BEON rapport nr. | 21 | Brug tussen Beleid en Onderzoek (Rapportage over het eerste BEON Meerjarenprogramma 1988-1992). | 1993 |
| BEON rapport nr. | 93-1 | Naar een duurzame ontwikkeling van de Noordzee. (Tweede Meerjarenprogramma BEON1993-1997). | 1993 |
| BEON rapport nr. | 93-2 | The appearance of scars on the shell of Arctica Islandica L. (Mollusca, Bivalvia) and their relation to bottom trawl fishery. | 1993 |
| BEON rapport nr. | 93-3 | BEON Jaarwerkplan 1993. | 1993 |
| BEON rapport nr. | 93-4 | BEON Beleidspresentatie "Zee en Wadvogels. "Voorkomen en invloeden daarop" d.d. 10 december 1993. | 1993 |

**1994**

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| BEON rapport nr. 94-1 | Effecten van verschuivingen van nutriëntenconcentraties op biota in de Nederlandse kustwateren. Philippart, C.J.M. & E.G. de Groodt, A.G. Brinkman, R.G. Jak, M.C.Th. Scholten (IBN 93 E 02). |
| BEON rapport nr. 94-2 | Vervalt; zie 96-3 |
| BEON rapport nr. 94-3 | Jaarwerkplan 1994. |
| BEON rapport nr. 94-4 | Jaarverslag 1993: Algenonderzoek in mesocosms en modelleringlering. Riegman, R. (NIOZ 93 E 01). |
| BEON rapport nr. 94-5 | Impact of anthropogenic activities on the productivity of the western Wadden Sea ecosystem. Veer, H.W. van der. (NIOZ 93 E 02). |
| BEON rapport nr. 94-6.1 | Bentic nutriënt generation in the ERSEM ecosystem model of the North Sea. Ruardij, P. and W. van Raaphorst. (NIOZ 93 E 03) |
| BEON rapport nr. 94-6.2 | The EcoWasp model and it's environment. Smit, J.P.C., A.G. Brinkman, E.G.M. Embsen, P. Ruardij, and W. van Raaphorst. (NIOZ 93 E 03) |
| BEON rapport nr. 94-7 | Risico-analyse Mariene Systemen (RAM*2 project) Eindrapport van de RAM-Auditgroep. |
| BEON rapport nr. 94-8 | Comparison of models describing species composition of marine phytoplankton Michielsen, H & Berg, A. van den & Joordens, J., et al.(project MANS-FYFY, WL 93 E 01). |
| BEON rapport nr. 94-9 | Verslag BEON Workshop Risico-analyse, d.d. 27 april 1994, Den Haag. |
| BEON rapport nr. 94-10 | BEON Beleidspresentatie "Microverontreinigingen: effecten en trends", d.d. 21 juni 1994. |
| BEON rapport nr. 94-11 | De epi- en endofauna van de Nederlandse, Duitse en Deense kustzone: een analyse van 20 jaar bijvangsgegevens. Buijs, J., J.A. Craeymeersch, P. van Leeuwen, A.D. Rijnsdorp. (BEONADD IV/V) |
| BEON rapport nr. 94-12 | De inductie van cytochroom P450 1 A in platvis door blootstelling aan polyaromatische koolwaterstoffen in de Noordzee. INP-programma 1991- 1992. Boon, J.P., H.M. Sleiderink, M.L. Eggens, A.D. Vethaak (NIOZ 93 M 05) |
| BEON rapport nr. 94-13 | Directe effecten van de visserij met de 12m en 4m boomkorren op het bodemleven in de Nederlandse sector van de Noordzee. Bergman, M.J.N. en J.W. van Santbrink. (NIOZ 93 V 07) |
| BEON rapport nr. 94-14 | Scavenging seabirds at beamtrawlers in the southern North Sea, distribution, relative abundance, behaviour, prey selection, feeding efficiency, kleptoparastism and the possible effects of the establishment of protected areas'. Camphuysen, C.J. (BEONADD IV/V) |
| BEON rapport nr. 94-15 | The relationship between food supply, reproductive parameters and population dynamics in Dutch Lesser Black-backed Gulls Larus fuscus: a pilot study. Spaans, A.L., M. BukaciÑska, D. BukaciÑska. (BEONADD IV/V) |
| BEON rapport nr. 94-16 | Pilot study on the influence of feeding conditions at the North Sea on the breeding results of the Sandwich Tern Sterna sandvicensis. Brenninkmeijer, A. & E.W. M. Stienen. (BEONADD IV/V) |
| BEON rapport nr. 94-17 | BEON-studie naar de effecten van de teruglopende nutriëntenbelasting van de Nederlandse kustzone. Boddeke, R. en P. Hagel. (RIVO 93 E 03) |

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| BEON rapport nr. 95-1 | Effecten van de schelpdiervisserij op het bodemleven in de Voordelta. Van der Land, M.A. (RIVO 94 V 06). |
| BEON rapport nr. 95-2 | Jaarwerkplan 1995. |
| BEON rapport nr. 95-3 | Trends in het voorkomen van vissen en epibenthische evertebraten in de Noordzee: Een vergelijking van datasets. Van der Veer, H.W., J.A. Craeymeersch, J. Van der Meer, A.D. Rijnsdorp, J.IJ. Witte. (NIOZ 93 A 04) |
| BEON rapport nr. 95-4 | De ontwikkeling van een in vitro assay voor de bepaling van de invloed van biotransformatie op de bioaccumulatie van lipofiele organohalogeen verbindingen in mariene toppredatoren.<br>I. Validatie van de assay met PCBS en de eerste resultaten met Toxafeen. Boon, J.P., van Schanke, A., Roex, E., de Boer, J., Wester, P. (NIOZ 94 M 01) |
| BEON rapport nr. 95-5 | BEON beleidspresentatie "Ontwikkelingen in het beleid", d.d. 9 december 1994. |

BEON rapport nr. 95-6 BEON beleidspresentatie "Modellering: de stand van zaken en het belang voor beleid en beheer", d.d. 31 maart 1995.

BEON rapport nr. 95-7 Wetenschappelijke discussie. De visserij-intensiviteit van de Nederlandse boomkorvisserij op de Noordzee mede in het licht van de milieu effecten en gesloten gebieden.

BEON rapport nr. 95-8 Antropogene eutrofiëring en natuurlijke variaties. Consequenties voor de produktiviteit van de Noordzee. INP-MOORING/PELAGIC FOOD WEB/STED/ STRAECOS. Van Raaphorst, W., F.C. van Duyl, H. Ridderinkhof, R. Riegman, P. Ruardy. (NIOZ 94 E 01)

BEON rapport nr. 95-9 Effecten van antropogene aktiviteiten op de produktiviteit van het ecosysteem in de Westelijke Waddenzee. Van der Veer, H.W., J.J. Beukema, G.C. Cadée, J. Hegeman, B. Mom, W. Van Raaphorst, J. IJ.. Witte (NIOZ 93 E 02)

BEON rapport nr. 95-11 Biomarkers of Toxic effects chemoreceprion: effects of contaminated dredge spoil on chemoreception acuity in whelks.Ten Hallers-Tjabbes, C. and C.V. Fisher. (NIOZ 93 M 05)

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1996

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BEON rapport nr. 96-2 Spisula subtruncata als voedselbron voor Zeeëenden in Nederland. Leopold, M.F. (IBN 95 V 29).

BEON rapport nr. 96-3 BENTOX. Toxische effecten van verontreinigde sedimenten voor marien benthos. 1e fase: Verkennend onderzoek met 'natuurlijk' verontreinigde sedimenten , 2e fase: Benzo(a)pyreen en fluoranteen, 3e fase: BaP concentratiereeks. Kaag, N.H.B.M., J.P. Boon, K. Booij, C.V. Fischer, E.M. Foekema, M.T.J. Hillebrand, H. Hummel, H. Kralt, M.C. Th. Scholten, B.M.H. Timmermans, A.P.M.A. Vonck, M. de Vries, E. van Weerlee. (TNO 93 M 04,TNO 94 M 06, TNO 95 M 16).

BEON rapport nr. 96-4 Algenbegrazing: Een nadere analyse van de invloed van toxicanten op het ontstaan van eutrofiëringsproblemen. Jak, R.G., Michielsen, B.F. (TNO 95 E 07).

BEON rapport nr. 96-5 Habitatkartering en beschrijving Nederlandse kustwateren (IBN 95 H 36)

BEON rapport nr. 96-6 Onderzoek naar de invloed van fluctuaties in de lokale voedselbeschikbaarheid op de populatiedynamiek van de grote stern Sterna sandvicensis: tussentijdse resultaten. Stienen, E.W.M. & A. Brenninkmeijer. (IBN 95 H 24).

BEON rapport nr. 96-7 Resultaten BEON Workshop NW4.

BEON rapport nr. 96-8 Thema bijeenkomst Boomkorvisserij.

BEON rapport nr. 96-9 Jaarwerkplan 1996.

BEON rapport nr. 96-10 SCREMOTOX (WL 95 M 21).

BEON rapport nr. 96-11 Effecten van de schelpdiervisserij op het bodemleven in de Voordelta: De schelpdierbestanden in de Voordelta in 1995. Van der Land, M.A. (RIVO 95 V 30).

BEON rapport nr. 96-12 Verslag van de BEON workshop ter voorbereiding van de Nederlandse inbreng van de tussenconferentie van Noordzee- en Visserijministers (IMM 97).

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BEON rapport nr. 96-14 Evaluatierapport BEON 1996. Tussentijdse evaluatie Tweede Meerjarenprogramma BEON 1993-1997. Rapport naar aanleiding van de BEON evaluatieworkshop d.d. 2 februari 1996, Den Haag.

BEON rapport nr. 96-15 Onderzoek naar mogelijkheden tot vermindering van discard produktie door technische aanpassing van boomkornetten (NIOZ 95 V 05). Fonds, M. & W. Blom

BEON rapport nr. 96-16 INP-Mooring 94-96: Antropogene eutrofiëring en natuurlijke variaties in de open Noordzee: metingen op een verankeringsstation in de Oestergronden (NIOZ 95 E 01)

**1997**

BEON rapport nr. 97-1 Fluctuaties in de lokale voedselbeschikbaar in relatie tot de populatiedynamiek van de Grote Stern Sterna sandvicensis: resultaten 1995-1996 (IBN 95 H 24). Stienen, E.W.M. en A. Brenninkmeijer.

BEON rapport nr. 97-2 Vervallen.

BEON rapport nr. 97-3 Jaarwerkplan 1997.

BEON rapport nr. 97-4 De betekenis van het zout- en silicaatgehalte in Nederlandse kustwateren voor het zeegrasareaal. Kamermans, P., M.A. Hemminga, D. de Jong, K.S. Dijkema. (NIOO 96 EH 07).

BEON rapport nr. 97-5 GiftigeAlgen en de Reductie van de Nutriëntenbelasting (BEON-GARdeN) Competitie tussen algen. Jaarverslag 1996. Riegman, R., K. Peeters, H. Los.(NIOZ 95 E 02).

BEON rapport nr. 97-6 In vitro biotransformatie van organohalogeenverbindingen in zeezoogdieren en vogels. Mogelijke gevolgen voor bioaccumulatie en genotoxiciteit. III: Gebromeerde vlamvertragers (Polybroom difenylethers & polybroom bifenylen). Boon, J.P., M.J. Greve, J.B. Bouma, M.K. de Boer, W.E. Lewis, H.J.C. Klamer, D. Pastor, P. Wester, J. de Boer (NIOZ 95 M 03).

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