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Rijksinstituut voor Kust en Zee/*RIKZ*

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

Final report:
results of a long term mesocosm experiment with three nutrient reduction scenarios

Rapport RIKZ -97.035
NIOO/CEMO-1997.05

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

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Samenvatting

In dit eindrapport wordt verslag gedaan van een onderzoek dat in het kader van het project BEON*EUTRO bij het Rijksinstituut voor Kust en Zee/RIKZ is uitgevoerd in de periode 1992-1995, in samenwerking met het Centrum voor Estuariene en Mariene Oecologie van het Nederlands Instituut voor Oecologisch Onderzoek. In dit rapport worden de resultaten van onderzoek met experimentele ecosystemen beschreven.
Het doel van het onderzoek was het opstellen van beleidsadviezen over duurzaam beheer van het zeemilieu ter bestrijding van de nadelen van eutrofiëring. Het beleid zoals afgesproken door de Noordzee Ministers Conferentie streeft een halvering van de stikstof en fosfaat belasting van de Noordzee na in 1995, vergeleken met de belasting in 1985. Voor fosfaat is dit beleid gerealiseerd want de belasting is inderdaad met 50% gedaald. De stikstof belasting is nog niet noemenswaardig verminderd. Er is kritiek op het beleid gericht op het terugdringen van de stikstof en fosfaat toevoer omdat gevreesd wordt voor verminderde visserij opbrengsten. De centrale vraag in het onderzoek luidde dan ook: in hoeverre is terugdringen van de toevoer van fosfaat en stikstof naar zee nodig om de nadelen van eutrofiëring te voorkomen zonder daarmee de productiviteit aan te tasten?

Om deze vraagstelling te beantwoorden is een aanpak gevolgd waarbij gebruik is gemaakt van experimentele ecosystemen, de zogeheten 'mesocosms'. Deze bestaan uit 3 meter hoge cilindrische vaten van 3 m^3 die zijn opgesteld in de open lucht. Er zijn aparte doorstroomcellen aan bevestigd waarin mosselen het water kunnen filtreren en kunnen groeien, en er is apparatuur voor continue registratie van zuurstofgehalte, temperatuur en fluorescentie. Na een aantal tests in 1992 is in 1993 een serie experimenten uitgevoerd met een viertal mesocosms, waarin de gevolgen van verminderde fosfaat belasting en verhoging van de mosselgraas is bestudeerd. In 1994 is dit herhaald met een zestal mesocosms waarbij in tweevoud de gevolgen van vermindering van de stikstof belasting en verhoogde graas werden bestudeerd. Al deze experimenten hadden een duur van 4 tot 6 weken.
Hieruit kwam het volgende naar voren:

1. De waarden van de belangrijkste variabelen in de mesocosms waren vergelijkbaar met waarden die in de Noordzee waren gemeten op 10-20 km uit de kust van Noordwijk. Ook waren de uitkomsten van de duplo mesocosms goed vergelijkbaar. De resultaten van de mesocosms kunnen dus worden vertaald naar de situatie in zee.
2. Een lage stikstof en fosfaat belasting was gunstig voor de dominantie van diatomeeën over flagellaten. De schuimvormende alg *Phaeocystis* domineerde in de meeste experimenten alleen bij hoge stikstof en fosfaat belasting,

hetgeen wordt toegeschreven aan het opraken van silicaat en bijgevolg de afname van diatomeeën.

3. In kortdurende experimenten waarin of de fosfaat- of de stikstof-belasting werd gemanipuleerd, was de invloed van zooplankton- graas in het algemeen gering. Het chlorofyl-gehalte en de primaire produktie vertoonde een lineair verband met de nutrienten belasting.
4. Een matige graasdruk door de mosselen ging gepaard met een verschuiving in dominantie van flagellaten naar diatomeeën.
5. Hoge aantallen mosselen veroorzaken een afname van de fytoplankton hoeveelheid en een toename van de voorraad opgeloste voedingsstoffen.

In 1995 is een experiment van 7 maanden uitgevoerd in de mesocosms, waarbij de effecten van een aantal scenario's voor terugdringing van de stikstof en fosfaatbelasting is uitgetest:
H(oog): de bestaande situatie (1995), d.w.z. een afname van de fosfaat belasting met 50% en van de stikstof belasting met 10 % ten opzichte van de periode 1980-1987;
M(iddel): de huidige internationale afspraken, d.w.z. een afname in de fosfaat én de stikstof belasting met 50 % t.o.v. 1980-1987;
L(aag): dit scenario vertegenwoordigt het lange termijn beleidsdoel, namelijk een 75 % afname van de fosfaat en stikstof belasting.
In dit onderzoek stonden de volgende vragen centraal:

1. Wat is het gevolg van deze scenario's voor de primaire produktie en de biomassa en samenstelling van het fytoplankton.
2. Wat is het gevolg van de scenario's voor de groei van bodemdieren en zooplankton, en voor de zooplankton samenstelling.
3. Welke mate van reductie van de stikstof en fosfaat belasting is mogelijk zonder de productiviteit van het ecosysteem aan te tasten.

In dit eindrapport wordt in hoofdzaak ingegaan op de resultaten van het lange-termijn experiment van 1995, waarbij de resultaten zullen worden vergeleken met die van de eerdere experimenten. De opzet is beschreven in hoofdstuk 1.

In hoofdstuk 2 wordt ingegaan op de werkwijze in de experimenten met de mesocosms, onder meer wat betreft de voorbehandeling van de bodem, de aangroeibestrijding, de toevoeging van de nutriënten en de bemonsterings- en analyse procedures. Aparte experimenten zijn uitgevoerd om de bodem-water uitwisseling te kwantificeren, en om de graasaktiviteit van het microzooplankton te bepalen. Noordzee gegevens bleken een goede overeenkomst te vertonen met de mesocosms wat betreft watertemperatuur, lichtinstraling, en voor het **H** scenario ook voor de chlorofyl concentraties en primaire produktie.

In hoofdstuk 3 wordt ingegaan op de ontwikkelingen van het fytoplankton. Biomassa en primaire produktie waren significant verschillend bij de verschillende scenario's. De voorjaarsbloei was fosfaat-gelimiteerd, terwijl in de zomer stikstofbeperking optrad. Er zijn aanwijzingen dat begrazing door micro-zooplankton beperkend was voor kleine fytoplankton soorten, waaronder *Phaeocystis* cellen die nagenoeg afwezig waren in de mesocosms in dit experiment.

In hoofdstuk 4 wordt ingegaan op de ontwikkeling van het zooplankton. Daar blijkt dat er inderdaad hoge dichtheden micro-zooplankton zijn aangetroffen, met name van de soort *Oxyrrhis*. De hoeveelheden volwassen mesozooplankton bleven evenwel laag ten opzichte van de veldsituatie. De biomassa van het microzooplankton vertoonde een positief verband met de stikstof-belasting. Er was geen verband tussen de stikstof of fosfaat belasting en de biomassa van het mesozooplankton.

In hoofdstuk 5 wordt verslag gedaan van een methode om de graasactiviteit van het micro-zooplankton te kwantificeren d.m.v. de zeewater-verdunnings methode. Hieruit komt naar voren dat er inderdaad overgrazing is opgetreden in enkele mesocosms.

De groei van mosselen en de biomassa van overige bodemdieren, als maatstaf voor de secundaire produktie, wordt besproken in hoofdstuk 6. In het voorjaar was de mosselgroei niet voedselbeperkt; in de zomer was er in het lage scenario L vrijwel geen groei. Over het gehele experiment was er een significant verband tussen primaire en secundaire produktie, en was de secundaire produktie in scenario L lager dan in de andere twee scenario's.

In hoofdstuk 7 worden de stikstof en fosfaat balansen gepresenteerd. Op basis hiervan is de interne kringloop en het vasthouden (retentie) van de nutriënten in de mesocosms geanalyseerd. Stikstof retentie was gerelateerd aan de fytoplankton ontwikkeling; fosfaat retentie vertoonde geen seizoenspatroon en wordt waarschijnlijk bepaald door binding aan het sediment. Ondanks een hoge N:P verhouding in de toevoer was er in de zomer sprake van stikstof beperking als gevolg van fosfaat teruglevering en denitrificatie.

In hoofdstuk 8 wordt ingegaan op het systeem-metabolisme aan de hand van zuurstof produktie en -opname in de tanks. Produktie en respiratie in de waterkolom vertonen een overeenkomstig patroon, met een significant verband met de stikstof toevoer. Ongeveer de helft van de produktie overdag wordt 's nachts verademd door fytoplankton en bacteriën. Zuurstof verbruik door de bodem vertoont geen verband met de nutriënten toevoer. De netto systeem produktiviteit is afhankelijk van de nutriënten toevoer, doch minder sterk dan het geval is voor de primaire produktie.

In hoofdstuk 9 wordt de stand van zaken beschreven van de modellering. Het bestaande mesocosm model is grondig aangepast, o.m. om de korte-termijn dynamiek in de mesocosms beter te kunnen beschrijven: de tijdstappen zijn verkort van 1 dag naar 1 uur. Daarmee zijn dus variaties binnen een dag te simuleren. Verder zijn verschillende fytoplankton groepen onderscheiden, en is er gewerkt aan de koppeling met de multi-species module BLOOM. De boekhouding van de fluxen is geüniformeerd voor de verschillende modules. Voor calibratie en validatie van het model moeten nog verschillende problemen worden opgelost.

Tot slot wordt in hoofdstuk 10 een synthese van het gehele mesocosm onderzoek gepresenteerd.

De belangrijkste conclusies van het onderzoek naar de effecten van de scenario's, zijn:

1. In het voorjaar is er een rechtlijnig verband tussen fosfaat toevoer en primaire produktie; in de zomer is stikstof de belangrijkste beperkende factor voor de primaire produktie. Er was geen invloed van de nutriënten toevoer op de samenstelling van het fytoplankton.
2. Wat betreft de secundaire produktie was er in het voorjaar geen verband met de scenario's, terwijl er in de zomer alleen een effect is opgetreden in het laagste scenario.
3. Een reductie van de fosfaat en stikstof toevoer naar de mesocosms van 50 % heeft geen effect op de secundaire produktie, terwijl een reductie met 75 % wel een afname van de secundaire produktie teweeg brengt.

De algemene conclusie van dit onderzoek is dat een afname van de toevoer van stikstof en fosfaat naar de Noordzee eerder een voordeel dan een nadeel is voor de visserij. Immers, het voorkomen van plaagalgen bloeien, met name *Phaeocystis*, vereist een verdere terugdringing van de fosfaat toevoer, waarbij geldt dat fosfaat niet beperkend is voor de secundaire produktie in het voorjaar. Het voorkomen van plaagalgenbloeien in de zomer vereist een drastische vermindering van de stikstof toevoer, teneinde de N:P verhouding in balans te houden en de primaire produktie te beperken. Een reductie van de stikstof belasting met 50 % tast de produktiviteit van het systeem, en daarmee de visserij opbrengst, niet aan.

Summary

This is the final report of a study carried out in the period 1992-1995 by the National Institute for Coastal and Marine Management/RIKZ in cooperation with the Centre for Estuarine and Coastal Ecology of the Netherlands Institute of Ecology, in the framework of the BEON*EUTRO project. The objective of this study was to prepare an advice for the Dutch government on sustainable management of marine ecosystems with respect to eutrophication and productivity. In this report the results of a series of mesocosm experiments are presented.
The actual policy in The Netherlands, as in other countries bordering the North Sea, is to reduce the nutrient loads to the North Sea by 50 % of the values in 1985. Indeed, phosphorus inputs to the Dutch coastal zone have decreased dramatically and were in 1995 about half of the 1985 inputs. For nitrogen, however, no significant decrease of the inputs has been achieved till now. The decision to reduce the nutrient inputs has been criticized because a decrease in productivity and subsequent fishery yield was foreseen. Therefore, the main research question in this project was formulated as: what degree of reduction of nutrient inputs to the North Sea is required to prevent adverse effects meanwhile maintaining a productive ecosystem?

To understand the effects of nutrient loading reduction ("de-eutrophication") in relation to system productivity, an ecosystem approach was chosen and mesocosm facilities were used for experiments. The mesocosms used were land-based, MERL-like tanks with a volume of 3 m^3, separate benthic chambers connected to each tank and an on-line monitoring system. After a pilot study in 1992, short-term experiments with four mesocosms were done in 1993, to study the effects of reduction of phosphorus loadings and of increased bivalve grazing pressure. In 1994 this approach was repeated with 6 mesocosms where the effects of reduced nitrogen loads and increased grazing pressure were tested in duplicate systems. The experiments lasted for 4-6 weeks.
The conclusions of these experiments were:

1. Values of key variables in the mesocosms were similar to those in the coastal North Sea, and were reproducible in duplicate mesocosms: the mesocosm methodology allows for the extrapolation of conclusions to the field situation with respect to dominant processes and variables.
2. Reduction of N and P loads favoured the dominance of diatoms over flagellates. *Phaeocystis* dominated in most experiments only at higher N or P loads, owing to depletion of silicate under these conditions and subsequent diatom decrease.
3. In short-term experiments where either phosphate or nitrogen loadings were manipulated, the influence of zooplankton grazing was limited. The chlorophyll

concentrations and primary production showed a linear relation with nutrient loading.

4. A moderate grazing pressure by mussels caused a shift towards the dominance of diatoms over flagellates.
5. Increased densities of mussels caused a decrease of phytoplankton biomass and an increase of the dissolved nutrient pool.

In 1995 a 7-month experiment was carried out aiming at the evaluation of the following nutrient reduction scenarios:
H(igh): the actual situation with a 50% and a 10% N reduction relative to the reference period 1980-1987,
M(edium): the actual policy objectives for 1995 with a 50 % reduction of both P and N, and
L(ow): the long-term policy objective with a 75% reduction of P and N.
The research questions were:

1. What is the effect of N and P loading reduction scenarios on primary production and on biomass and composition of the phytoplankton community.
2. What is the effect of the reduction scenarios on secondary production of macrobenthos and zooplankton, and on zooplankton composition.
3. To what extent is N and P reduction possible while maintaining productivity of the system.

This final report deals mainly with the results of the 1995 experiment, but results are compared with the findings of the short-term mesocosm experiments, as explained in the introduction (chapter 1).

In chapter 2, a detailed description of the methods is given, including pre-treatment of sediment and mesocosms, temperature control, fouling prevention, nutrient addition, and sampling and measurement procedures. In this experiment special attention was given to measuring sediment-water exchange rates and microzooplankton grazing rates. Comparison with North Sea field data showed that mesocosm data were within ranges of field data for water temperature, mean water column irradiance, and chlorophyll-a concentration and primary production per m^2 in the highest nutrient scenario.

Chapter 3 presents the phytoplankton dynamics. There were significant responses of phytoplankton biomass and production to the treatments. The spring bloom was P-limited, while in the summer period N was limiting biomass and production. There were indications of grazing-control of small sized phytoplankton by microzooplankton. This may have been a limiting factor for the development of *Phaeocystis*, that was almost absent in this experiment.

In chapter 4, zooplankton dynamics are described. Microzooplankton, and in particular *Oxyrrhis marina*, showed high densities compared to field data. Densities of adult mesozooplankton were relatively low. The biomass of microzooplankton showed a positive correlation with nitrogen loading. There was no relation between phosphate or nitrogen loading and biomass of the mesozooplankton.

Chapter 5 present the results of a pilot study of microzooplankton grazing rate estimations, by using the seawater dilution method. The results confirm the idea that microzooplankton affected the phytoplankton by grazing in some of the mesocosms.

In chapter 6, the growth of mussels in the benthic chambers, and of other benthic animals that settled in the tanks, is presented as an indication of the secondary productivity. There was no growth limitation in spring, but in summer mussel growth was almost absent in the low nutrient scenario. There was a significant relation between primary and secondary production over the entire experiment. Secondary production of the macrobenthos in scenario L was lower than in the other two scenarios.

In chapter 7, budgets for N and P are presented, and used for analysis of cycling and retention of nutrients as a function of loading. N retention was related to phytoplankton development, while P retention showed little seasonal variation, presumably due to storage in sediment. N-limitation of primary production in summer, despite a high N:P input ratio, was ascribed to regeneration of P and loss of N through denitrification.

In chapter 8, the system metabolism in the mesocosms is evaluated by analyzing oxygen production and respiration in the tanks. Pelagic production and respiration were closely coupled, and there was a significant correlation with the N-input. Nearly half of the net daytime production was respired in the water column at night, mainly by algae and other microorganisms. Benthic respiration was not related to external nutrient loads. Although net system production was positively related with nutrient loading, the effects of de-eutrophication (reduction of nutrient loads) on net system production were less dramatic than for primary production.

Chapter 9 describes the state-of-the-art of the simulation model. Adaptations of existing model formulations were introduced in order to fit the model to the dynamics of the mesocosm processes. Therefore a model structure was developed where different modules could operate independently from each other. The time scale was adapted to the time scale of the experiments, i.e. from days to hours, which means that within-day variability could be simulated. Different groups of phytoplankton are distinguished, and formulations are developed to include a multispecies module into the mesocosm model. Improvements were made in administration of the fluxes in a uniform way for the various modules.

Finally, chapter 10 gives a short synthesis of the whole project.

The main conclusions of the research reported here, are:

1. In spring, a linear relationship between P input and primary production was observed, while in summer N was the main limiting factor for phytoplankton production. No effect of the nutrient input on the composition of the phytoplankton community was observed.
2. In spring, there was no relation between nutrient input and secondary production, while in summer a decrease in

secondary production was only observed in scenario L, with the lowest nutrient input.

3. Reduction of N and P input to the mesocosms by 50 % does not affect the secondary production; only a reduction scenario of 75 % has shown a decrease in secondary production.

As a general result of the project it can be concluded that reduction of anthropogenic P and N loadings to the North Sea are beneficial rather than a threat to fisheries. Prevention of harmful algal blooms, i.e. *Phaeocystis* requires a further reduction of P input, and P is not limiting the secondary production in spring. Prevention of harmful algal blooms in summer requires a dramatic reduction of the N input, in order to keep the N:P ratio in balance and to limit primary production. A reduction up to 50 % is possible without decrease in secondary production and subsequent fish yield.

1 General introduction

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In this report results are presented of a 7-month mesocosm experiment conducted in 1995, aiming at the evaluation of three nutrient reduction scenarios. Nitrogen and phosphorus were added to the mesocosms at rates mimicking present and future conditions in the North Sea coastal zone. Scenarios for the reduction of anthropogenic nutrient loadings, relative to the nutrient loading to the North Sea in the period 1980 - 1987, were as follows:

nutrient reduction(%):	**N**	**P**
scenario 1 (H)	-10	-50
scenario 2 (M)	-50	-50
scenario 3 (L)	-75	-75

Silicate was supplied at equal rates in all scenarios. Each scenario was replicated in identical mesocosms.
Results of the scenario experiments will be compared with previous mesocosm experiments, carried out in the period 1992-1994. In 1992 a one-month pilot study was done with high nutrient loads (Peeters at al, 1993; Escaravage et al., 1995a); in 1993 two series of experiments were done with 4 mesocosms; in one series the effects of different phosphorus inputs were tested, and in another series increased mussel grazing was imposed (Smaal et al., 1994; Prins et al., 1995a, 1995b, 1995c; Escaravage et al, 1996). In 1994 one series of experiments was done with different nitrogen loadings, and one series with increased grazing pressure (Escaravage et al., 1995b, 1997; Peeters et al., 1995). In contrast to the 1995 experiment, these experiments all lasted for 4-6 weeks.

1.1 SCENARIOS FOR THE REDUCTION OF ANTHROPOGENIC N AND P LOAD TO THE NORTH SEA

In order to prevent harmful effects of marine eutrophication, it was decided at the Second and the Third North Sea Ministers Conference in 1987 and 1990 respectively, that the anthropogenic input of N and P to the North Sea in 1995 should be reduced to about 50% of the 1985 input. Much effort has been put into sewage treatment and other measures to achieve the required nutrient input reduction. Indeed P loads have decreased dramatically, and in 1995 a 50% reduction of the 1985 loads was observed in the Dutch coastal zone (De Vries et al., 1997). For nitrogen, measures have been less effective, and no significant decrease of N loads was achieved till now.

As anthropogenic loads only contribute partly to the nutrient concentrations in the North Sea, P concentrations have decreased in a small part of the North Sea only: significantly lower concentrations were observed in the Dutch coastal zone up to 30 km off-shore. As N loadings were maintained, the N:P ratio has increased at these stations from 25 - 30 up to 40 - 55 (De Vries et al., 1997). Owing to the relatively fast regeneration of phosphorus, the reduced concentration is only apparent in spring; in summer there is a relative surplus of P.
In the off-shore zone effects are difficult to detect, because the anthropogenic contribution to the amounts of inorganic nutrients is lower.

The effect of measures to prevent eutrophication effects can be summarized for different areas of the North Sea, and for different seasons, as:

coastal zone in *spring*:
- decreased P concentrations, and P-limitation at the end of the spring blooms
- high N:P ratio

coastal zone in *summer* and **offshore zone** in *spring and summer*:
- P regeneration, and N rather than P limitation
- relatively low N:P ratio

The Ministers Conference decision to reduce nutrient loads has been criticized because a decrease in fisheries yield was foreseen (Boddeke & Hagel, 1991). This was based on the observation that the increase in nutrient loads in the period 1955 - 1980 correlated with increased fish yields. Indeed, growth rates of flatfish seem to have decreased in recent years, corresponding with the period of P load reduction. However, various other factors have been mentioned as interfering with fish growth (Rijnsdorp & Van Leeuwen, 1996). Recent observations have demonstrated an increase of flatfish growth in 1996 and 1997 (Daan, pers. comm.). No evidence on causal relationships between fish stocks and nutrient input reduction is available at present. Yet, more knowledge on food web processes in the North Sea is required to make quantitative predictions of bottom-up control of higher trophic levels. In addition to the effects on benthic suspension feeders (see Smaal, 1997; Smaal & Twisk, 1997; Scholten & Smaal, 1998), particular attention should be given to the role of zooplankton in the food chain, because knowledge gaps exist of food chain efficiencies of zooplankton.

The question still remains *to what extent* anthropogenic nutrient loads to the North Sea have to be reduced in order to prevent harmful effects of eutrophication, meanwhile maintaining the productivity of the system.

To provide an answer to this question we have done a series of short-term mesocosm experiments in the period 1992 - 1994 and a long-term experiment in 1995, reported in this document (also see Prins et al., 1997, 1998).
The conclusions of the previous mesocosm experiments were:

1. Values of key variables in the mesocosms were similar to those in the coastal North Sea, and were reproducible in duplicate mesocosms: the mesocosm methodology allows for the extrapolation of conclusions to the field situation with respect to dominant processes and variables.
2. Reduction of N and P loads favoured the dominance of diatoms over flagellates. *Phaeocystis* dominated in most experiments only at higher N or P loads, owing to depletion of silicate under these conditions and subsequent diatom decrease.
3. In short-term experiments where either phosphate or nitrogen loadings were manipulated, the influence of zooplankton grazing was limited. The chlorophyll concentrations and primary production showed a linear relation with nutrient loading.
4. A moderate grazing pressure by mussels caused a shift towards the dominance of diatoms over flagellates.
5. Increased densities of mussels caused a decrease of phytoplankton biomass and an increase of the dissolved nutrient pool.

1.2 RESEARCH QUESTIONS

The experimental setup in 1995 was different from the previous experiments with respect to the duration of the experiment and the pre-treatment of the sediment. Natural sediment was incubated prior to the experiment with water containing the nutrient concentrations of the treatment of that tank. The experiment started in March and ended in September. In one of the H treatment tanks a leak occurred in July; it was refilled within 2 days. Special attention was given to sediment-water flux measurements and to quantifying microzooplankton grazing rates. Mussels were added to the benthos chambers at low densities, and no separate grazing experiments were conducted.

The research questions of this experiment were:

1. What is the effect of N and P load reduction scenarios on primary production and on biomass and composition of the phytoplankton community.
2. What is the effect of the reduction scenarios on secondary production and zooplankton composition and activity.
3. To what extent is N and P reduction possible while maintaining productivity of the system.

1.3 OUTLINE OF THE REPORT

In chapter 2 the methods of the mesocosm experiment and additional measurements are presented. Also, a comparison is made with North Sea conditions. In chapter 3 phytoplankton dynamics are presented over the whole period in the 3 treatments. In chapter 4 results on meso and microzooplankton dynamics are given. In chapter 5 results of a pilot study are presented on the grazing activity of microzooplankton. In chapter 6 results are presented on mussel growth and secondary production of the benthos compartment. Chapter 7 presents the results of N and P cycling and retention, based on analysis of the nutrient budgets. In chapter 8 the metabolism and productivity of the system is analyzed, based on the on-line dataset. Chapter 9 presents the state-of-the-art of the simulation modelling. In chapter 10 a synthesis is presented of the whole project.

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2 Description of mesocosms and methods, and a comparison with North Sea conditions

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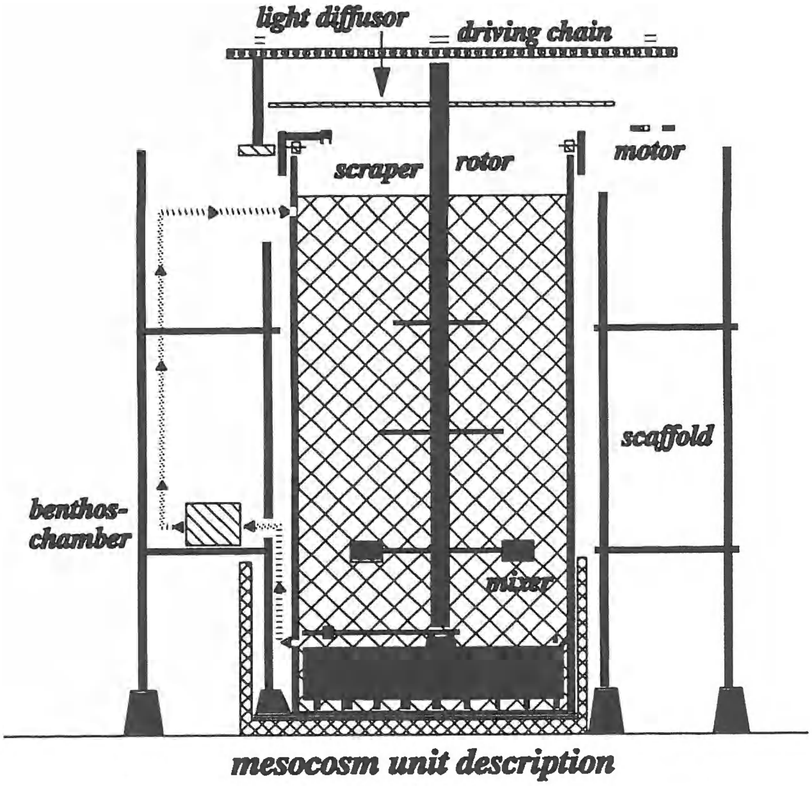
2.1 GENERAL MESOCOSM DESCRIPTION

All experiments were carried out with 6 land-based mesocosms, located at the field station of R.I.K.Z. near the mouth of the Oosterschelde estuary (SW Netherlands). The mesocosms consisted 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, resulting in a well-mixed water column with only minor vertical gradients. A scraper, made of a blade of polyethylene, 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 diffusor of structured plexiglass (Groenendijk, Pl 20070 TK) was installed to ensure a homogeneous light climate 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 30 days.
Each of the 'pelagic' tanks was connected to two 16 l Perspex benthos chambers. The benthos chambers were designed especially to contain a benthic compartment with macro fauna, 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. Water was pumped from an outlet at 2.6 m depth in the pelagic tank with a 701 VB/R Watson-Marlow tubing pump at a rate of 70 $l \cdot h^{-1}$ per chamber. The water entered the pelagic tank again just below the water surface, after passing through the benthos chambers. With the use of 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 insolation of the tanks with foil.
A sediment container of 150 l was placed on the bottom of the tanks and filled with sediment. Azoic sand with a median grain size of 210 μm was mixed with a silt suspension of surface sediment from the Oesterput (Oosterschelde) sieved on a 500 μm mesh net. The processed sediment had a silt content of 5% DW corresponding

Figure 2.1
Schematic view of mesocosm unit with benthos chamber.



to the mean value for the Dutch coastal area used as reference site for this study.
Figure 2.1 gives a schematic view of a mesocosm unit. An extensive description of the mesocosms is given in Prins et al. (1994).

2.2 DESCRIPTION OF THE EXPERIMENT

2.2.1 Experimental design

In 1995, one long-term experiment (7 months) has been carried out from March to September. On 6 December the sediment was introduced into the sediment container of each mesocosm. During the following three months the mesocosms were continuously flushed with Oosterschelde water. The mesocosms were kept in the dark by covering them with black foil, to prevent growth of algae. On 8 February 1995 a continuous loading with inorganic nutrients was started. Three different treatments were used with replicate mesocosms in each treatment. Treatments were randomly assigned to each of the mesocosms. 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).

On 6 March 1995 the experiment was started. The foil was removed from the mesocosms and 40 mussels were added to each of the mesocosms. The mussels were collected one week before the experiment, from the low tide level at a site close to the field station of RIKZ at Jacobahaven. Mussels with a shell length of 17-20 mm were selected, and 40 mussels were added to each of the mesocosms. On 31 May 1995 the number of mussels in the mesocosms was reduced to 20, in order to limit mussel biomass and grazing impact on the phytoplankton. A random selection of 20 mussels was left in the mesocosms, all other mussels were removed and used to determine ash-free dry weights (see §2.2.11).

2.2.2 Sampling procedures for chemical parameters

On the first day of the experiment, the water column of each mesocosm was sampled to determine the initial concentrations of particulate and dissolved substances and phytoplankton. During the experiment samples of the water column were collected regularly, to follow the development of water column concentrations of particulate and dissolved matter (2x week^{-1}), phytoplankton composition (2x week^{-1}), primary productivity (1x week^{-1}) and bacterial production (1x week^{-1}). Samples were taken before the inflow of the benthos chambers. Zooplankton was sampled once a week.
The exchange of dissolved materials between the water column and the benthos chambers was measured by sampling in- and outflow of the benthos chambers (2x week^{-1}). The in- and outflow of the benthos chambers was sampled 2x week^{-1} for the determination of chlorophyll-*a* . Fluorescence, oxygen, pH, temperature and conductivity were measured continuously. Chlorophyll-*a* data were used to calibrate fluorescence measurements.
The sediment of the pelagic tanks was sampled once a month for the determination of sedimented particulate matter. A stratified method was used to sample three sectors of the sediment: sector A was the outer sector (area within 0.2 m of wall; surface area 0.56 m^2), sector C was the central sector (diameter 0.4 m; surface area 0.11 m^2) and sector B was the halfway sector (width 0.2 m; surface area 0.33 m^2). Samples were subsampled for the analysis of particulate organic carbon (POC), particulate nitrogen (PN), particulate phosphorus (PP), chlorophyll-*a* and phaeophytin-*a*.
Once a month cores were taken from the sediment of the pelagic tanks. Samples were immediately stored at -20°C. Subsamples were used to determine the total volume of the interstitial water in the sediment. The pore water was collected by squeezing the samples with compressed nitrogen gas. Samples were analysed for DOC and inorganic nutrients.
Inorganic nutrient fluxes at the sediment-water interface of the pelagic tank were measured at five occasions in April, May, June, July and September by incubating sediment cores in a through flow systems. Oxygen fluxes were measured jointly with nutrient fluxes in May, July and September (see § 2.2.5).

2.2.3 Determination of the mean water column daily irradiance

Daily irradiance was recorded directly under the optical diffusor 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, 1975) 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.

$$\mathrm{Ln}\ \left(\frac{I_z}{I_0}\right) = -K_d \cdot z \qquad (1)$$

where:

I_0 = incident irradiance at surface (W m^{-2})
I_z = incident irradiance at z metre depth (W m^{-2})
K_d = irradiance attenuation coefficient (m^{-1})
z = depth of the water column (m)

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

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

where:

$\bar{I}$ = daily irradiance averaged over the water column (mol m^{-2} d^{-1})
$\bar{I}_0$ = daily irradiance at surface (PAR in mol m^{-2} d^{-1})

2.2.4 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 70 °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-}.

2.2.5 Sediment-water exchange of dissolved nutrients and oxygen

Principles of the method. Fluxes of dissolved nutrients and oxygen between the sediment of the pelagic system and the water column were estimated by measuring the concentration difference between the in- and outflow of an undisturbed sediment core in a flow-through system. For a given flux, depending on sediment area and activity, the difference between in- and outflow of the sediment core is a linear function of the flow rate. Provided that the sediment activity does not vary over time during the incubation, the concentration difference measured between in- and the outflow will reach a steady state after a certain period. Steady state concentrations in the incubator will be reached faster when the water circulation through the core is stopped for a period of time before measurements. The period needed to reach steady state concentration equals the dead volume of the incubator divided by the flow rate. After this period of pre-conditioning, the pump can be started and concentrations in the outflow should be constant. From that moment on, every measurement of the concentration difference between in- and outflow is an independent estimate of the sediment-water flux. Fluxes were determined following equation 3:

$$F = \frac{Q \cdot ((C_{o,s} - C_i) - (C_{o,c} - C_i))}{S} \qquad (3)$$

where:

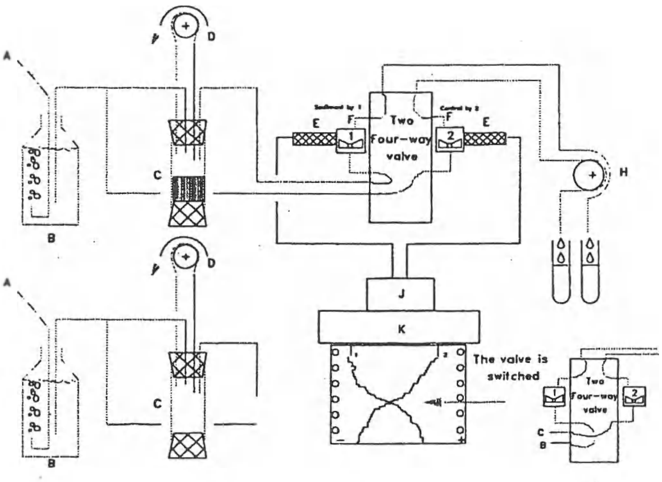
F = sediment-water flux (μmol m^{-2} d^{-1})
Q = water flow rate through the cores (l d^{-1})
$C_{o,s}$ = outflow concentration sediment core (μM)
C_i = inflow concentration cores (μM)
$C_{o,c}$ = outflow concentration control core (μM)
S = surface area of the cores (m^2)

Sampling and measuring set-up (Figure 2.2). Sediment sampling was carried out by scuba diving. Perspex cores (diam. = 5cm, length = 15cm) were pushed into the mesocosm sediment to a depth of *circa* 8 cm, and then both ends were hermetically closed with rubber stoppers. In order to cope with the high patchiness of the newly deposited detritus on the surface of the sediment one core was taken in a zone with a thin detritus layer and the second core was taken in the centre of the tank, a zone with a strong accumulation of detritus. After sampling, the cores were placed in a thermostated water bath at *in situ* temperature. Two cores without sediment were used as controls. The upper stopper of the cores was gently removed and replaced with a stopper provided with four injection needles through it, then the cores were closed again without air bubbles. The volume of water above the sediment surface was kept near 100 ml. The homogenization of the overlaying water was performed with a peristaltic pump inserted between two of the injection needles. The flow rate of the peristaltic pump was set below the level at which sediment

resuspension occurred (≈100 ml h^{-1}). The two other injection needles were used for the through flow system. One needle was connected to a one litre vessel immersed in the water bath and containing oxygen-saturated mesocosm water. A peristaltic pump connected to the fourth needle ensured the flushing of the sediment core with the mesocosm water contained in this vessel. All connections were made with Tygon © tubing, which is chemically and biologically inert and not permeable to oxygen.
Samples for dissolved nutrient concentrations were taken in the outflow of the sediment core and from the mesocosm water vessel. Oxygen concentrations were measured with a Cyclobios © respirometer inserted between the sediment cores and the peristaltic pump. The respirometer included two Orbisphere polarographic probes used to successively measure the outflow and the inflow of the sediment Karawatha switch from one measuring position to the other one was made by activating two four-way valves inserted between the entry ports and the polarographic probes (Figure 2.2). For each core, at least three switches were made, which means that four measurements were made of both the inflow and the outflow of each sediment core.

Practical considerations The perfusion flow was set to meet the following criterion: The concentration difference to be measured between the inflow and the outflow should be higher than the analytical uncertainty (DIN ±0.05 μM; DIP ±0.03 μM, Schreurs, comm. pers.). Computer simulations made with dissolved nutrient flux ranges derived from the literature (van Raaphorst et al., 1991; van Duyl et al., 1993) (DIN ±0.09 μmol h^{-1} $core^{-1}$, DIP ±0.009 μmol h^{-1} $core^{-1}$) indicated that it should be possible to measure significant differences between the inflow and the outflow of the sediment cores at a perfusion flow of 50 ml h^{-1}. It was decided to use a 40 ml h^{-1} flow rate which implies that steady state should be reached after 100/40= 2.5 hours.
The experimental procedure was as follows:
Sampling
Inserting cores in the measuring system
Cores left isolated for 2.5 hours
Circulation through the cores for 2 hours
First sample for nutrient concentrations in inflow and outflow
Second sample idem (one hour later)
Third sample idem (one hour later)
Oxygen concentrations measured the day after
Due to the size of the thermostated bath and to the time needed for the oxygen measurements, three mesocosms could be measured simultaneously.

Figure 2.2
Experimental set-up for the measurement of sediment-water exchange of dissolved nutrients and oxygen. A: Compressed air, B: filtered (GF/C) mesocosm water, C: Perspex cores closed with rubber stoppers and filled with mesocosm water with or without mesocosm sediment, D: Peristaltic pump for circulation of the overlaying water (homogenization), E: Polarographic sensors, F: O_2 measurement chambers, G: Two four-way micro valve, H: Peristaltic pump for through-flow of the system, I: Sampling tubes for further nutrient analysis, J: Electronic amplifier with four ranges for full scale amplification of the O_2 signal, K: Potentiometric chart recorder.



2.2.6 Phytoplankton abundance, biomass and production

Chlorophyll-*a* and phaeophytin-*a* were extracted on GF/C filters according to Gieskes & Kraay (1984) and analysed by HPLC method with a 85-100% acetone/water-water gradient, using 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). Counts were made in 5 ml concentrated samples (x10) on an inverted microscope (magnification x160 and x400). Dense samples were counted in a haemacytometer.

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-bicarbonates (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. (1991). Irradiance (I, in W·m^{-2}) and production (P), in mg C·mg Chl^{-1}·h^{-1}) were fitted to the model (Eilers & Peeters, 1988):

$$P(I) = \frac{I}{aI^2 + bI + c} \qquad (4)$$

Maximum production (P_{max}), irradiance half saturation constant (I_k) and daily integrated production were estimated by nonlinear regression (Wetsteijn et al., 1990).

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. Due to technical problems with the incubator, no ^{14}C-incubations were carried out in July.

2.2.7 Bacterial production

Pelagic bacterial production was estimated by measuring the incorporation of methyl-^{3}H-thymidine (2.92-3.18 TBq·mmole^{-1}, Amersham Ltd.) into DNA (Fuhrman & Azam, 1982). Samples of 5 ml were placed in glass vials. A diluted solution of tritiated thymidine was added to a final concentration of 19 nM. Control incubations were fixed with 1.5% formaldehyde (final concentration) before the addition of tritiated thymidine. After an incubation in the dark for 45-60 minutes the experimental vials were fixed with formaldehyde. The samples were filtered on cellulose nitrate filters (0.2 μm pore size) and rinsed with 5% trichloroacetic acid (Ellenbroek & Cappenberg, 1991). Radioactivity on the filters was determined by liquid scintillation counting using external standardization. Bacterial production was calculated from the following conversion factors: 11 fg C·cell^{-1}, $2 \cdot 10^{18}$ cells produced per mol of thymidine incorporated (Ducklow & Carlson, 1992).
Bacterial production was not measured in July.

2.2.8 Characterization of the light limitation

Irradiance and nutrients are the main limiting factors for the 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. (1991) 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 4) as $I_k = P_{max} / \alpha$, where P_{max} is the peak and α 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: $P/P_{max} = I_{av}/I_k$.
In the mesocosm experiments, the ratio I_{av}/I_k was used as an index of daily mean light limitation. In their typography of the limiting factors for the primary production on the Dutch continental shelf, Peeters et al. (1993b) considered phytoplankton with a P/P_{max} ratio of less then 0.5 as light limited. The same convention was used in this experiment.

2.2.9 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):

$$\frac{V}{V_{max}} = \frac{S}{(S+K_{su})} \qquad (5)$$

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

The degree of nutrient limitation was estimated from the water column nutrient concentrations using the following K_{su} 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.

2.2.10 Bivalve filtration rates

From the fluorescence recordings of inflow and outflow of the benthos chambers clearance rates of the mussels were calculated. As a consequence of the decrease in particle concentrations in the water flowing through the chambers, the actual clearance rate of the mussel population in the chambers was lower than the sum of individual clearance rates. The population clearance rate represented the total volume of water cleared free of particles by the mussels, and was calculated from:

$$CR_{mussels} = Q \cdot \frac{(C_i - C_{o,mussels})}{C_i} \qquad (6)$$

where:
$Cr_{mussels}$ = clearance rate mussel population in l h^{-1}
Q = water flow through chamber
C_i = inflow fluorescence
$C_{o,mussels}$ = outflow fluorescence mussel chamber

Fluorescence values were corrected for background fluorescence when necessary.

Individual clearance rates were calculated, assuming an exponential decrease of the particle concentration in the water flowing through the chamber and after correction for sedimentation measured in the control chamber:

$$CR_{ind} = \frac{Q}{n} \cdot [\ln(\frac{C_i}{C_o}) - \ln(\frac{C_i}{C_o, control}] \qquad (7)$$

where:
Cr_{ind} = individual clearance rate in l h^{-1}
n = number of mussels
$C_{o,control}$ = outflow fluorescence control chamber

2.2.11 Mussel biomass and growth

At the start of the experiment on 6 March 1995, 40 small mussels were added to each of the mesocosms. Mussels had been collected one week before the experiment, from the low tide level at a site close to the field station of RIKZ at Jacobahaven. Mussels with a shell length of 16-20 mm were selected. The mussels were put into separate benthos chambers, that were connected to the mesocosm and were supplied with mesocosm water at a rate of 70 l hour^{-1}.
A random sample of 40 mussels was used to determine initial length, dry weight and ash-free dry weight. Dry weights were determined after drying the mussels for 48 hours at 70 °C. Ash-free dry weights were determined from weight loss after incineration for 4 hours at 540 °C in a muffle furnace.
To determine growth rates, numbers and shell lengths of the living mussels were measured at 4-5 week intervals. At 31 May 1995 the number of the remaining mussels in the mesocosms was reduced to 20, by randomly selecting and removing a subsample of the mussels. Shell lengths, dry weights and ash-free dry weights were determined as described above. At the end of the experiment dry weights and ash-free dry weights of the remaining mussels were determined.
Growth was determined as increase in shell length, and instantaneous growth rates were calculated from the increase in ADW:

$$\mu_w=\frac{\ln(W_t-\ln(W_0)}{\Delta t}) \qquad (8)$$

where:
μ_w = growth rate in day^{-1}
W_0, W_t = ADW at begin and end of the experiment.
Δt = duration of the experiment in days

2.2.12 Assessment of macrofaunal composition and biomass

At the end of the experiment, the sediment of the mesocosms was sampled to determine the biomass of the macrobenthos that had colonized the systems. The sediment of the pelagic system was sampled by collecting 15 random cores (diameter 56 mm) of the sediment. The cores were filtered on a 1 mm sieve. The species composition of the macrobenthos was determined, and the ash-free dry weight was established by drying and burning, as described in 2.2.11. Sediment samples of the benthos chambers were collected for macrofauna determination. Macrofauna was collected by sieving the entire sediment of the benthos chambers through a 500μm mesh sieve. The samples were fixed with 5% formaldehyde. Biomass was determined as ash-free dry weight for the abundant species.

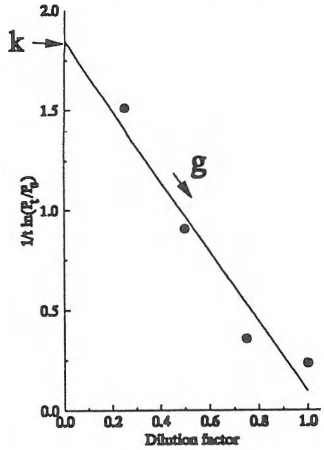
2.2.13 Zooplankton sampling and analysis

Two groups of zooplankton were sampled and analysed: microzooplankton (20-200 μm, mainly ciliates and heterotrophic dinoflagellates) and mesozooplankton (200-2000 μm, mainly copepods).

Microzooplankton:
The samples used for the phytoplankton analyses were used to analyse microzooplankton as well. Samples (1 l) were taken twice a week (Monday and Thursday, always between 7 and 8 a.m.) from outlets at 2.6 m depth.
Samples were fixed with acid Lugol's iodine solution (Throndsen, 1978) to a final concentration of ca. 0.3 %. In the laboratory samples were concentrated (ca. 10x) by sedimentation. After sedimentation 5 ml was taken from the residu and stained with Bengal rose after decolorizing with sodium thiosulphate. Microzooplankton numbers, dimensions and species composition were determined using an inverted microscope. The used magnification was 200x and sometimes 500x for a better distinction between the genera *Gyrodinium* and *Gymnodinium*. Depending on organism abundance 200 or 400 fields of view were counted. The diameter of aloricate ciliates was measured, and length and width of loricate ciliates was determined. 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; sp.4 : >60 μm. Unidentifiable ciliates without a lorica are referred to as ciliate sp. 1-4. For species identification the zooplankton sheets (from Conseil International pour l'Exploration de la Mer) 117 (1969), 120 (1969), 121 (1969), 123 (1969), 126 (1969) and 127 (1969) and Thomsen (1992) were frequently used. In general, sample analysis was done within 6 months after sampling.

Mesozooplankton:
Depth-integrated samples for mesozooplankton analysis were taken using a long PVC-tube (inner diameter 58 mm), provided with a ball valve at the lower end (see Kuiper, 1977). Lowering the tube eight times at different positions resulted in a total sample of ca. 50 l. The collected water was filtered through a 55 μm zooplankton net; in this way also the smallest naupliar stages and eggs were retained. The filtered water was thrown back into the mesocosm. In general, samples were taken twice 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. Zooplankton numbers, dimensions and species composition in each sample were determined in a subsample of 25 ml in a 50 ml cuvet under a stereomicroscope (magnification 40-50x). Occasionally, at great phytoplankton abundance, 5 ml subsamples were taken. Subsamples were stained with Bengal rose. Copepod eggs, naupliar, copepodite and adult stages of each species were counted. Beginning at copepodite stage IV a distinction was made between males and females. Body-lengths of naupliar stages and cephalo-thorax lengths of calanoid copepodite and adult stages were measured. Length of harpacticoid and cyclopoid copepodites and adults was measured as body-length. For other groups, like larval stages of *Mollusca* and *Annelida*, body-lengths were measured. For species identification the zooplankton sheets (from Conseil International pour l'Exploration de la Mer) 4 (1943), 7 (1969), 12 (1948), 50 (1953), 84 (1960), 85 (1960) and 107 (1965), Smith (1977) and Klein Breteler (1982) were frequently used. In general, sample analysis was done within 6 months after sampling.

Figure 2.3
Theoretical example illustrating the relationship between the phytoplankton net specific growth (measured by the log of the fluorescence ratio after/before the incubation period) and the dilution applied to the batches used for the incubations (each batch represents one level of dilution of the same original mesocosm water sample.



2.2.14 Estimation of the microzooplankton grazing

The estimation of microzooplankton grazing was performed by the dilution method (Landry and Hasset, 1982). It expresses change in phytoplankton biomass by

$$P_t=P_0e^{(k-g)t} \qquad (9)$$

or

$$\frac{1}{t}\ln\left(\frac{P_t}{P_0}\right)=k-g \qquad (10)$$

where P_0 and P_t are phytoplankton biomass at the beginning and end of the experiment; k = algal growth rate (d^{-1}); g = algal mortality rate due to grazing (d^{-1}); t = time (d). Values of k and g are calculated from changes in phytoplankton biomass during incubations of different dilutions of water containing the natural microzooplankton assemblage. The 'apparent phytoplankton growth rate' ($1/t \cdot \ln(P_t/P_0)$) at the different dilutions is linearly related to the dilution factor. In Fig. 2.3, a theoretical example has been plotted: the Y-axis intercept of this relationship is the phytoplankton growth rate, k, in the absence of grazers. The negative slope is the grazing coefficient, g. The dilution factor was calculated from the chlorophyll concentrations at t=0 in every dilution as a fraction of the undiluted sample.

This method assumes that phytoplankton growth rates are not density dependent, ingestion is a linear function of consumer density and that eq. (9) describes phytoplankton growth adequately. This implies that light and nutrient levels may not become limited

during the experiment and that every individual grazer will consume the same amount of phytoplankton in every dilution.
Mesocosm water was sampled in 15 liters buckets and back-filtrated with a 100 μm mesh sieve: the sieve was immersed in the bucket containing the mesocosm water, the water pouring through the sieve was drawn out by means of siphon into another bucket. Seawater used for dilutions was filtered using Whatman GF/C filters (47 mm ϕ, pore size app. 1 μm), oxygen saturation was restored by pressurised air. Mixtures were made in 5 liter bottles in ratios of 1/0, 3/1, 1/1, and 1/3 (mesocosm-/filtered water). Two liters of each dilution was drawn into two separate glass incubation bottles (Schott Duran, GL 45, 1000 ml). To prevent nutrients from becoming limited during the incubations, DIN, DIP and Si, if necessary, were added in excess in the same proportions as the nutrient concentrations in the mesocosm.
The bottles were sealed without airbubbles and attached to a rotating wheel contained within a 430 liter, water-cooled incubator (Tackx, 1983).

The estimation of microzooplankton grazing in mesocosms was performed from July 18 till August 7. During this period 18 experiments were done with samples from different mesocosms. The temperature (19-21 °C) and light conditions in the incubator were set at comparable levels as in the mesocosms.

2.3 EXPERIMENTAL TREATMENTS

The nutrient loading of the mesocosms aimed at reproducing the characteristics of the nutrient loading at a reference site of the Dutch coastal zone of the North Sea. Three levels of nutrient loading were used to simulate different scenarios for the reduction of the anthropogenic nutrient load to the Dutch coastal zone. The three levels of nutrient enrichment (High, Medium and Low) were defined in proportion to the level of the years 1980 to 1987 (Table 2.1). This latter period was considered as a period of high eutrophication.
Different levels of reduction of the anthropogenic N-load were used for each treatment (H>M>L), whereas the P-load was the same in treatments H and M and lower in treatment L. Silicate loading of the North Sea was considered not to be influenced by eutrophication, and Si was supplied at equal rates in all treatments (also see chapter 1).
The actual levels of nutrient loading used in each treatment were defined with the help of the mathematical simulation model DYNAMO, developed within the MANS (Management Analysis of the North Sea) project. Runs of DYNAMO were carried out by RIKZ. The DYNAMO model is a two dimensional model of the North Sea, divided in compartments of 16x16 km. The model calculated the net nutrient loading of a box of 112 (North-South) by 26 (East-West) km along the Dutch coast off Noordwijk, for each month of the year 1987. Simulations were made for each scenario (1,2 and 3 in Table 2.1), with 4.5 m s^{-1} southwesterly winds (dominant wind direction above the North Sea).
The DYNAMO simulation outputs were used to define the nutrient loadings of the mesocosms, after adaptation to the mesocosm experimental design. The year 1987 used as reference by DYNAMO

differed from the previous years (over the period 1980-1987) by a higher riverine discharge. Therefore, the DYNAMO simulation results were corrected for the higher discharge in 1987. With southwesterly winds, the residence time of the water in the modelled box was 18 days. In previous experiments the residence time of the water in the mesocosms was 30 days. In order to keep most experimental conditions comparable to previous experiments, the nutrient loadings predicted by DYNAMO were corrected for an increase of the residence time from 18 to 30 days. DYNAMO simulations were given as average loadings per month. These values were interpolated to weekly averages for the entire experimental period. The average actual nutrient loading in each treatment is shown in Table 2.2.

Table 2.1
The three scenarios for the reduction of the anthropogenic nutrient loading to the Dutch coastal area (in proportion to the period 1980-1987), used to define the three treatments (High, Medium, Low) in the mesocosm experiment. Silicate was supplied at equal rates in all treatments.

	Reduction in anthropogenic nutrient loading compared to the period 1980-1987 (%)	
	Nitrogen	Phosphorus
Scenario 1 (Treatment H)	-10	-50
Scenario 2 (Treatment M)	-50	-50
Scenario 3 (Treatment L)	-75	-75

Table 2.2
Average actual nutrient loadings in the 1995 mesocosm experiment.

Treatment	**DIN (mmol m^{-2} d^{-1})**	**phosphate (mmol m^{-2} d^{-1})**	**silicate (mmol m^{-2} d^{-1})**	**N:P**
H	11.7	0.31	2.85	38
M	6.9	0.31	2.85	22
L	4.0	0.22	2.85	18

Fig 2.4
Planned (broken lines) and actual loadings (full lines) of dissolved inorganic and total N and P.

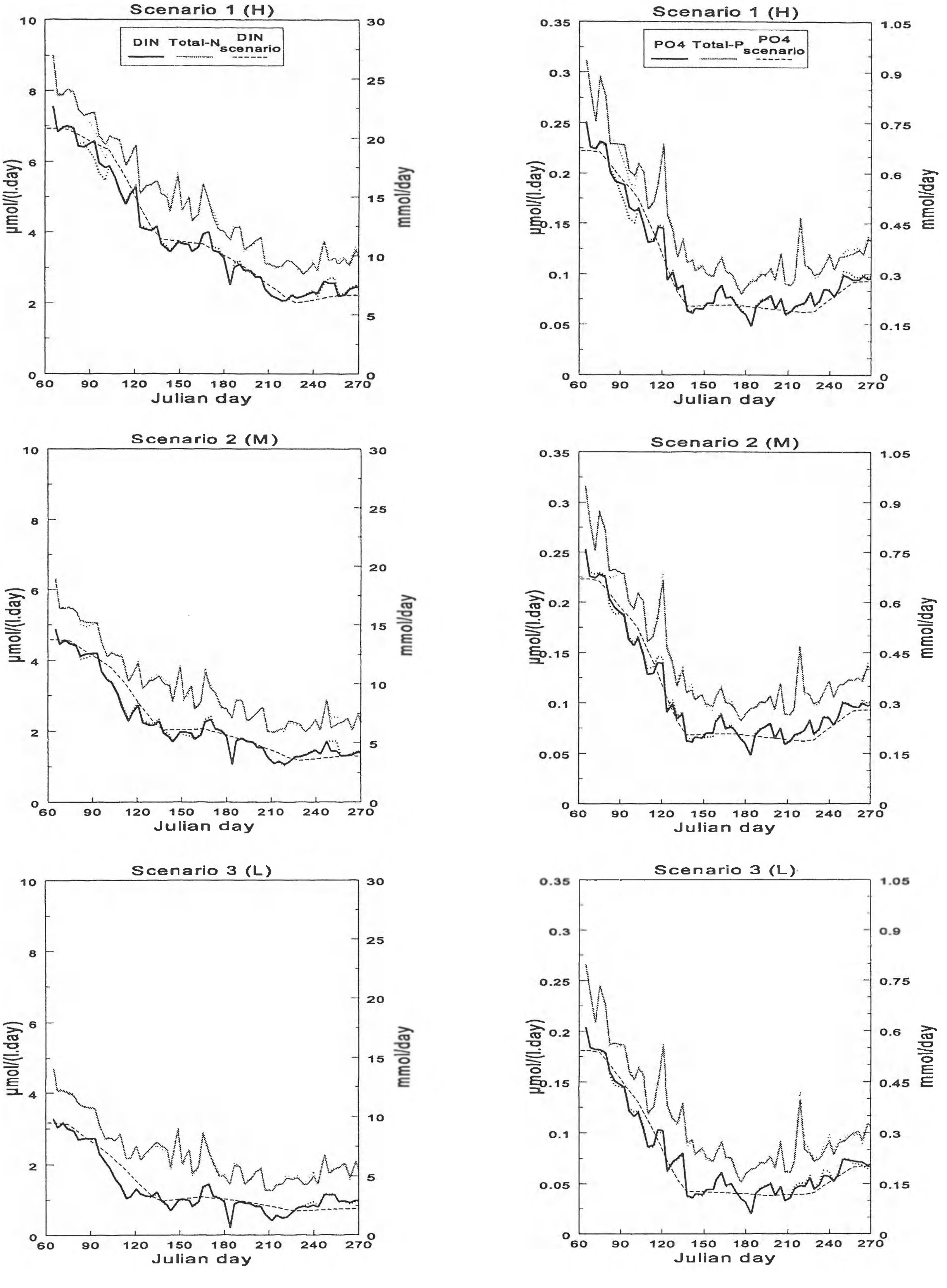
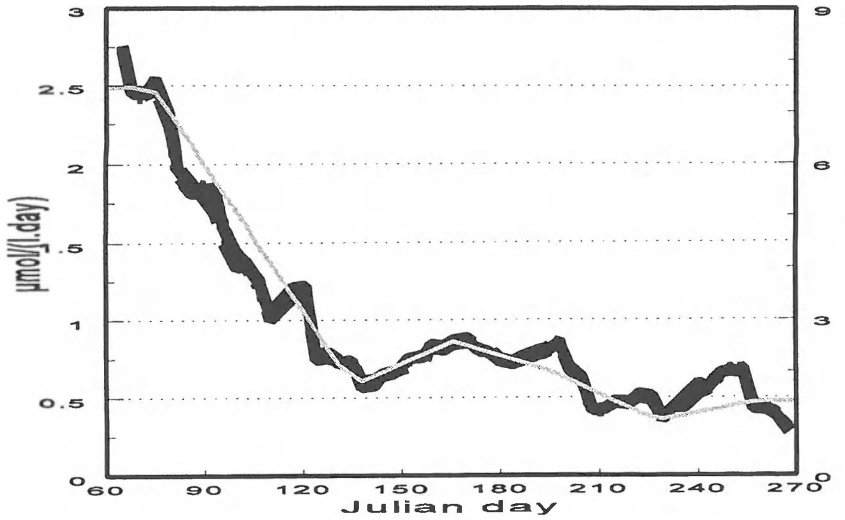


Figure 2.5
Planned (grey line) and actual (black line) Si-loadings in the six mesocosms.



Final nutrient inputs were the sum of inorganic nutrient additions and input of particulate and dissolved nutrients through the sea water. Planned and actual nutrient loadings of the six mesocosms are shown in Figure 2.4 and 2.5.

2.4 MESOCOSM CONDITIONS COMPARED TO *IN SITU* NORTH SEA CONDITIONS

2.4.1 Temperature

Temperatures in the mesocosm showed a seasonal cycle comparable to *in situ* temperatures in the Oosterschelde estuary (Fig. 2.6).
Highest temperatures were observed in August. The average daily temperature range was ±2 °C.

Fig 2.6
Daily average water temperature in the mesocosms (full line). The dotted line shows the the daily average *in situ* water temperature in the Oosterschelde mouth.

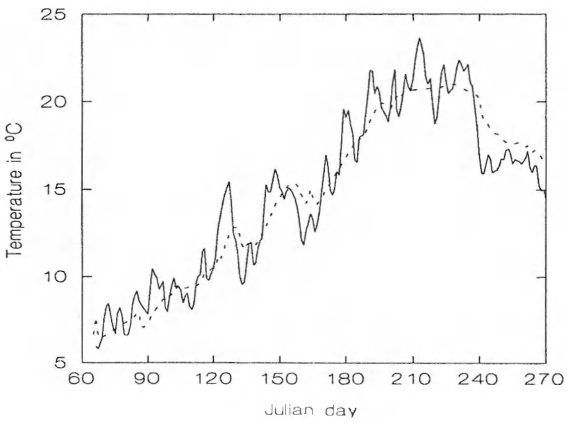
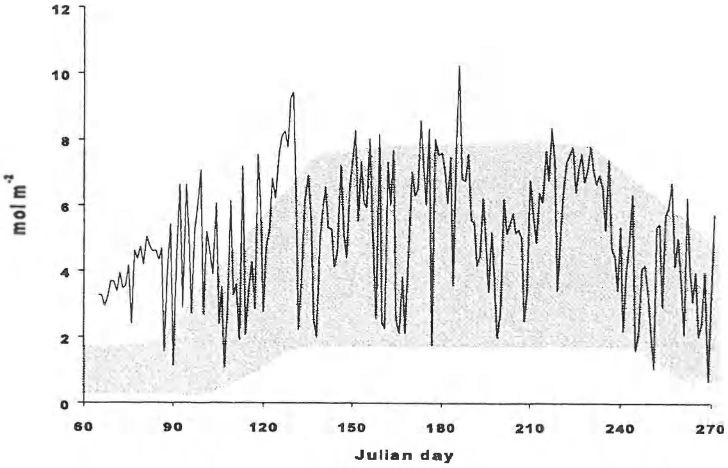


Figure 2.7
Mean water column irradiance in mesocosm H1. The shaded area shows the range of values observed at North Sea stations NW2 and NW20.



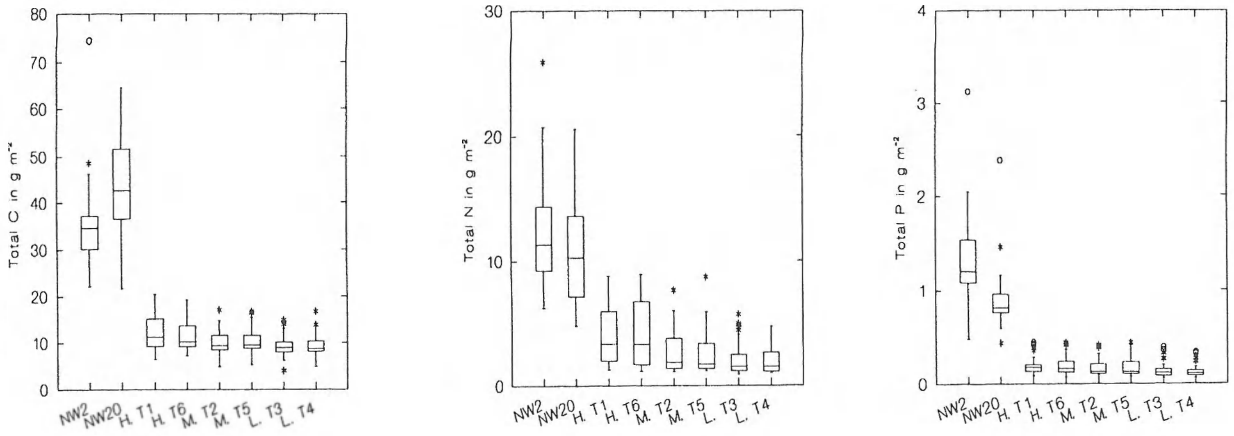
2.4.2 Light

Irradiance in the mesocosms was measured under the light diffusor of mesocosm H1. Measured surface irradiance was combined with observed light extinction values to estimate mean water column irradiance according to formula 2. The observed mean water column irradiance in mesocosm H1 was compared to data of North Sea stations NW2 and NW20. The latter data were collected during EUZOUT cruises in the years 1988-1990 (Fig. 2.7).
Irradiance at NW2 was generally lower than at NW20 as a consequence of higher turbidity near the coast. Light conditions in the mesocosm were slightly better in spring, probably as a consequence of lower turbidity. In general, values observed in the mesocosm fell within the range observed at the North Sea stations. The mean water column irradiance in the other mesocosms showed values comparable to mesocosm H1 (also see Chapter 3).

2.4.3 Nutrients

Data on inorganic dissolved nutrient concentrations and total nutrient concentrations at North Sea stations NW2 and NW20 were collected during routine monitoring cruises in the years 1988-1990. Box plots of total carbon, nitrogen and phosphorus concentrations at the two North Sea stations and in the mesocosms are shown in Figure 2.8.

Fig 2.8
Total amounts of carbon, nitrogen and phosphorus in the water column of the mesocosms, compared to values observed at NW2 and NW20 in the periode March-September 1988-1990.



The box contains the middle 50% of the data, and the horizontal line through the box represents the median. Extending from either side of the box are the 'whiskers', representing data points within 1.5 times the interquartile range (length of the box) beyond the edges of the box on either side. The ends of the whiskers mark the inner fences. Asterisks represent data points within 3 times the interquartile range (the outer fences). Data points falling outside the outer fences are shown as circles. For an extensive description of box plots see e.g. Kirby (1993), Sokal & Rohlf (1995) and Tukey (1977).

Total amounts of C, N and P in the water column above 1 m^2 of sediment were lower in the mesocosms than at the North Sea stations. This is similar to the results of previous experiments. Nutrient loading to the mesocosms was similar to the nutrient loading to the North Sea when expressed in units per surface area. The much lower total nutrient stock in the water column of the mesocosms was probably caused by the fact that the mesocosms contained less detritus.

2.4.4 Phytoplankton biomass and production

Data on phytoplankton biomass at North Sea stations NW2 and NW20 were also collected during a number of cruises in the years 1988-1990. The data from the North Sea cruises were compared to the phytoplankton biomass data from the mesocosms in the H treatment, as the latter treatment resembled the nutrient loadings in the late 1980's most closely.

Figure 2.9 shows that the phytoplankton biomass in the mesocosms fell within the range of observations from the North Sea stations. The phytoplankton spring bloom in the mesocosms started in early April, and this timing was identical to the situation in the North Sea. Phytoplankton biomass in late summer was lower in the mesocosms than at the North Sea stations. A possible explanation for this divergence is differences in zooplankton grazing.

Primary production in the period March-September in the North Sea is approximately 90% of total year production (Table 2.3). Primary production at NW20 is higher than at NW2 because of better light conditions. Primary production in the mesocosms generally fell between the values for NW2 and those for NW20 (Figure 2.10). There was also a general agreement between the ratio of spring versus summer production in the mesocosms and the North Sea stations.

Figure 2.9
Chlorophyll-*a* concentrations (g m^{-2}) in treatment H (lines) compared with the concentrations measured at NW2 and NW20 during the years 1988-1990 (box-and-whisker plots).

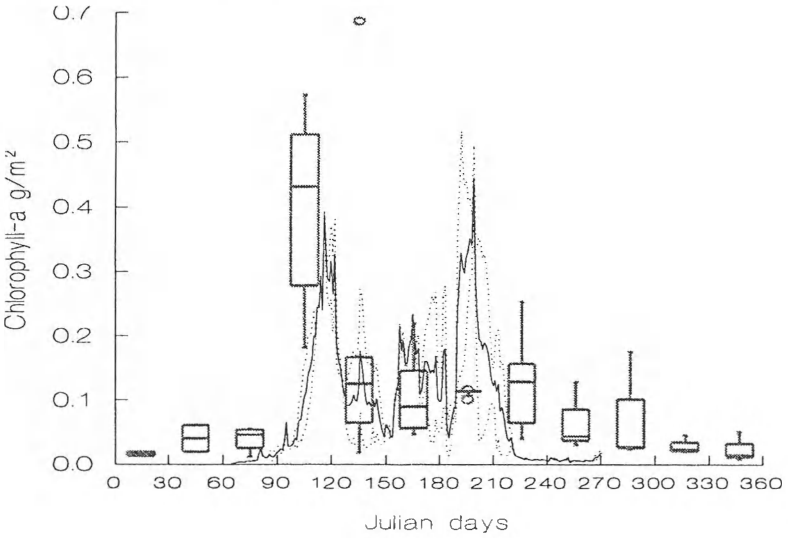
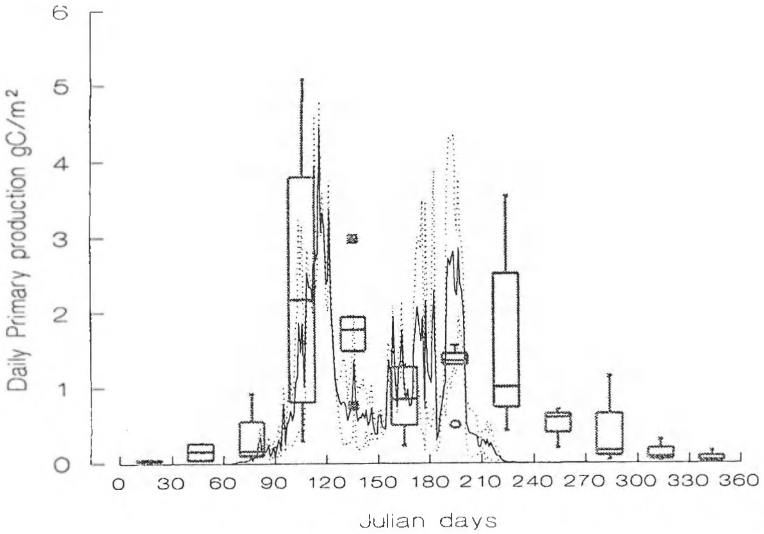


Table 2.3
Primary production in mesocosms H1 and H2 compared to values measured at NW2 and NW2 in the periode March-September 1988-1990.

		total production in g C m^{-2}			
Location	year	March-May	June-September	March-September	Annual production
NW2	1988	48	56	104	108
	1989	112	114	226	258
	1990	68	42	110	135
	average =	76	70	147	167
NW20	1988	129	124	253	263
	1989	140	112	252	288
	1990	281	271	553	572
	average =	183	169	352	374
mesocosm H1		99	108	207	-
mesocosm H2		64	51	114	-

Figure 2.10
Daily primary production (g C m^{-2}) in treatment H (lines) compared with the values measured at NW2 and NW20 during the years 1988-1990 (box-and-whisker plots).



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3 Phytoplankton dynamics in response to three nutrient scenarios in mesocosms

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ABSTRACT

In a 7 month experiment, carried out in 1995, 3 different nutrient loading scenarios were applied to 6 land-based marine mesocosms, representing the present condition in the Dutch coastal zone (N and P loadings in 1995 being equal to respectively 90% and 50% of the loadings in 1985), the situation after implementation of a 50% reduction in anthropogenic N- and P-loading, and a more extreme situation with a 75% reduction in N- and P-loading. Each nutrient load was slightly varied in time to reproduce the seaonal variations actually observed in the field. Phytoplankton biomass and production showed significant responses to the treatment and reproduced a seasonal pattern similar to what was inferred for the field from bio-assays experiments: P-limitation of the spring bloom followed by a more extended period dominated by a nitrogen limitation of both phytoplantkon biomass and production. This experiment did not succeed to reproduce the spring/summer *Phaeocystis* bloom. The hypothesis is put forward that the lack of predation on the micro/mesozooplankton mainly due to the relative small scale of our mesocosms combined to the long duration of experiment could have been responsible for an overgrazing of the small size fraction of the phytoplankton.

3.1 INTRODUCTION

In the last decades eutrophication of the North Sea has increased considerably as witnessed by the development of large-scale oxygen deficiencies in the German Bight, overall increase of phytoplankton biomass and bloom duration and possible shifts in its specific composition (Smayda 1990, Vollenweider et al., 1992). Those phenomenons are known to be related to the large anthropogenic inputs of phosphorus and nitrogen to the North Sea. This prompted the decision of the Second North Sea Conference to halve the anthropogenic load of phosphorus and nitrogen by 1995.

In order to get background information on the possible effect of the programmed nutrient reductions on the natural environment, a series of experiments was carried out in 1993 and 1994 in land based mesocosms. Natural bodies of sea water (mouth Oosterschelde estuary) were incubated during approximately one month and submitted to variable nutrient treatments. In 1993, two phosphorus-gradient experiments (spring, summer) were carried out in four mesocosms (Escaravage et al., 1996). In 1994, two nitrogen-gradient experiments

(early and late summer) were carried out in six mesocosms used in duplicate (Escaravage et al., 1995).
In each of the gradient experiments a proportional relationship could be established between the load of the first limiting nutrient (N or P) and the phytoplankton biomass and production and changes in phytoplankton species compostition were observed along the nutrient gradient. The significance of those results was however partially hampered since, the initial nutrient pool first had to be consumed to allow the treatment (nutrient-load gradient) to become operational. Consequentely, responses to the treatment could only been observed at the very end of the experiments.
Another drawback of the relatively short duration of the experiments was the inconsistency between the life history of the phytoplankton introduced in the mesocosms at the first day of the experiment and the conditions in the mesocosms. When entering the mesocosms, the phytoplankton community faced conditions radically different from those in the field and showed rapid changes in concentrations and composition.The treatment (nutrient load gradient among the mesocosms) acted but as a secondary constraint for the phytoplankton community which first had to adapt to the new conditions prevailing in the mesocosms.
In order to cope with those limitations inherent to the short duration of experiments it was decided to run a long term experiment between March and September 1995 allowing to follow the development of phytoplankton communities (biomass, production, species composition) over the whole growth period. The nutrient input was varied among the six mesocosms and reproduced in replicate three scenarios of anthropogenic nutrient input reduction to the Dutch coastal zone: 10%N, 50%P (achieved during the 90's); 50%N, 50%P (originally planned for 1995 but not yet realized for N) and 75%N, 75%P (a more extreme case of sanitation). Phytoplankton biomass, production and species composition was monitored and related to the gradient in the nutrient treatment.

3.2 MATERIAL AND METHODS

Analytical procedures are described in Chapter 2.
The nutrient additions actually realized during the experiment are shown in Table 3.1. In what follows, the mesocosms are encoded with the letters H, M and L corresponding to a high, medium or low N-load combined with the number 1 or 2 to identify the replicates within each treatment.
The present experimental set-up combined both nitrogen- and phosphorus- load reduction. A decreasing gradient in N-load was established between treatments H, M and L. The P-load was similar in treatments H and M and lower in treatment L.
At julian day 183 a leakage occurred in mesocosm H1. The mesocosm was then filled again with Oosterschelde water enriched with nutrients to reach the concentrations in this mesocosm before the leakage. In order to promote the likeness between both mesocosms, mesocosm H1 was flushed with the overflow from mesocosm H2 the whole week following the leakage.

Table 3.1
Actual nutrient loads to the mesocosms (including the initial pool at day 1) in mmol

Treatment	Net loads (mmol) DIN	DIP	Si
H1	2388	67	595
H2	2386	66	593
M1	1376	66	592
M2	1392	67	598
L1	799	47	587
L2	801	47	588

3.3 RESULTS

3.3.1 Irradiance and potential light limitation for primary production

Irradiance evolution could be decomposed into seasonal trends and meteorologically driven stochastic variations. As a result of seasonal trends, irradiance increased until julian day 150 (Spring), rather stabilized between julian days 170 and 235 (Summer) and decreased after julian day 235 (Autumn). Meteorological events induced mainly short term increases or decreases of irradiance and had minor effect on its long term evolution except between julian days 150 and 170 when an extended period of bad weather conditions induced a pronounced decrease of irradiance (Figure 3.1).

Figure 3.1
Daily surface irradiance (PAR, mol photons.m^{-2}.d^{-1}) measured under the optical diffusor on top of the mesocosms. Daily measurements were smoothed (weighed moving average, 15 days period) to underline the main long term trends.

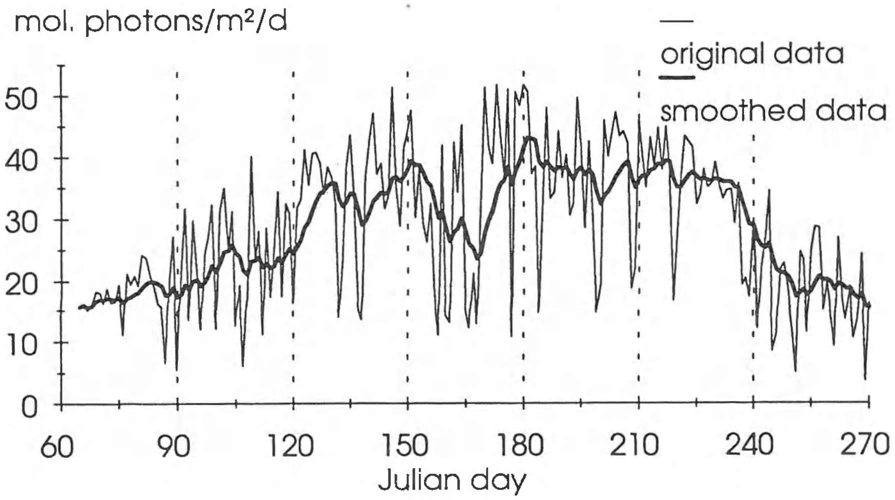
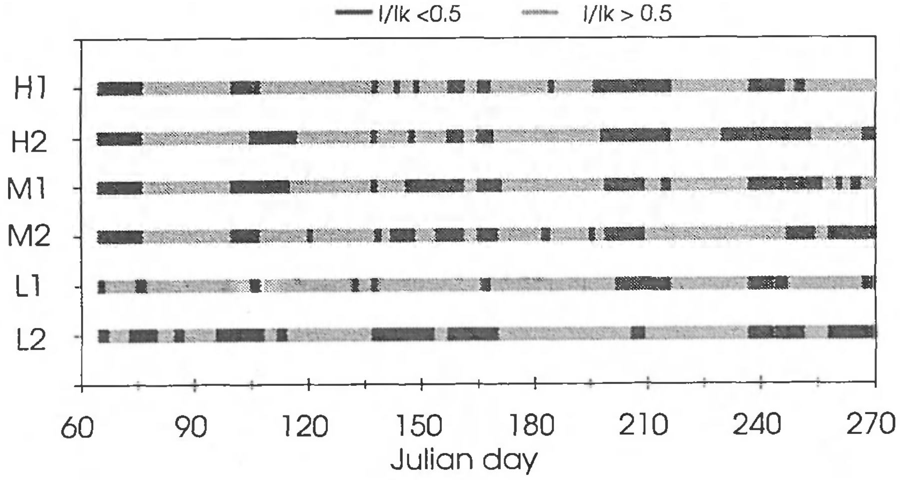


Figure 3.2

Index of light limitation (I/I_k) estimated in the six mesocosms (H1, H2, M1, M2, L1, L2). I is the mean daily irradiance available to the phytoplankton, I_k the photosynthetic characteristic irradiance ($I_k = P_{max} / \alpha$, with P_{max} the highest point and α the slope of the P/I curves obtained by the C_{14} incubations). When I/I_k is lower than 0.5, light limitation is potentially occuring (See Chapter 2.2.6 for further explanation).



The I/I_k ratio was used as an indication of light limitation for the primary producers (as described in Chapter 2).The intensity of light limitation did not significantly differ among the mesocosms (ANOVA F_{ratio}=0.980, p=0.470) (Figure 3.2). Averaged for all mesocosms, periods when light limitation occurred represented 37% of the total time of the experiment. Four periods of intensification of the light limitation measured in all mesocosms corresponded to lowered surface irradiance (Figure 3.1) between days 60 and 75, 100 and 110, 135 and 170 and after day 235.

3.3.2 Dissolved inorganic nutrient concentrations and potential limitation for primary production

Initial DIN concentrations ranked following the N-load gradient (Figure 3.3). DIN concentrations decreased very sharply in mesocosms L1 and L2 between days 90 and 120, were just above 2 µM between days 120 and 200 and below 2 µM until day 260. In treatment M, the initial decrease of DIN was smoother than in treatment L. Limiting concentrations were measured in M2 from day 180 on, DIN concentrations were generally higher in M1 than in M2. DIN concentrations decreased very smoothly in mesocosm H2 the whole experiment through and always remained above the limitation threshold. DIN concentrations were similar in H1 and H2 until day 200. Between days 200 and 215 (one week after the leakage), DIN concentrations sharply decreased in mesocosm H1. After day 230, DIN concentrations were higher in H1 than in H2.

Initial DIP concentrations ranked following the P-load gradient, higher in H and M treatments than in the L treatment (Figure 3.4). DIP concentrations decreased similarly in all mesocosms until day 115 and then were just above 0.1 µM. Between days 120 and 160 DIP concentration increase took place in all mesocosms but

Figure 3.3
Dissolved inorganic nitrogen in the six mesocosms (L1, L2, M1, M2, H1, H2). The Y-axis was log-scaled to highlight the low concentration level. For more clearness at low concentration levels, data were smoothed (moving average, 1½ week period). When concentrations decrease below 2 µM (Ks value from literature sources, see Chapter 2), DIN becomes potentially limiting for the primary production.

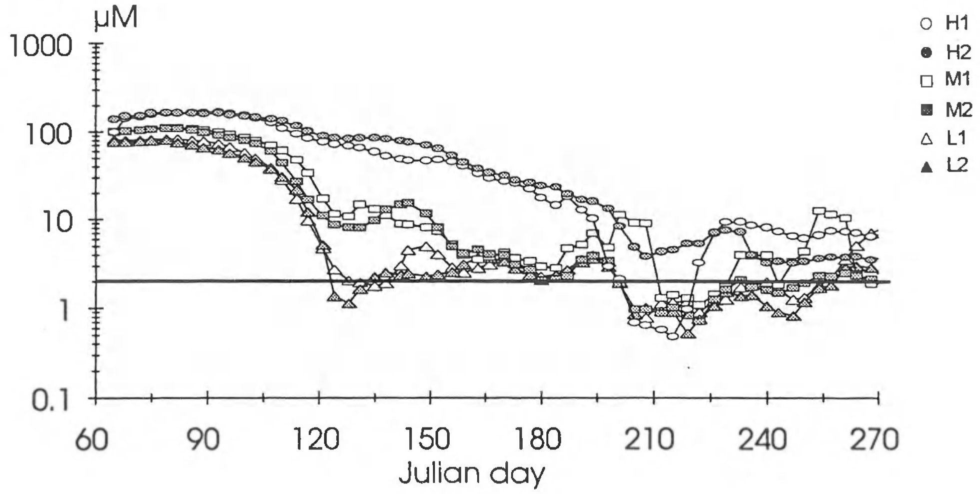
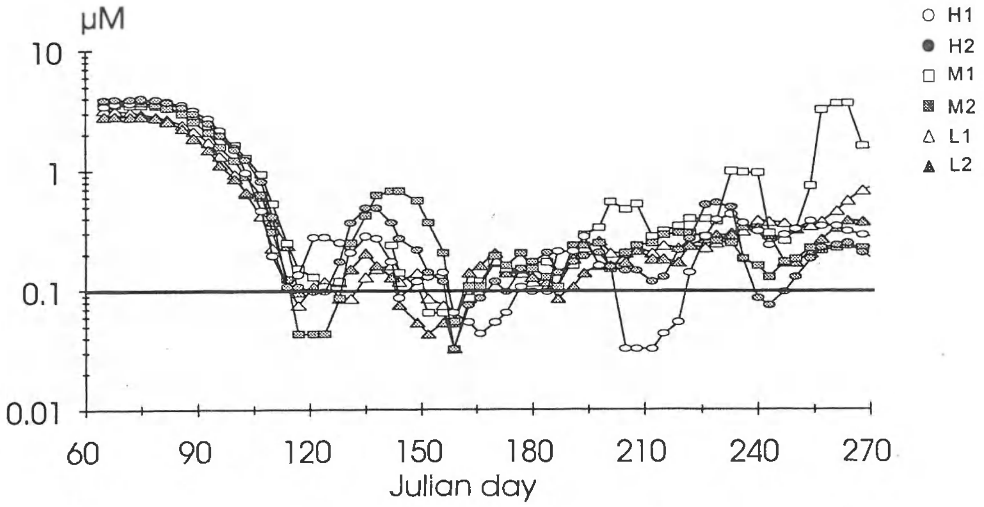
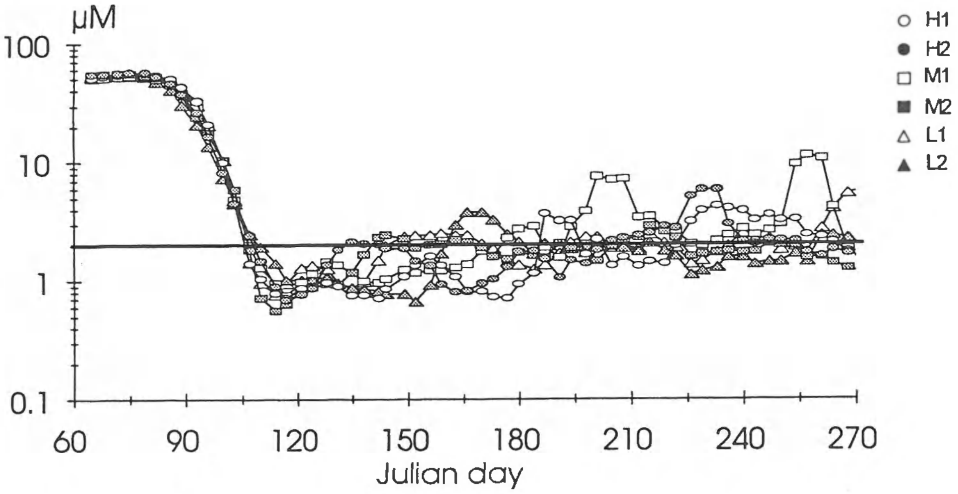


Figure 3.4
Dissolved inorganic phosphorus in the six mesocosms (L1, L2, M1, M2, H1, H2). The Y-axis was log-scaled to highlight the low concentration level. For more clearness at low concentration levels, data were smoothed (moving average, 1½ week period). When concentrations decrease below 0.1 µM (Ks value from literature sources, see Chapter 2), DIP becomes potentially limiting for the primary production.



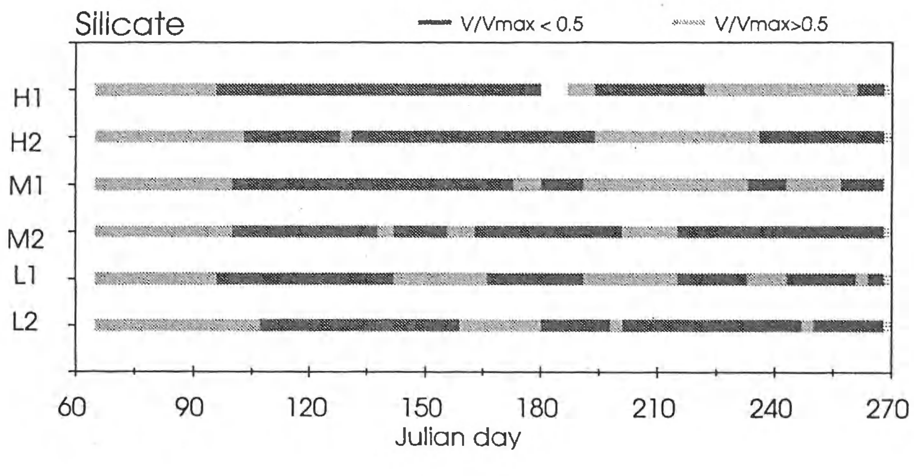
concentrations remained below 1 µM. On day 160, DIP concentrations were below the limitation threshold in all mesocosms. From this day on, concentrations tended to increase in all mesocosms. This trend was more complex for some mesocosms: concentration increases in M1 on days 200, 230 255, concentration decreases in H1 and H2 on days 200 and 230 respectively.

Figure 3.5
Dissolved silicate in the six mesocosms (L1, L2, M1, M2, H1, H2). The Y-axis was log-scaled to highlight the low concentration level. For more clearness at low concentration levels, data were smoothed (moving average, 1½ week period). When concentrations decrease below 2 µM (Ks value from literature sources, see Chapter 2), Silicate becomes potentially limiting for the primary production.



Initial silicate concentrations were similar in all mesocosms (Figure 3.5). Silicate concentrations began to decrease from day 85 on and became lower than 2 µM between days 105 and 130 in all mesocosms. From this day on, silicate concentrations increased occasionally above 2 µM in some mesocosms.
The occurrence of silicate, DIP and DIN potential limitations for primary producers is shown on figures 3.6 and 3.7. ANOVA (within/among treatments, mesocosms nested within treatments as error term) was used to test wether the intensity of nutrient limitation significantly differed among the treatments.

Figure 3.6
Potential silicate limitation for primary producers in the six mesocosms (L1, L2, M1, M2, H1, H2) estimated by the procedure given in chapter 2). Silicate is not limiting when $V/V_{max}>0.5$ and potentially limiting when $V/V_{max}<0.5$.



Silicate became potentially limiting for diatoms from day 100 on in all mesocosms. There was no simple time-pattern in the occurrence of the silicate limitation and no significant difference in the intensity of the silicate limitation was found between the treatments (ANOVA, F_{ratio}=0.538, p=0.631).

DIP became limiting for primary producers from day 105 on in all mesocosms except M1. A second period of intensification of the potential DIP limitation took place between day 150 and 180. After day 190 DIP was potentially limiting only in treatment H. Considered over the whole period, no significant difference in the intensity of the DIP limitation could be found among the treatments (ANOVA, F_{ratio}=3.979, p=0.143).

DIN was potentially limiting for the primary producers in mesocosms L1 and L2 between days 120 and 150. After day 195, DIN became limiting in all mesocosms except in mesocosm H2. A significant effect of the treatment on the intensity of the DIN limitation was found (ANOVA, F_{ratio}=28.2, p=0.011). A paired comparison following the ANOVA concluded that the difference was significant between L and H (p=0.015), not significant between M and H (p=0.057) and between L and M (p=0.208).

At several occasions silicate and phosphorus (before day 180) or silicate and nitrogen (after day 180) limitations occurred simultaneously. Diatoms were then potentially limited by silicate and phosphorus or nitrogen. Molar ratios are frequently used to determine which nutrient

Figure 3.7

Potential phosphorus and nitrogen limitation for primary producers in the six mesocosms (L1, L2, M1, M2, H1, H2) estimated by the procedure given in chapter 2). Silicate is not limiting when $V/V_{max}>0.5$ and potentially limiting when $V/V_{max}<0.5$.

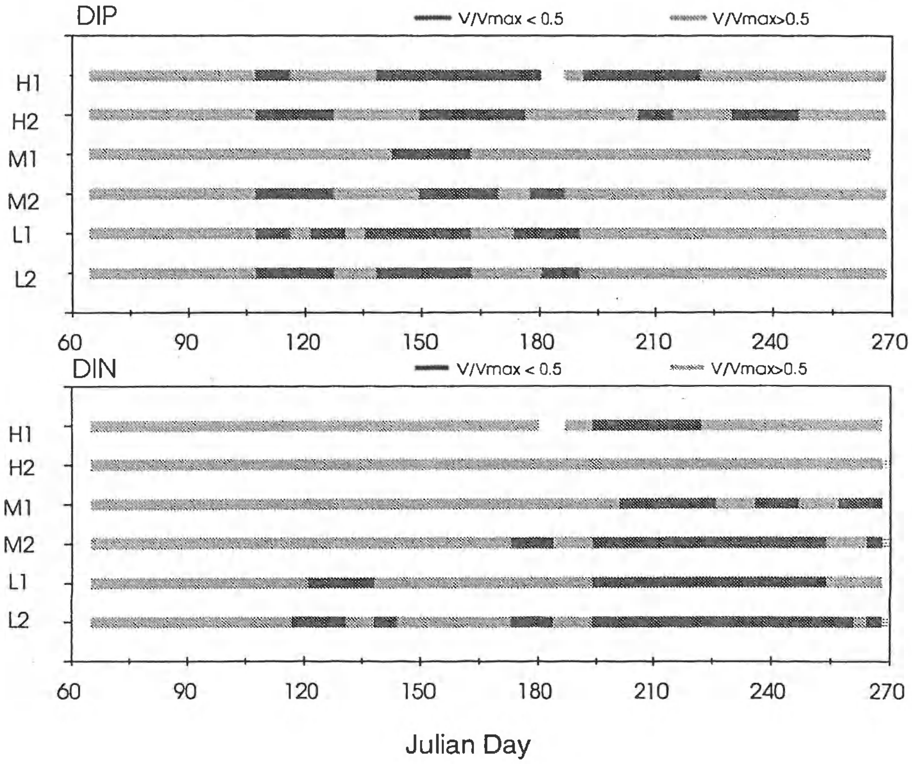
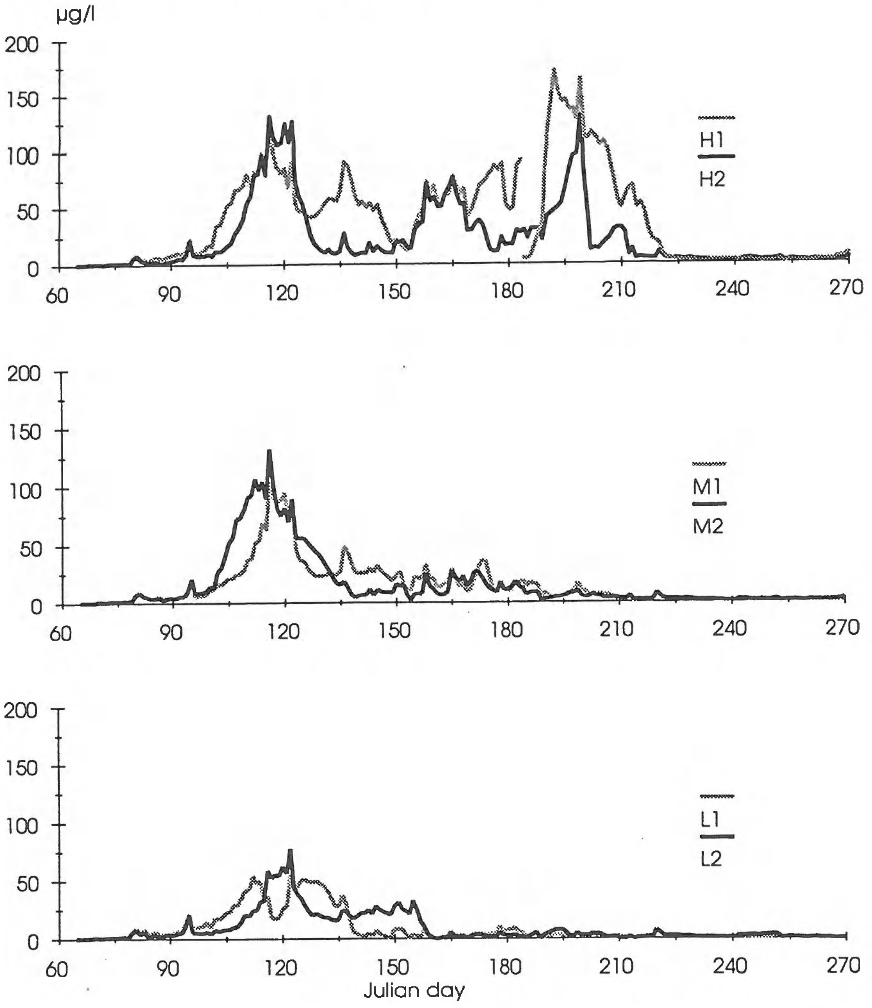


Figure 3.8

Chlorophyll-*a* concentrations in the six mesocosms (H1, H2, M1, M2, L1, L2).



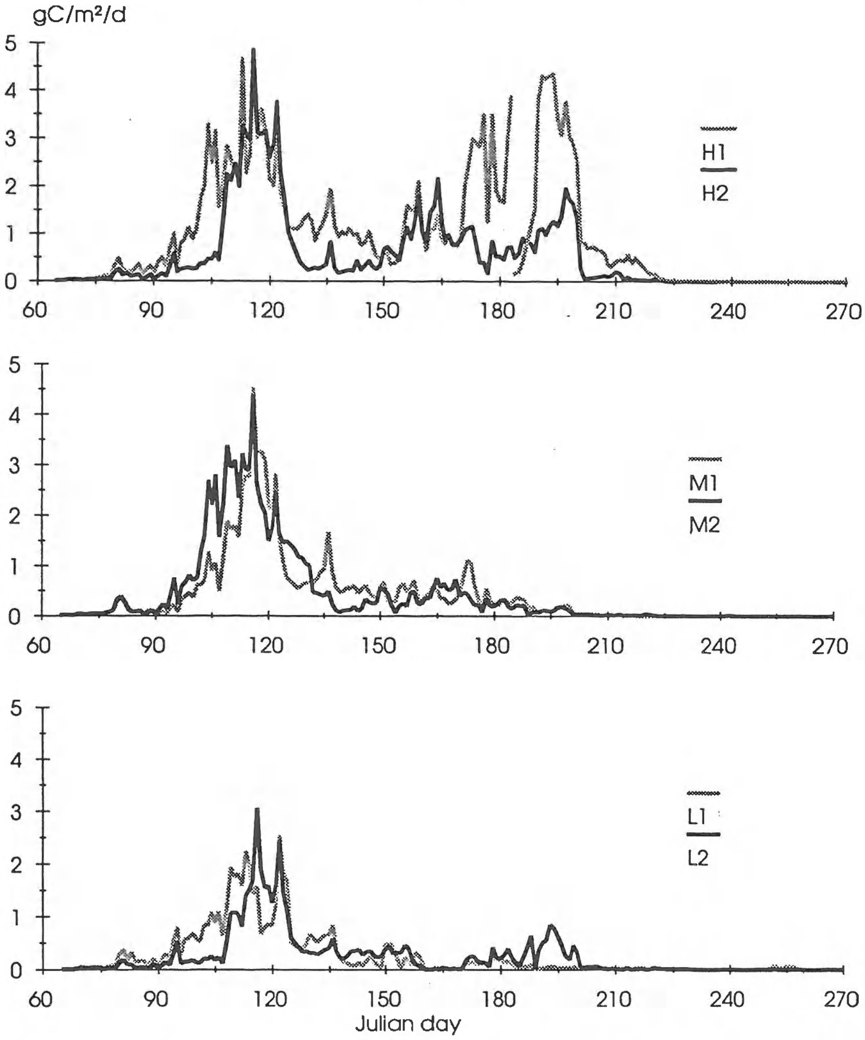
in a mixture should be the first limiting nutrient for phytoplankton. In the present case, since both nutrients are at concentrations below the theoretical limitation threshold for phytoplankton uptake, it seems reasonable to assume that diatoms were simultaneously limited by silicate and phosphorus (before day 180) and later by silicate and nitrogen (after day 180). There was not much overlap between nitrogen and phosphorus limitations except in mesocosms L1 and L2 between julian days 120 and 150. When treatment L is omitted, it can be concluded that non silicon-using phytoplankton was phosphorus limited in the first half of experiment (days 100 to 180) and nitrogen limited in the second half of experiment (after day 180).

3.3.3 Phytoplankton biomass and production

Main changes in chlorophyll-a concentrations

The height of the chlorophyll-*a* peaks and the duration of the bloom periods differed greatly between treatments (Figure 3.8). In treatment H four main peak periods were observed. Treatments M and L shared the first bloom period only with treatment H (peak near julian day 120) since no further significant development was observed in those mesocosms. The first peak was of similar height in treatments H and M but lower in

Figure 3.9
Daily primary production in the six mesocosms (H1, H2, M1,M2,L1,L2)



treatment L. Marked differences were observed between the replicates within treatment H. At two occasions (julian days 130-150 and 170-180) chlorophyll-*a* concentrations behave in opposite manner in H1 and H2. In both cases, chlorophyll-*a* concentrations increased in H1 whereas they were decreasing in H2. Chlorophyll-*a* concentrations also greatly differed in mesocosms H1 and during the last peak period (after day 180) following the leakage and subsequent filling of mesocosm H1.

Main changes in primary production
Primary production reproduced an evolution similar to the chlorophyll-*a* concentrations. Differences in primary production observed between the mesocosms were similar to those observed for chlorophyll-*a* (Figure 3.9).

Response of chlorophyll-a concentration and primary production to the treatment
Analysis of Variance (mixed model ANOVA with replicate mesocosms nested within treatment) was performed to test whether the treatment

had a significant effect on the chlorophyll-*a* concentration and on the primary production. In order to account for possible interferences between the experimental treatment and the seasons, two periods, Spring (until julian day 150) and Summer (after julian day 150) were distinguished. The N-load and P-load effects were tested separately. Homogeneity of the variance was obtained after a log-transformation of the chlorophyll-*a* concentration and primary production.
Results obtained for the chlorophyll-*a* concentration and the primary production were similar (Table 3.2). Before julian day 150 the chlorophyll-*a* concentration and the primary production were significantly controlled by the P-load whereas the N-load effect was not significant (treatments H and M not different). After julian day 150, the N-load effect became significant ($H \neq M \neq L$).
The cumulated primary production measured in each mesocosm during the spring and summer periods and over the whole experiment is showed in Table 3.3. In the mesocosms with the highest nitrogen load, the yields obtained in spring and summer were in the same order of magnitude. In treatments M and L most of the yield (two third on average) was produced during the spring period.

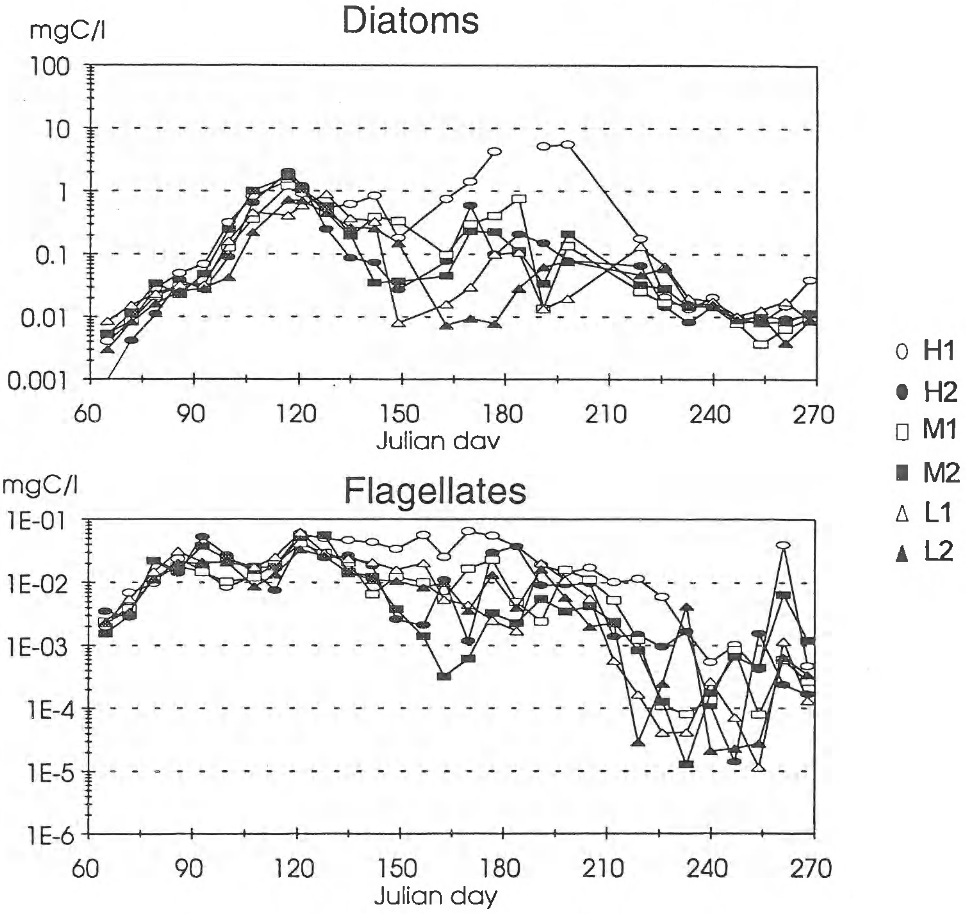
Table 3.2
F-values from a nested ANOVA for the treatment effect (P-load and N-load gradients) on chlorophyll-a concentration and primary production in spring (before julian day 150) and summer (after julian day 150).
*p<0.050; ** p<0.010.

Treatement	**P-load gradient**		**N-load gradient**	
Period	**Spring**	**Summer**	**Spring**	**Summer**
Chlorophyll-*a*	10.8*	5.4	5.2	25.26**
Primary Production	11.7*	3.5	4.9	11.4*

Table 3.3
Cumulated primary production (gC.m^{-2}) measured in spring, summer and over the whole experimental period

Period **Treatment**	**Spring**	**Summer**	**Whole experiment**
H1	99.0	108.3	206.6
H2	63.9	51.2	114.4
M1	67.3	19.7	86.4
M2	76.6	15.6	91.6
L1	48.6	6.7	54.8
L2	38.5	13.8	51.9

Figure 3.10
Total biomass of diatoms and flagellates biomass (mgC/l) in the six mesocosms (H1, H2, M1, M2, L1, L2).



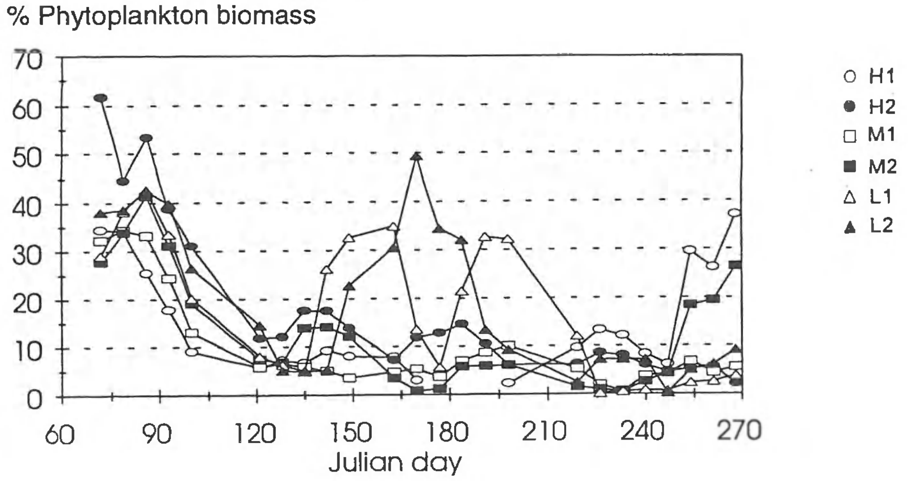
3.3.5 Phytoplankton species composition

Main groups evolution and relative importance

Two different functional groups were distinguished among the phytoplankters: flagellates and diatoms (respectively non-silicon and silicon using phytoplankton). Flagellates consisted mainly of three subgroups: autotrophic nanoflagellates (2 to 20 μm), cryptophyceae and *Phaeocystis sp.*

Both diatom and flagellate biomass increased exponentially until julian day 90 without noticeable difference between the mesocosms (Figure 3.10). Diatom biomass continued to increase.until julian day 120 in most mesocosms whereas flagellate biomass sharply decreased after julian day 90.

Figure 3.11
Percentage of phytoplankton biomass due to the flagellates (H1, H2, M1, M2, L1, L2).



After the initial period of growth, large differences in phytoplankton biomass began to appear between the mesocosms but, most of the time, these differences apparantly did not result from the treatments since they did not reproduce in the replicates. Between julian days 170 and 180 however, treatment may have had an effect on phytoplankton concentrations since diatom concentrations were significantly lower in mesocosms L1 and L2 than in the other mesocosms.
Due to the initial diatom growth, the biomass fraction due to the flagellates decreased from ≈50 to below 10% between julian days 65 and 120. The flagellate biomass percentage remained in most mesocosms below 20% until julian day 240 except in mesocosms L1 and L2 where it exceeded 30% at several occasions between julian days 150 and 200 (Figure 3.11).

Main components of the flagellate biomass and evolution
The contributions of the three sub-groups, autotrophic nanoflagellates, cryptophyceae and *Phaeocystis sp.* to the total flagellate biomass was estimated and plotted in Figure 3.12.
Cryptophyceae biomass increased during the first three weeks of the experiment in all mesocosms. Afterwards, cryptophyceae biomass levels and evolution differed greatly among the mesocosms. No clear trend along time or treatment was observed.
Nanoflagellate biomass increased in all mesocosms from day 80 on.and peaked twice at julian days 90 and 120. From julian day 120 on, the nanoflagellate biomass sharply decreased in all mesocosms (until julian day 150). At several occasions between julian days 150 and 200 nanoflagellate biomass was higher in H1 and to a less extent in H2 than in the other mesocosms. After julian day 200, nanoflagellate biomass remained very low in all mesocosms until the end of experiment
Phaeocystis biomass increased from julian day 100 on and culminated in all mesocosms around day 130. *Phaeocystis* biomass decreased in all mesocosms except H1 between julian days 120 and 150 and remained generally very low

Figure 3.12

Biomass of autotrophic nanoflagellates cryptophyceae and *Phaeocystis sp* in the six mesocosms (H1, H2, M1, M2, L1, L2).

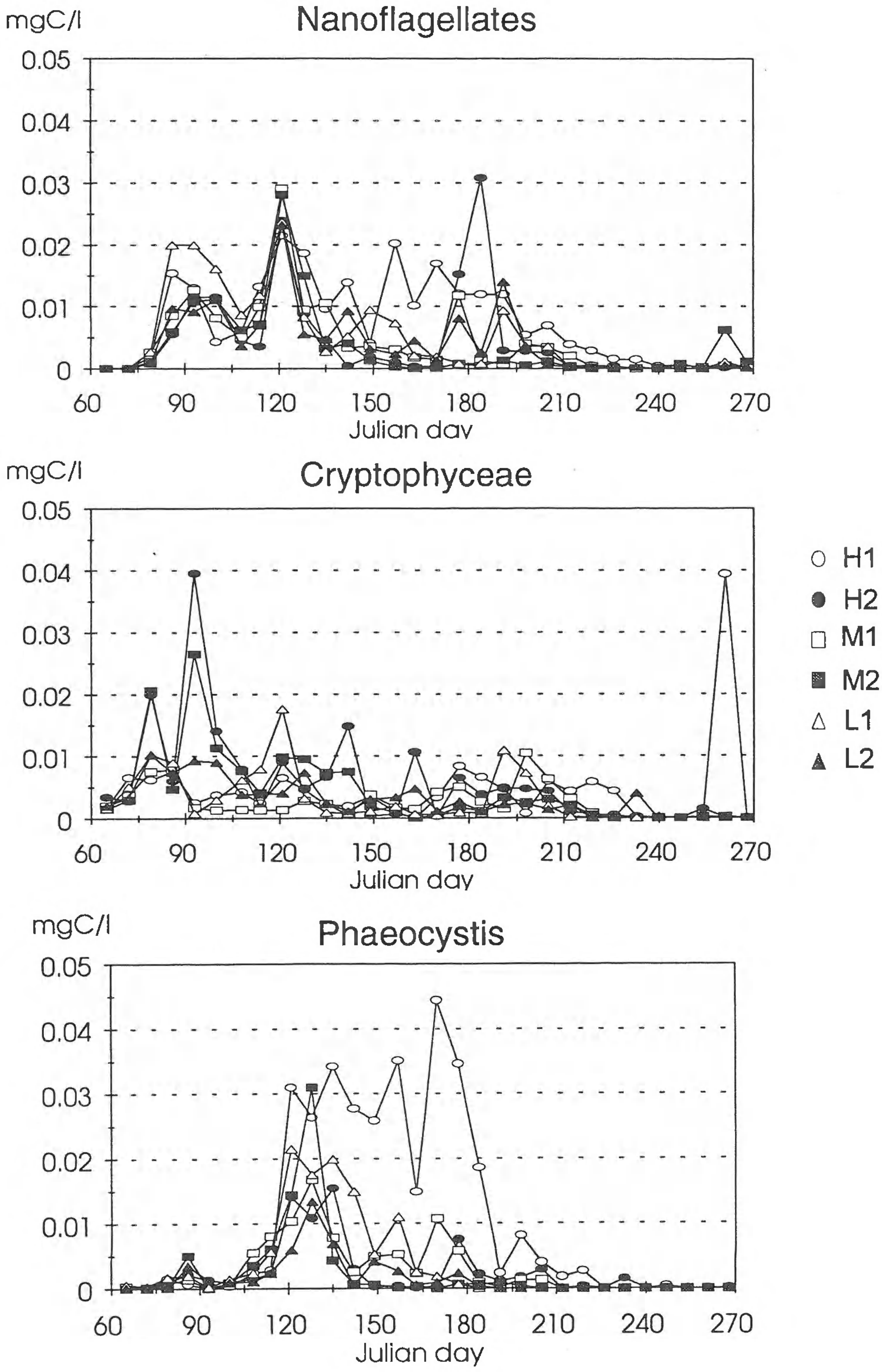
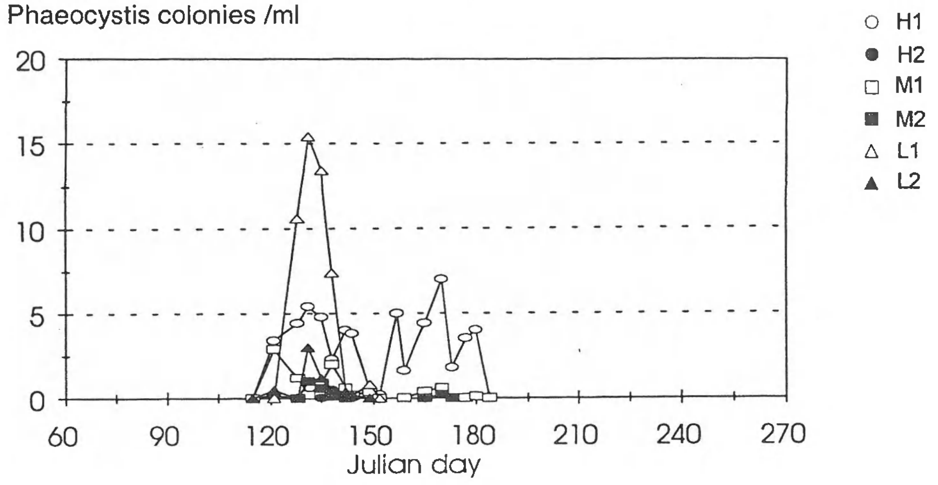


Figure 3.13

Phaeocystis sp. colony concentration in the six mesocosms (H1, H2, M1, M2, L1, L2).



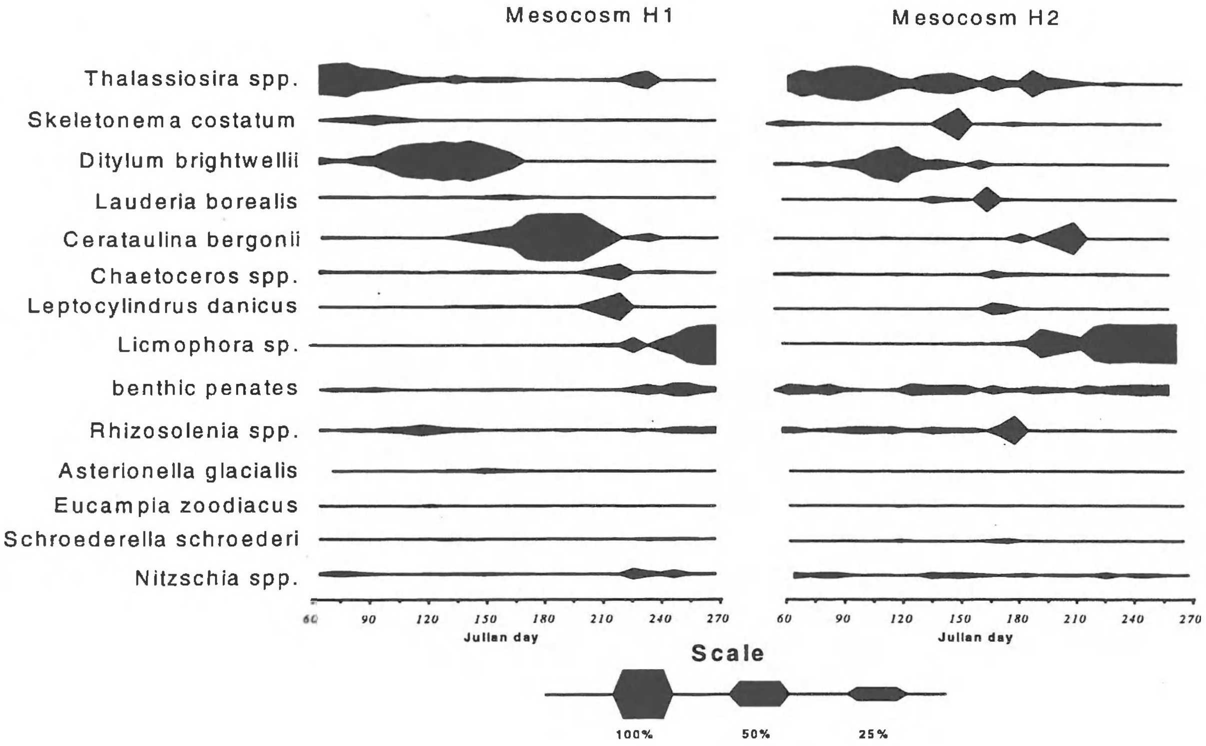
until the end of experiment. *Phaeocystis* biomass remained higher in H1 than in the other mesocosms between julian days 140 and 190. After julian day 200, *Phaeocystis* biomass remained very low in all mesocosms.

Phaeocystis sp. colonies were mainly observed in mesocosms :L1 and H1 (Figure 3.13). In L1 a peak-shaped colonial bloom reaching 15 colonies.ml^{-1} occurred between julian days 120 and 140. In H1 two colonial developments were observed both culminating around 5 colonies.ml^{-1} around days 135 and 175 respectively.

Main components of the diatom biomass and evolution

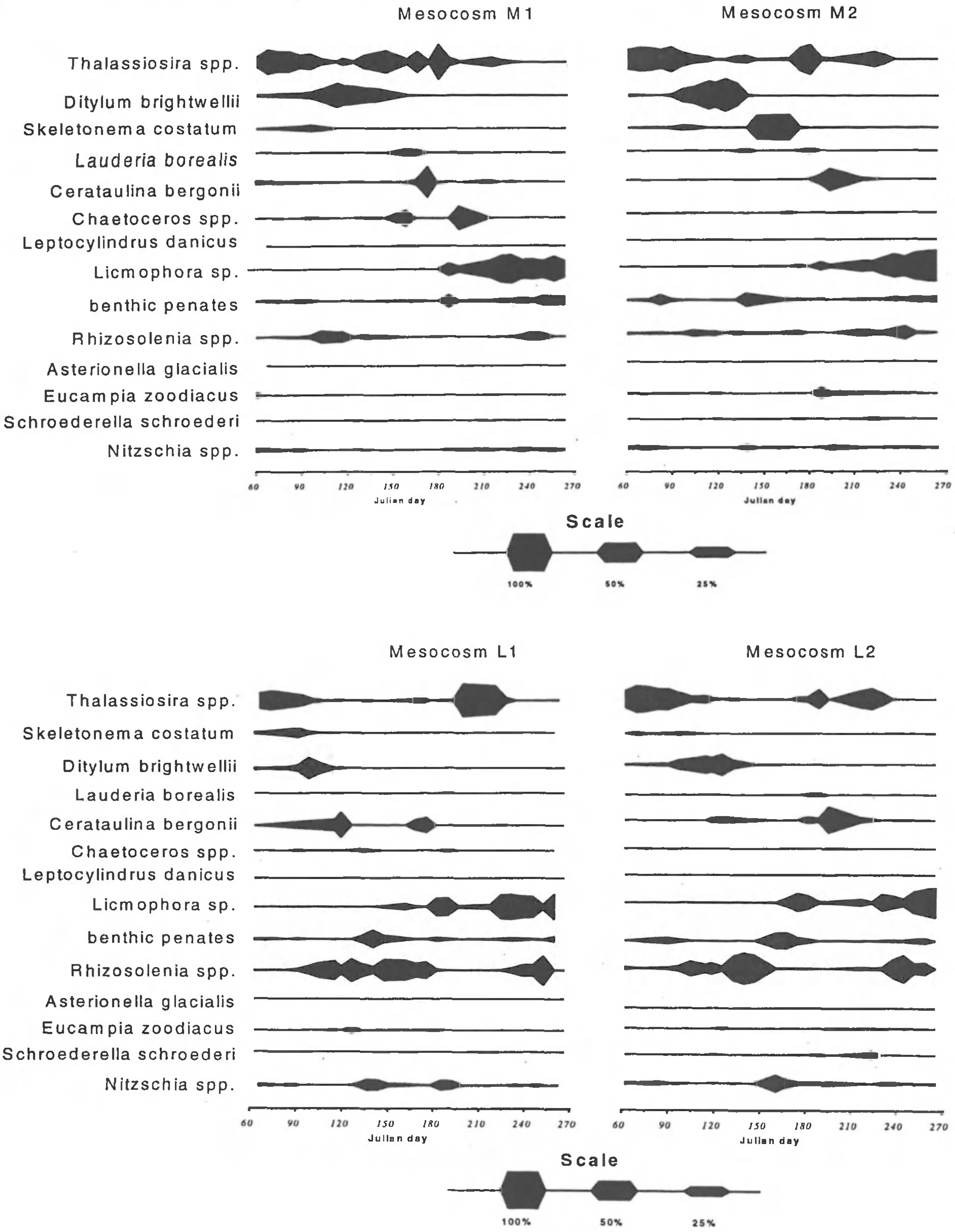
For a better readibility of the graphs, only diatom species reaching 0.005 mg C l^{-1} at least once during the experiment are shown. 14 species or group of species were selected by this way. The relative contribution of the different species or group of species to the diatom biomass is illustrated by the Kite diagrams in Figures 3.14 a,b,c.

Figure 3.14a
Kite diagrams of the diatom biomass composition in mesocosms H1 and H2. The width of the black area is proportional to the contribution of the corresponding species or group of species to the total diatom biomass (See scale).



Until julian day 120 (initial period of diatom bloom, Fig.3.10) the same species dominated the diatom community in mesocosms H1 and H2. In both mesocosms, *Thalassiosira spp.* (mainly *T. nordenskioldii*) contribution to the biomass decreased progressively to the profit of *D. brightwellii*. After julian day 120 (and until julian day 200) diatom communitites diverged radically within treatment H. In mesocosm H2, *D. brightwellii* collapse (biomass decrease on Figure 3.10) was accompanied by a passive increase in the contribution of the other species to the diatom biomass. In mesocosm H1 *D. brightwelli* remained dominant until julian day 150 when it began to decline to the profit of *C. bergonii* (second biomass increase on Figure 3.10). After julian day 200, *C. bergonii* collapsed (last biomass decrease on Figure 3.10) and the low diatom biomass measured in both mesocosms became dominated by diatoms with benthic affinities.

Figure 3.14b-c
Kite diagrams of the diatom biomass composition in mesocosms M1 and M2 and L1, L2. The width of the black area is proportional to the contribution of the corresponding species or group of species to the total diatom biomass (See scale).



As previously observed in treatment H, *Thalassiosira* species and *D. brightwellii* were dominant in both mesocosms M1 and M2 during the initial phytoplankton growth, until julian day 120. Between julian days 120 and 200, no species succeeded to remain dominant for a long time. During this period, the best represented species were *Thalassiosira* species, *S. costatum, C. bergonii* and *Chaetoceros spp.* After julian day 200, benthic diatoms (*Licmophora sp.* mainly) gained in importance and became the dominant algae in both mesocosms the two last weeks of experiment. A similar diatom species succession was observed in treatment L and M except for *Rhizosolenia* species that contributed to a greater fraction of the diatom biomass in treatment L than in treatment M.
At the end of the experiment *Licmophora sp.* dominated the diatom biomass in mesocosms L similarly as in the other treatments.

3.4. Discussion

3.4.1 Nutrient limitations in mesocosms

The nature of the nutrient limitation varied markedly according to the season: a P-limitation developed in spring (treatments M and H) followed by a N-limitation in summer (all treatments). In addition, periods of N-limitation occurred in spring in the mesocosms with the lowest nutrient loading (treatment L). This pattern resembled field observations made by Peeters & Peperzak (1990) that in most locations of the Dutch coastal shelf (except the central North Sea), P-limitation occurs in spring and N-limitation in summer.

3.4.2 Phytoplankton biomass and production

The nutrient limitations pointed out by the low nutrient concentration appeared to actually control both phytoplankton biomass and production. In spring, phytoplankton biomass and production were similar in treatment H and M, limited by the phosphorus availability (same P-loads in H and M) and lower in mesocosms L due to P and/or N limitation (see overlap between N and P-limitations on Fig.3-7). In summer, phytoplankton biomass and production ranked following the nitrogen gradient (L<M<H).
Those results were compared with previous mesocosm experiments dealing with phosphorus (1993) (Escaravage et al., 1996) and nitrogen (1994) (Escaravage et al., 1995) loading reduction. In those experiments, positive relations were observed between nutrient loading and phytoplankton biomass and production. During the experiments in 1993 and 1994, only one nutrient loading was varied (phosphorus or nitrogen) and turned out to be responsible for most of the limitation of phytoplankton biomass and production. Comparisons were made between experiments sharing similar mean surface irradiances (Table 3.4).
Covariance analysis was done to investigate to which extent the relationships established between phytoplankton biomass and production and nutrient loading during those experiments run in different years were comparable. Results of the covariance analysis are given in Table 3.5.
The effect of phosphorus was investigated by comparing measurements made in treatments L and M in spring 1995 with those obtained in summer 1993 (except treatment H where phytoplankton was overgrazed by a rotifer population (cf. Escaravage et al., 1996).

Both chlorophyll-*a* concentrations and primary production were, for a similar P-load, three to five times lower in 1995 than in 1993 and due to the low number of observations (7 mesocosms), regressions were not found to be significant (Table 3.5 and Figure 3.15). The higher phytoplankton biomas and production measured in 1993 when compared to spring 1995 could have been partly caused by the high initial chlorophyll concentration (10 µg.l^{-1}) observed in the former experiment.

Table 3.4
Mean surface irradiance measured during mesocosm nutrient gradient experiments run since 1993.

Experiment	Treatment	Mean Irradiance (mole photons/m^2/d)
Spring 1993	P-load gradient	14
Summer 1993	P-load gradient	30
Spring 1994	N-load gradient	20
Summer 1994	N-load gradient	28
Spring 1995	N-/P-load gradient	26
Summer 1995	N-/P-load gradient	30

Table 3.5
Results of the covariance analysis performed between nutrient gradient,experiments run in 1993, 1994 and 1995.

Treatment	Experiments	Variable	p(regression)	slopes differences
Phosphate	Spring 95 /Summer 93	Chl.a	0.05	n.s.
		Prim.P.	n.s.	n.s.
Nitrate	Spring 95 /Summer 94	Chl. a	<0.001	n.s.
		Prim. P.	<0.001	n.s.
Nitrate	Summer 95 / Summer 94	Chl. a	<0.001	n.s.
		Prim. P.	<0.001	0.013

Figure 3.15

Chlorophyll-*a* and production against the phosphorus loading in spring 1995 and summer 1993.

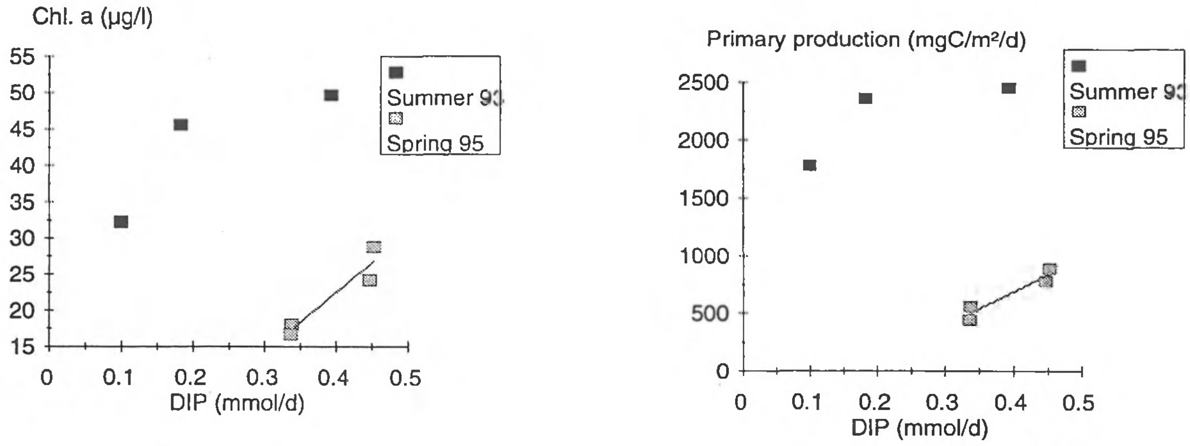
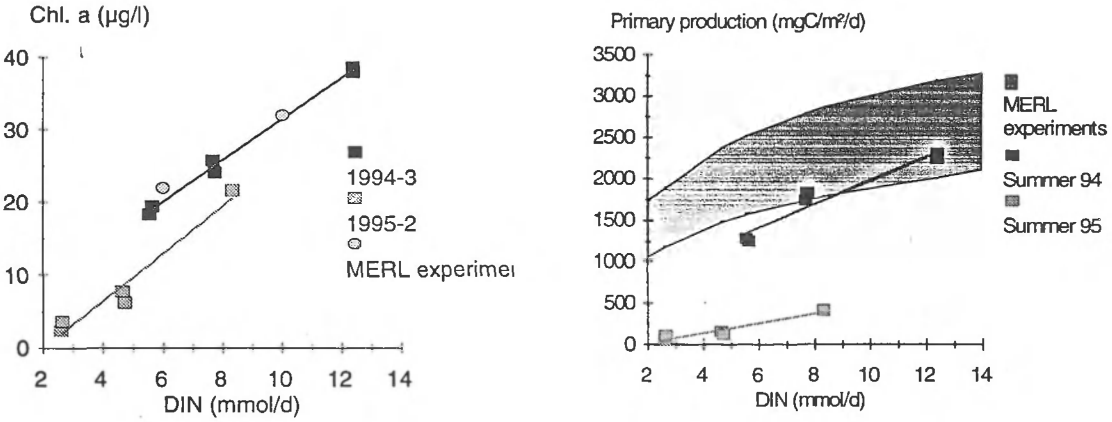


Figure 3.16

Chlorophyll-*a* and production against the nitrogen loading in summer 95 and summer 1994. Data from MERL experiments (references in text) are plotted for comparison.



Chlorophyll-a concentrations and slopes against the nitrogen load were similar in 1995 and 1994; primary production level and slope against the nitrogen load were significantly lower in 1995 than in 1994 (Table 3.5 and Figure 3.16).

Outcomes of similar experiments performed in the MERL mesocosms in 1982-1983 (Oviatt et al., 1986; Keller, 1988) were compared with the previous results. For nitrogen loads equalling 6 and 10 mmol N m^{-2} d^{-1}, mean chlorophyll-a concentrations in the MERL mesocosms were ca 22 and 32 µg.l^{-1} (extracted graphically from Keller (1988) and rescaled for the mesocosm size). Those values were close to the results obtained in 1994 and 1995 (Fig.3.16) and reproduced a similar response of the chlorophyll-a concentrations to the increase in nitrogen load.(+10 and +15 µg Chl l^{-1} per mmol N l^{-1} added in MERL and 1994/1995 respectively).

From the same MERL experiment, Oviatt et al. (1986) extracted a log-linear relationship between the water column production (PP, g O_2 m^{-2} yr^{-1}) and the DIN load (DIN, mmol $m^{-2}d^{-1}$) as: PP=386.Ln(DIN)+491. For comparison with our results, yearly O_2 productions were transformed into daily C productions by using two assimilation coefficients (dO_2/dCO_2 1.25 and 2) estimated from previous experiments as boundaries for our mesocosms (Prins et al., 1994). Predicted primary productions were estimated over the N-load range used in our mesocosm experiments (2 to 14 mmol $m^{-2}d^{-1}$). The ranges for the primary production predicted from the MERL model showed a good agreement with the productions measured during summer 1994 (Fig. 3.16). Primary production in 1995 was lower and its response to the nitrogen load was weaker when compared with 1994 or with the MERL experiment.

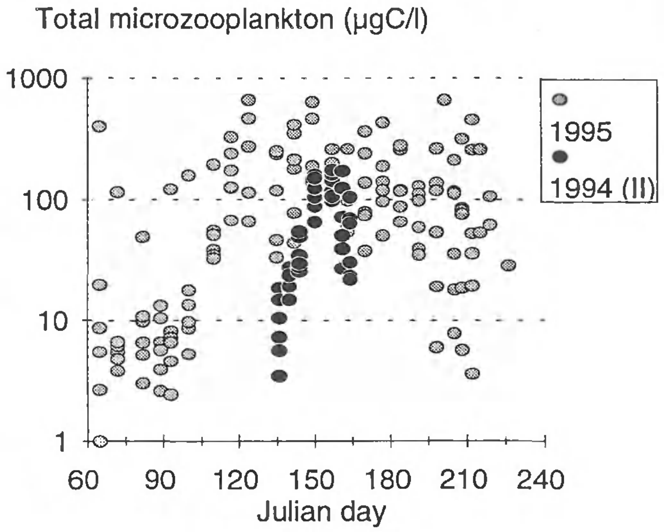
In both 1993 and 1994 experiments the response to the nutrient reduction was mainly due to small algae (*Phaeocystis* colonial bloom, L. danicus) whereas in 1995, large diatoms (*Thalassiosira* sp., *Rhizosolenia* sp., *D. brightwellii*) dominated phytoplankton and *Phaeocystis* development was poor. Several studies agree on the negative correlation between growth rate and cell size under limitation of either nutrients or light (Banse, 1982; Thingstad & Sakshaug, 1990; Falkowski, 1980; Chan, 1978). The large cell size in 1995 could be responsible for the relatively low production levels measured in 1995 when compared with 1993 and 1994.

3.4.3 Phytoplankton abundance and species composition

Whereas both phytoplankton biomass (expressed as µg chlorophyll-a) and production significantly reacted to the gradient in the nitrogen load, phytoplankton cell numbers and species composition did not show any consistent trend along this gradient. A similar lack of response of phytoplankton species composition and cell concentrations to the N-load gradient (considered between the same extremes as in the present experiment) appeared in the MERL experiments (1982-1983, Oviatt et al., 1989) whereas both phytoplankton biomass and primary production were also tightly controlled by the nitrogen availability in that experiment (see above). Microscopic observations indicated that the chloroplast cell content of the phytoplankton increased with the nitrogen load; however, quantification of this process was not possible. A similar feature was observed in a previous mesocosm experiment (1994) and could explain discrepancies between phytoplankton cell numbers and chlorophyll-a concentrations. Those observations agree with results by Tett et al. (1975) indicating that nutrient deficient phytoplankton (in cultures and in the field) show a decrease in the chlorophyll/carbon ratio.

The fact that phytoplankton species composition did not show much changes among the treatments could result from the relatively narrow range covered by the nutrient load gradient in the present experiment. For comparison, the whole range of nutrient loads used during the 1982-1983 MERL experiment was about fifteen times broader than the gradient used in our study in 1995 (Oviatt et al., 1989). However, in a previous experiment (summer 1994), similar ranges in nutrient loading induced significant differences among the treatments (Escaravage et al., 1995.

Figure 3.17
Microzooplankton concentrations measured in summer 1994 and during the present experiment in all mesocosms.



In the latter experiment, the species responsible for the positive response to the treatment were mainly *Phaeocystis* sp.and *Leptocylindrus danicus*, which are relatively small species (respectively 20 and 100 pgC/cell). When compared to summer 1994, the development of both species was very poor in 1995 and more generally speaking, phytoplankton in 1995 was dominated by large diatoms such as *Thalassiosira sp., D. brightwellii* and *Rhizosolenia sp.* with individual biomass around 500 pgC/cell. Riegman et al. (1993) ascribed comparable shifts from small towards larger phytoplankton as possible responses to size differential grazing: large algae are relatively bad competitors for nutrient uptake but are less edible than small algae. In order to check wether this hypothesis could apply for the species shift observed between 1994 and 1995, the zooplankton biomass were compared between both years. A complete description of the zooplankton development during the present experiment is given in chapter 4.

The biomass of microzooplankton, the dominant zooplankton component in those experiments, was at a low level during the first month of the present experiment, increased tremendously after julian day 90 and remained relatively high afterwards. Initial microzooplankton biomass in 1994 (julian day 135) was far lower than levels observed in 1995 for the same period. Microzooplankton biomass sharply increased in 1994 but only reached levels comparable with those measured in 1995 during the second half of experiment. The microzooplankton species dominating in those mesocosm experiments were potential grazers for phytoplankton, due to their relatively large size (*Protoperidinium, Oxyrrhis, Peridinium, Strombidium*). The initial period in 1994 with moderate nutrient limitation, optimal light conditions (Escaravage et al., 1995) and low grazing pressure could allow the succesful development of a *Phaeocystis* bloom mainly dependent on the nitrogen availability. In 1995, when light conditions became favorable to *Phaeocystis*, the microzooplankton community had already established itself and could have prevented the development of significant blooms of *Phaeocystis* and other small algae.

3.5 Conclusion

The realistic nutrient loadings employed for this long term experiment allowed the reproduction of spatial and seasonal patterns of nutrient limitation in our mesocosms that were also observed in the North Sea from bioassay experiments (Peeters & Peperzak (1990). Moreover, the actual control of phytoplankton by the nutrient availability observed in this experiment constitute a validation for those *in situ* measurements.

Nitrogen appeared to be the main limiting nutrient during this experiment. This result points at the crucial importance of the reduction of the nitrogen load to the sea to reduce eutrophication effects.

The long term experiment allowed the development of a florishing microzooplankton community apparently free of intensive predation. This fact may have resulted from to the relatively small size of our mesocosms which makes them inadequate for the development of higher trophic levels (predators). Strong grazing of small sized phytoplankton could have caused the poor development of *Phaeocystis* bloom and other small diatoms and the consequently lower primary production, compared to the short term experiments or in to the MERL eutrophication study where higher trophic levels were present.

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4 Long-term zooplankton development in nutrient-manipulated mesocosms

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ABSTRACT

Micro- and mesozooplankton development in 6 nutrient-manipulated mesocosms was studied during a long-term (March-September) experiment in 1995.
The microzooplankton community was dominated by heterotrophic dinoflagellates (*Gyrodinium* spp., *Protoperidinium* spp. and *Oxyrrhis marina*); ciliates (mainly ciliate sp.1, *Strobilidium* spp. and *Strombidium* spp.) were of minor importance. The estimated microzooplankton carbon biomass values were comparable with published values from more productive systems. As a consequence of the high microzooplankton biomass severe grazing was directed towards the smaller phytoplankton size fraction. An ANOVA analysis showed a significant effect of the treatments on mean biomass for the whole period ($P=0.016$), but no significant effect for spring or summer. For the whole period a pairwise comparison showed significant differences in mean biomass between treatments L and H ($P=0.007$) and between treatments M and H ($P=0.025$) and no significant difference between treatments L and M ($P=0.099$).
Calanoid nauplii (mainly *Temora longicornis* and *Acartia* sp.) developed slowly in all mesocosms; in one of the mesocosms calanoid nauplii were replaced by cyclopoid and harpacticoid nauplii. Calanoid *Temora* nauplii did not develop further than the earliest copepodite stages. This bad development could be attributed to poor food conditions due to the severe microzooplankton grazing pressure, leading to a dominance of greater diatom species, which were not grazed by the smaller calanoid stages. Cyclopoid and harpacticoid naupliar, copepodite and adult stages outnumbered the corresponding calanoid copepod stages. Mesozooplankton carbon biomass values in the mesocosms were much lower than values known from Dutch coastal North Sea waters and one to two orders of magnitude smaller than the microzooplankton biomass values. An ANOVA analysis showed no significant effect of the treatments on mean biomass for the spring, summer or the total period.

4.1 INTRODUCTION

Eutrophication in coastal areas usually leads to an increase in phytoplankton primary production and biomass and shifts in phytoplankton species composition. This has been reviewed for coastal waters and inland seas all over the world by Smayda (1990). For many North Sea coastal areas eutrophication effects have been

documented by Cadée (1990), Cadée & Hegeman (1986), Gillbricht (1988) and Lancelot et al. (1987). From enclosure experiments eutrophication effects on phytoplankton biomass (Nixon et al., 1984; Riemann et al., 1988), primary production and community structure (Escaravage et al., 1994) were reported.
Zooplankton plays an important role in the marine environment by channelling phytoplankton primary production to higher trophic levels like ctenophores (Fraser, 1970; Kuipers et al., 1990) and fish (Ryland, 1964; Last, 1978; Fransz, 1980). Eutrophication effects on zooplankton have also been described. For example, eutrophication stimulated the productivity and development of the copepod *Temora longicornis* in the Marsdiep (Fransz et al., 1992). Another example is the increase of larval polychaetes in the plankton along a nutrient gradient in a mesocosm experiment (Nixon et al., 1984).
Mesocosms offer the opportunity to study the response of zooplankton to phytoplankton development resulting from nutrient manipulations. During short-term (1 month) experiments in 1994 a fast growing microzooplankton community developed in the mesocosms, almost immediately responding to the increase of phytoplankton (see Wetsteyn & Vink-Lievaart, 1995). During these experiments copepods did not develop as a consequence of the short experimental period and the lack of eggs and nauplii in the water used to fill the mesocosms; on the other hand appendicularians developed to much higher densities than known from North Sea coastal waters, probably as a consequence of the absence of predators (Wetsteyn & Vink-Lievaart, 1995).
The objective of the zooplankton work in 1995 was to quantify the micro- and mesozooplankton development in the mesocosms during the nutrient experiments, thereby expecting a better mesozooplankton development as a consequence of a longer experimental duration.

4.2 MATERIAL AND METHODS

4.2.1 General experimental conditions

A general description of the mesocosms and experiments is given in Chapter 2. The 1995 experiment was designed as a long-term (March-September) nutrient experiment. Treatment H(igh) represented a reduction of anthropogenic nutrient loadings (compared to the period 1980-1987) for N with 10 % and P with 50 %; treatments M(edium) and L(ow) reductions of 50 % for N and 50 % for P, respectively 75 % for N and 75 % for P (see also Table 2.1). All mesocosms received similar Si loadings. The nutrient loadings in mmol m^{-2} d^{-1} are summarized in Table 2.2. Each treatment was replicated (H1 and H2, etc.).
During the period March-May 40 mussels were added to each of the mesocosms; at the end of May the number of mussels in each mesocosm was reduced to 20, forming a basic, but small grazing pressure in each mesocosm (see also 2.2.1).
In each mesocosm a sediment container with a mixture of azoic sand and 'aged' Oosterschelde sediment was placed (see 2.1). At the start of the 1995 experiment the mesocosms were simultaneously filled with Oosterschelde water, filtered through 2 mm gauze. During the experiment each mesocosm was flushed with 100 l (unfiltered) Oosterschelde water day^{-1}, resulting in a residence time of the water of ca. 30 days.

4.2.2 Microzooplankton sampling and analysis

The samples used for the phytoplankton analyses were used to analyse microzooplankton (20-200 μm, mainly heterotrophic dinoflagellates, ciliates and rotifers) as well. Samples (1 l) were taken twice a week (Monday and Thursday, always between 7 and 8 a.m.) from outlets at 2.6 m depth. Only samples, taken simultaneously with the mesozooplankton samples, were analyzed; 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 %.
In the laboratory samples were concentrated (ca. 10x) by sedimentation. After sedimentation 5 ml was taken from the residu and stained with Bengal rose after decolorizing with sodium thiosulphate. Microzooplankton species composition and dimensions were determined using an inverted microscope. The used magnification was 200x and sometimes 500x for a better distinction between the genera *Gyrodinium* and *Gymnodinium*. Depending on organism abundance mostly 200 and sometimes 100 fields of view were counted. 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. In many samples a large ciliate, Ciliate sp. 120*79 μm, occurred. This ciliate was counted more reliable in the mesozooplankton samples (see 4.2.3).
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.
Biovolume was estimated from appropiate geometric approximations. To calculate carbon biomass from biovolume estimations a conversion factor of 0.19 pg C μm^{-3} for the ciliates (Putt & Stoecker, 1989) and 0.14 pg C μm^{-3} for the heterotrophic dinoflagellates (Lessard, 1991) was taken. Because cells shrink 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). All procedures and conversion factors are summarized in Wetsteyn et al. (1996).
In general, sample analysis was done within 6 months after sampling.
For species identification (a.o.) the zooplankton-sheets (from Conseil International pour l'Exploration de la Mer) 117 (1969), 120 (1969), 121 (1969), 123 (1969), 126 (1969) and 127 (1969), Hamburger & von Buddenbrock (1911), Kahl (1933, 1934), Bakker & Phaff (1976), Maeda & Carey (1985), Lynn & Montagnes (1988) and Thomsen (1992) were frequently used.

4.2.3 Mesozooplankton sampling and analysis

Depth-integrated samples for mesozooplankton (200-2000 μm, mainly copepods and appendicularians) analysis were taken using a long PVC-tube (inner diameter 58 mm), provided with a ball valve at the lower end (see Kuiper, 1977). Lowering the tube eight times at different positions resulted in a total sample of well over 50 l. The collected water was filtered through a 55 µm zooplankton net. In this way also eggs and the smallest naupliar stages were retained. The filtered water was returned to the mesocosm. The inlet water, used to flush the mesocosms (see 4.2.1.), was filtered in the same way. 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 were determined in a subsample of 25 ml in a 50 ml cuvet under a stereomicroscope (magnification 40-50x). Occasionally, at great phytoplankton abundance, 5 ml subsamples were taken. From calanoid copepods all stages from each species were counted.

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 4.2.2). For the same reason Ciliate sp. 120*79 µm and Egg sp. 59 µm were 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. All procedures and conversion factors are summarized in Wetsteyn et al. (1996).

In general, sample analysis was done within 6 months after sampling.

For species identification (a.o.) the zooplankton-sheets (from Conseil International pour l'Exploration de la Mer) 4 (1943), 7 (1969), 11 (1948), 12 (1948), 35 (1951), 37 (1951), 50 (1953), 84 (1960), 85 (1960), 107 (1965), 133 (1970) and 163 (1980), Smith (1977) and Klein Breteler (1982) were frequently used.

4.3 RESULTS

4.3.1 General experimental conditions

The daily averaged water temperature in the Oosterschelde mouth gradually increased from ca. 6.6 °C at Julian day 65 to ca. 20.8 °C at Julian day 220. In the mesocosms water temperature was similar, but showed greater fluctuations (see Fig. 2.6). Between the mesocosms there were no differences in water temperature.

After the weekend of 1-2 July (Julian days 182-183) mesocosm H1 was almost empty due to a leakage in one of the benthos chambers. After the leakage the mesocosm was refilled with Oosterschelde water (at Julian day 184) and, during one week, with the overflow water (100 l d^{-1}) from the replicate mesocosm H2.

4.3.2 Zooplankton development (densities)

In this chapter the development of the numerically most important zooplankton species or taxonomical groups in the mesocosms during the 1995 experiment is presented as separate figures with respect to microzooplankton (Figures 4.1a, 4.2a and 4.3a) or mesozooplankton (Figures 4.1b, 4.2b and 4.3b) for each pair of replicate mesocosms.

4.3.2.1 Microzooplankton development in mesocosms L1 and L2

In each mesocosm a small peak of *Gyrodinium* spp. developed between days 120 and 150; the most important species were *Gyrodinium* sp.3 and *Gyrodinium spirale*. *Gymnodinium* spp., mainly *Gymnodinium* sp.2, were not of importance during this experiment. *Protoperidinium* spp. in both mesocosms, mainly *Protoperidinium bipes* en *Protoperidinium* sp.2, reached maximum densities around day 120. The most abundant heterotrophic dinoflagellate was *Oxyrrhis marina*; in both mesocosms high concentrations of this species were observed at day 157. Other heterotrophic dinoflagellate species were hardly seen in these two mesocosms, only *Diplopelta pusilla*.

The number of loricate ciliates remained very low in both mesocosms; only on a few occasions *Tintinnopsis beroidea* and *Helicostomella subulata* were found. Aloricate ciliates, mainly Ciliate sp.1, *Strobilidium* sp.2 and *Strombidium* sp.2, were found in all samples from both mesocosms, but concentrations were not extremely high. Ciliates sp. 120*79 µm (data not presented in Figure 4.1a) were seen in samples from both mesocosms.

The rotifer *Trichocerca marina* was found in both mesocosms, but only once in mesocosm L2 with reasonable numbers. *Synchaeta* spp. (data not presented in Figure 4.1a) were seen in a few samples, but densities were very low.

Other observed microzooplankton species/groups (data not presented in Figure 4.1a) in mesocosms L1 and L2 were *Mytilus edulis* (larvae), peritrichous ciliates like *Acineta tuberosa*, *Corynophrya* sp. and *Vorticella* sp., *Amoeba* sp., Nematode sp. and eggs like Egg sp., *Trichocerca* eggs and Egg sp. 59 µm.

4.3.2.2 Mesozooplankton development in mesocosms L1 and L2

In the first samples only a few calanoid copepod stages were found: no eggs, small amounts of nauplii in both mesocosms, hardly any copepodites and no adults. After than nauplii (mainly *Temora longicornis*, the rest *Acartia* sp.) were found in all samples with maximum densities at day 135 in mesocosm L1 and at day 143 in mesocosm L2. Later, small amounts of copepodites (*Temora longicornis* and *Acartia* sp.) and adults (*Temora longicornis*) were found between days 117 and 185 in both mesocosms.

Cyclopoid and harpacticoid nauplii were observed in all samples (not in the first samples after filling) and copepodites and adults developed in both mesocosms with a great peak at day 120 in mesocosm L2.

Nematodes were found in all samples from both mesocosms, especially between days 170 and 210 in mesocosm L1. Polychaetes (larvae) were found in both mesocosms with maximum concentrations at day 180 in mesocosm L1.

The appendicularian *Fritillaria borealis* occurred in both mesocosms between days 120 and 150; the greatest peak was found in

Figure 4.1a
Microzooplankton development during the 1995 experiment in mesocosms L1 and L2. Data for heterotrophic dinoflagellates, ciliates and rotifers. Day 60 = 01/03/95, day 270 = 27/09/95. For species/groups not presented in the figure: see text. L1, L2: see text.

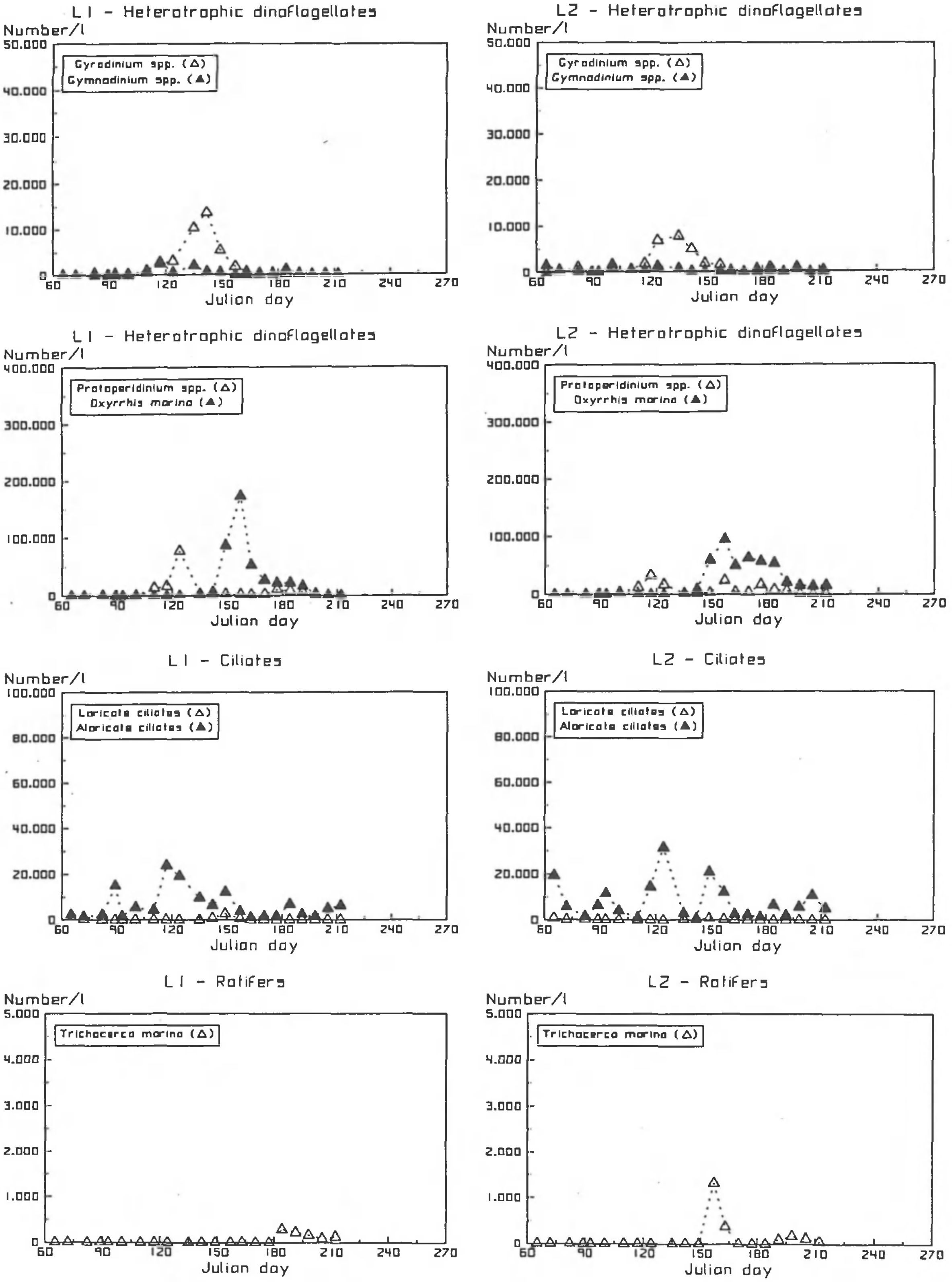
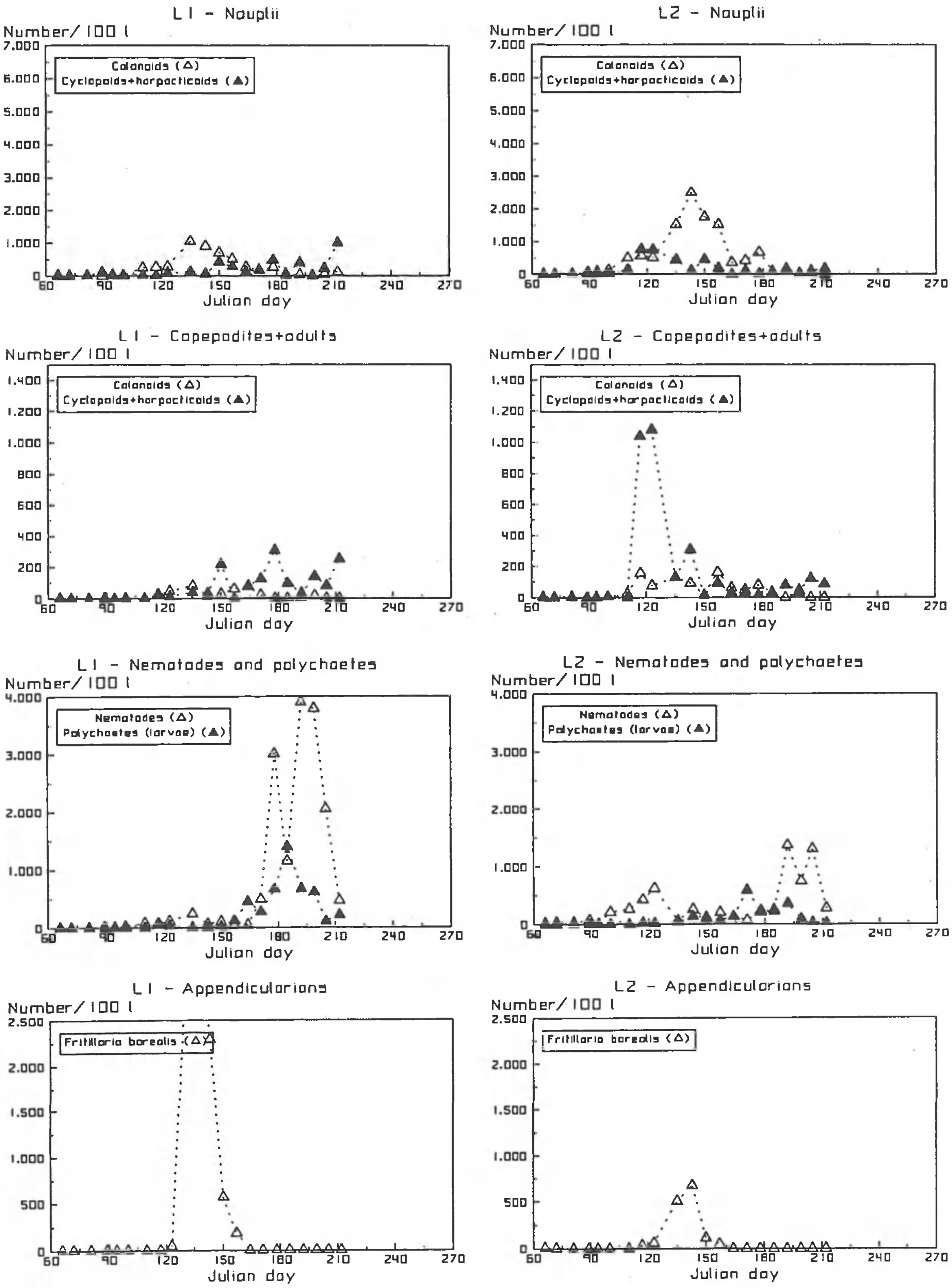


Figure 4.1b
Mesozooplankton development during the 1995 experiment in mesocosms L1 and L2. Data for copepods, nematodes, polychaetes and appendicularians. Day 60 = 01/03/95, day 270 = 27/09/95. For species/groups not presented in the figure: see text. L1, L2: see text.



mesocosm L1. *Oikopleura dioica* (data not presented in Figure 4.1b) was found in low concentrations in a few samples from both mesocosms.
The cirriped *Semibalanus* sp. (data not presented in Figure 4.1b) entered when filling the mesocosms, disappeared within three weeks and re-occurred with regular intervals.

4.3.2.3 Microzooplankton development in mesocosms M1 and M2

Gyrodinium spp. in both mesocosms peaked between days 120 and 150; the most important species were *Gyrodinium* sp.2 and *Gyrodinium spirale*. *Gymnodinium* spp., mainly *Gymnodinium* sp.2, occurred with low concentrations in both mesocosms. *Protoperidinium* spp. were found in each mesocosm; the most important species were *Protoperidinium bipes* and *Protoperidinium* sp.2. *Oxyrrhis marina* was the most abundant heterotrophic dinoflagellate species in both mesocosms; in mesocosm M1 this species peaked three times between days 150 and 200 with high concentrations and in mesocosm M2 a huge peak developed between days 120 and 150. Within the category other heterotrophic dinoflagellates only *Diplopelta pusilla* was seen in both mesocosms. The number of loricate ciliates, mainly *Tintinnus pectinus* and *Tintinnopsis beroidea*, remained low in both mesocosms. In all samples from both mesocosms aloricate ciliates, mainly Ciliate sp.1 and *Strombidium* sp.2, were found, but concentrations were not extremely high. Ciliates sp. 120*79 μm (data not presented in Figure 4.2a) were seen in samples from both mesocosms.
The rotifer *Trichocerca marina* peaked twice in mesocosm M1 and once in mesocosm M2. *Synchaeta* spp. (data not presented in Figure 4.2a) were found in only a few samples.
Other observed microzooplankton species/groups (data not presented in Figure 4.2a) in both mesocosms were *Mytilus edulis* (larvae), peritrichous ciliates like *Acineta tuberosa*, *Corynophrya* sp. and *Vorticella* sp., *Amoeba* sp., Nematode sp. and eggs like Egg sp., *Synchaeta* eggs, *Trichocerca* eggs and Egg sp. 59 μm.

4.3.2.4 Mesozooplankton development in mesocosms M1 and M2

Only a few calanoid copepod stages were seen in the first samples after filling the mesocosms: no eggs, a few nauplii, hardly any copepodites and no adults. Later on, nauplii (mainly *Temora longicornis*, the rest *Acartia* sp.) were found in almost all samples from both mesocosms. Copepodites (*Temora longicornis* and *Acartia* sp.) were found in both mesocosms and adults (*Temora longicornis* and *Acartia* sp.) in only a few samples taken in mesocosm M1. Cyclopoid and harpacticoid nauplii (almost absent after filling) were found in all samples from both mesocosms with a large peak in mesocosm M2 between days 105 and 150; copepodites and adults developed in both mesocosms and peaked at day 135.
Nematodes occurred in all samples from both mesocosms, especially after day 120. Polychaetes (larvae) were found in both mesocosms from day 150 onwards.
The appendicularian *Fritillaria borealis* was mainly found in mesocosm M1 and peaked around day 150.
The cirriped *Semibalanus* sp. (data not presented in Figure 4.2b) was found in the first samples from both mesocosms and re-occurred with regular intervals.

Figure 4.2a
Microzooplankton development during the 1995 experiment in mesocosms M1 and M2. Data for heterotrophic dinoflagellates, ciliates and rotifers. Day 60 = 01/03/95, day 270 = 27/09/95. For species/groups not presented in the figure: see text. M1, M2: see text.

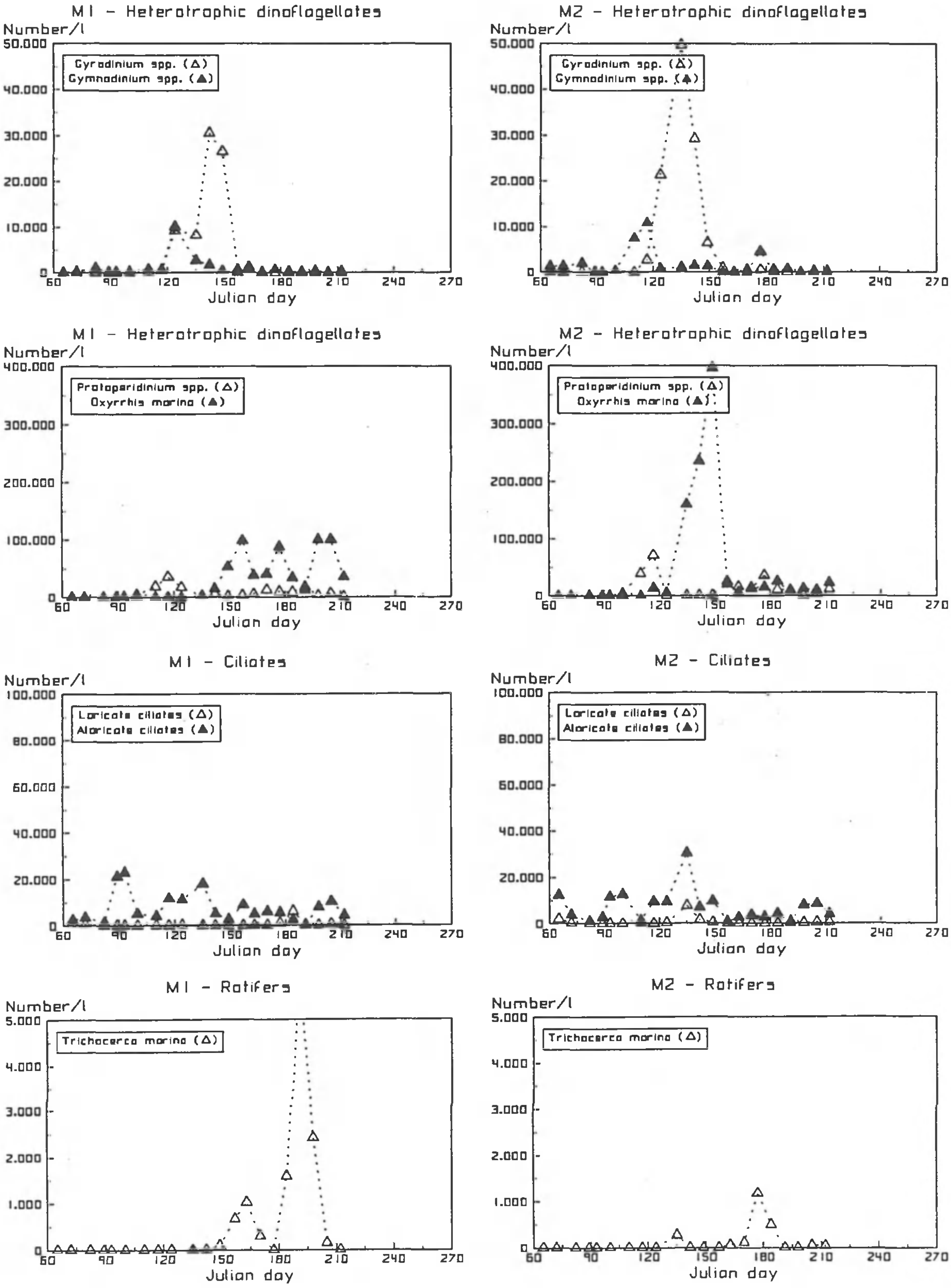
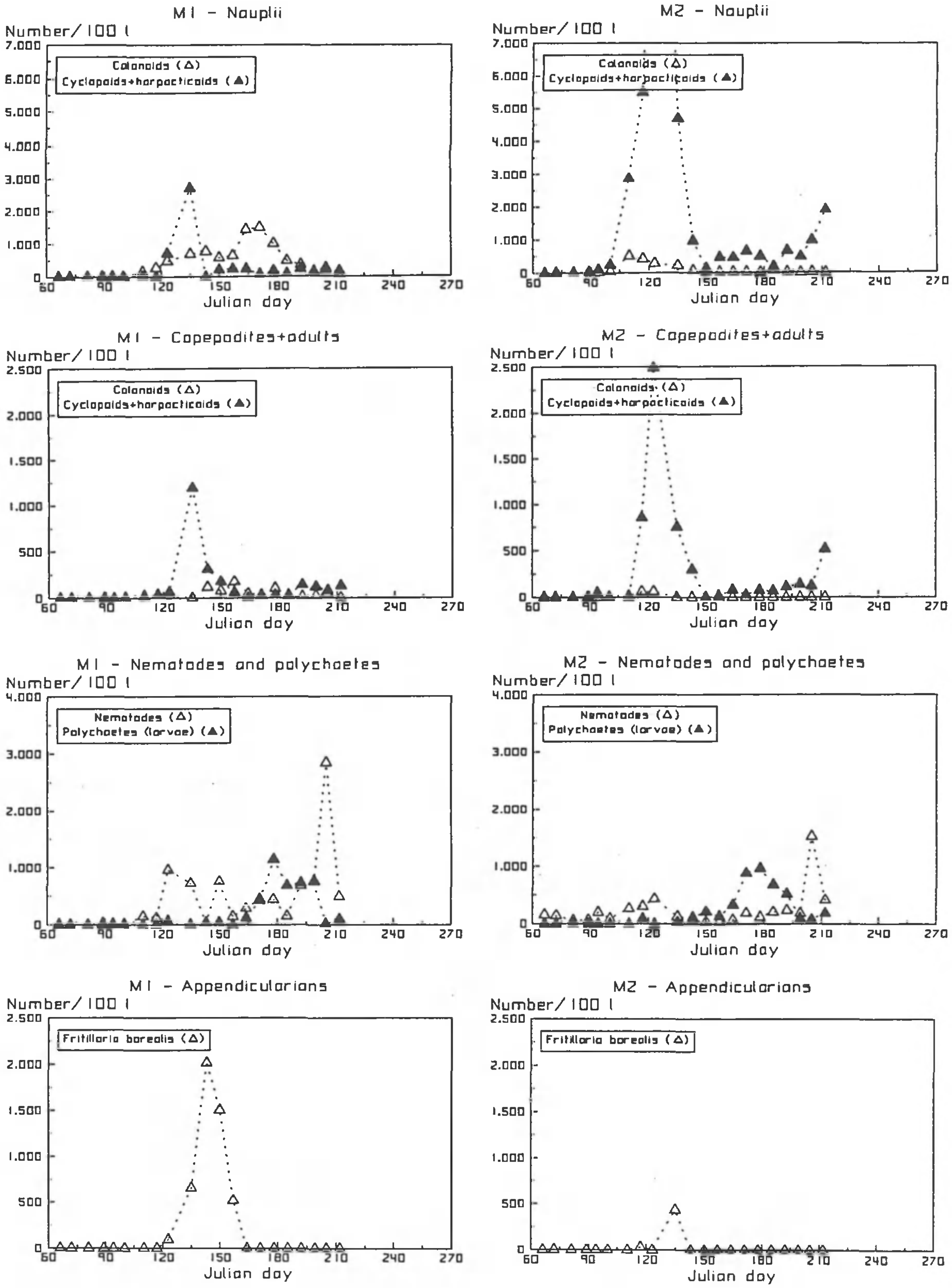


Figure 4.2b

Mesozooplankton development during the 1995 experiment in mesocosms M1 and M2. Data for copepods, nematodes, polychaetes and appendicularians. Day 60 = 01/03/95, day 270 = 27/09/95. For species/groups not presented in the figure: see text. M1, M2: see text.



4.3.2.5 Microzooplankton development in mesocosms H1 and H2

Gyrodinium spp. peaked between days 120 and 150 and at day 180 in both mesocosms; the most important species were *Gyrodinium* sp.2 and *Gyrodinium spirale*. *Gymnodinium* spp., mainly *Gymnodinium* sp.1 and *Gymnodinium* sp.2, peaked several times with low concentrations in both mesocosms. *Protoperidinium* spp. were found with small, but high peaks in both mesocosms; the most important species were *Protoperidinium* sp.2 and, but to a lesser account, *Protoperidinium bipes*. *Oxyrrhis marina* reached very high densities between days 120 and 150 in mesocosm H1 and had a longer lasting, but smaller, peak in mesocosm H2. The only other observed heterotrophic dinoflagellate species in both mesocosms was *Diplopelta pusilla*.

Loricate ciliates, mainly *Tintinnopsis beroidea* and *Tintinnus pectinus*, developed only at the end of the 1995 experiment.

Aloricate ciliates, mainly *Strobilidium* sp.1 and sp.2., were found in each sample. Concentrations were not extremely high, but higher than in the other four mesocosms. The large Ciliate sp. 120*79 μm (data not presented in Figure 4.3a) was found in samples from both mesocosms.

The rotifer *Trichocerca marina* peaked after day 180 in both mesocosms. In this period also *Synchaeta* spp. (data not presented in Figure 4.3a) were found in both mesocosms.

Other observed microzooplankton species/groups (data not presented in Figure 4.3a) in mesocosms H1 and H2 were *Mytilus edulis* (larvae), peritrichous ciliates like *Acineta tuberosa*, *Corynophrya* sp. and *Vorticella* sp., *Amoeba* spp. like *Amoeba* sp. and *Vahlkampfia guttula*, Nematode sp. and eggs like Egg sp., *Synchaeta* eggs, *Trichocerca* eggs and Egg sp. 59 μm.

4.3.2.6 Mesozooplankton development in mesocosms H1 and H2

In the first samples no calanoid eggs and only a few nauplii were seen. Later, calanoid nauplii (mainly *Acartia* sp., the rest *Temora longicornis*) were found in almost all samples from mesocosms H1 and H2. In mesocosm H1 calanoid nauplii reached a high maximum, then, between days 178 and 185, numbers dropped to a much lower value and reached again a high value. The cause of the fast decrease between days 178 and 185 must be found in the leakage of mesocosm H1 at days 182-183 (see 4.3.1). In mesocosm H2 the development of calanoid nauplii was very poor. Later, calanoid copepodites (mainly *Acartia* sp., the rest *Temora longicornis*) were observed between days 117 and 199 in mesocosm H1. In mesocosm H2 calanoid copepodites hardly developed. In mesocosm H1 calanoid adults (mainly *Temora longicornis*, but also *Acartia* sp.) occurred in only five samples; in mesocosm H2 adults from *Temora longicornis* were found in only one sample.

Cyclopoid and harpacticoid nauplii were observed in all samples from mesocosms H1 and H2. Cyclopoid and harpacticoid copepodites and adults (not present directly after filling) in samples from mesocosm H1 were present from day 117 to day 199 and in mesocosm H2 from day 110 to day 185.

Nematodes and polychaetes (larvae) were found in samples from both mesocosms.

Fritillaria borealis occurred in both mesocosms in only a few samples between days 135 and 150. *Oikopleura dioica* (data not presented

Figure 4.3a
Microzooplankton development during the 1995 experiment in mesocosms H1 and H2. Data for heterotrophic dinoflagellates, ciliates and rotifers. Day 60 = 01/03/95, day 270 = 27/09/95. For species/groups not presented in the figure: see text. H1, H2: see text.

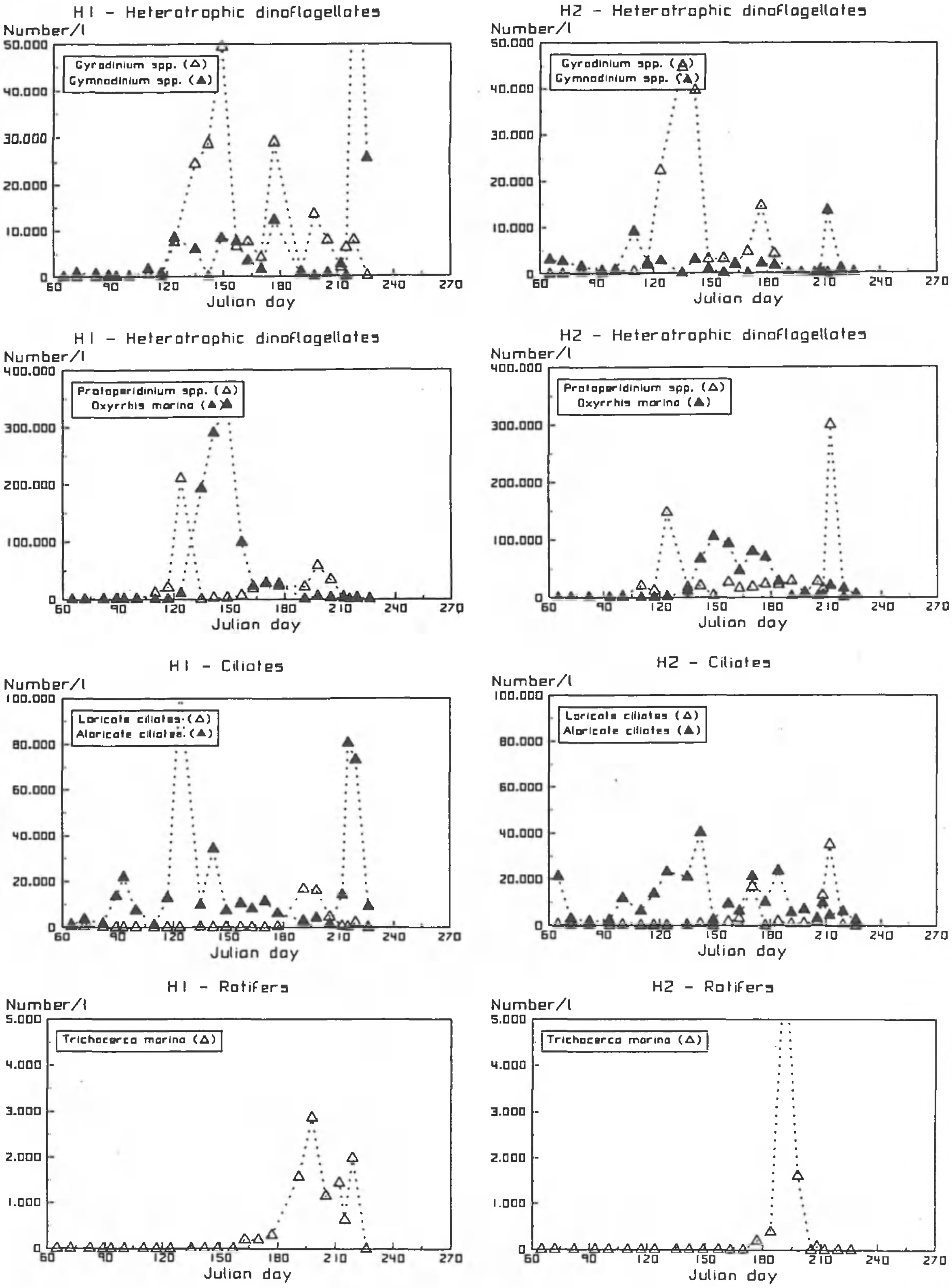
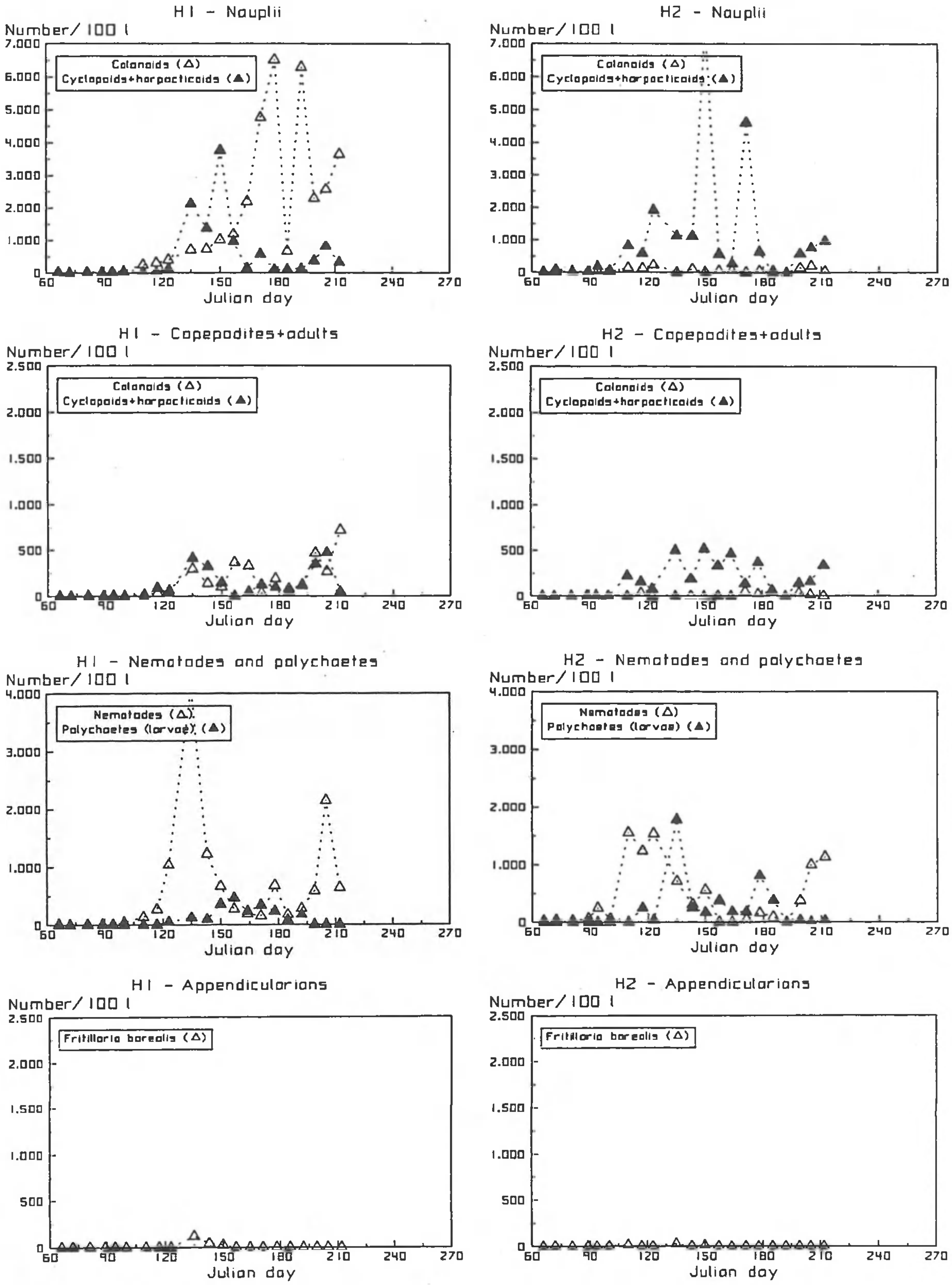


Figure 4.3b

Mesozooplankton development during the 1995 experiment in mesocosms H1 and H2. Data for copepods, nematodes, polychaetes and appendicularians. Day 60 = 01/03/95, day 270 = 27/09/95. For species/groups not presented in the figure: see text. H1, H2: see text.



in Figure 4.3b) was found in only one sample from mesocosm H2. The cirriped *Semibalanus* sp. (data not presented in Figure 4.3b) occurred with regular intervals in both mesocosms.

4.3.3 Zooplankton development (biomass)

4.3.3.1 Microzooplankton biomass

The development of microzooplankton carbon biomass (without eggs and cysts) in the mesocosms is shown in Figure 4.4a. In all mesocosms biomass increased after day 100 and peaked 2-4 times before low values were found again in mesocosms L1-M2 after day 210, respectively day 225 for mesocosms H1 and H2. In all mesocosms heterotrophic dinoflagellates were the major contributors to microzooplankton biomass.
Microzooplankton biomass in mesocosms L1 and L2 varied between 3 and 273 μgC/l, in mesocosms M1 and M2 between 1 and 444 μgC/l and in mesocosms H1 and H2 between 1 and 663 μgC/l. The mean biomass values for the spring, summer and spring + summer periods are given in Table 4.1. An ANOVA analysis showed no significant effect of the treatments on mean biomass for the spring or the summer periods. Considering the mean biomass values for the spring + summer period resulted in a significant effect of the treatments ($P=0.016$). A pairwise comparison with Fisher's Least Significant Difference Test showed significant differences in mean biomass between treatments L and H ($P=0.007$) and between treatments M and H ($P=0.025$). There was no significant difference at the 5% level between treatments L and M ($P=0.099$).

4.3.3.2 Mesozooplankton biomass

The development of mesozooplankton carbon biomass (without eggs and fish larvae) in the mesocosms is shown in Figure 4.4b.
In all mesocosms biomass sharply decreased after the mesocosms were filled. This sharp decrease was caused by the disappearance of *Semibalanus* sp. nauplii, which were quite abundant when the experiment started. Between days 100 and 110 biomass in all mesocosms increased and peaked 2-4 times until day 210. The low biomass in mesocosm H1 at day 184 was not caused by the leakage in this mesocosm at day 182-183, because biomass values were already decreasing from day 171 onwards.
Mesozooplankton biomass in mesocosms L1 and L2 varied between ca. 1 and 41 μgC/l, in mesocosms M1 and M2 between ca. 1 and 25 μgC/l and in mesocosms H1 and H2 between ca. 1 and 27 μgC/l. The highest value of 41 μgC/l (in mesocosm L2) was caused by a sudden increase of *Semibalanus* sp. nauplii. The mean biomass values for the spring, summer and spring + summer periods are given in Table 4.2. An ANOVA analysis showed no significant effect of the treatments on mean biomass for the spring, summer and spring + summer periods.

Figure 4.4a
Microzooplankton biomass (without eggs and cysts) in µgC/l during the 1995 experiment in all mesocosms. L1, L2, etc.: see text. GYRO+GYMNO = *Gyrodinium* spp. + *Gymnodinium* spp., PROTO = *Protoperidinium*.spp., OXYR = *Oxyrrhis marina*, CILI = loricate + aloricate + peritrichous ciliates, ROTIFE = *Trichocerca marina* + *Synchaeta* spp. and REST = rest.

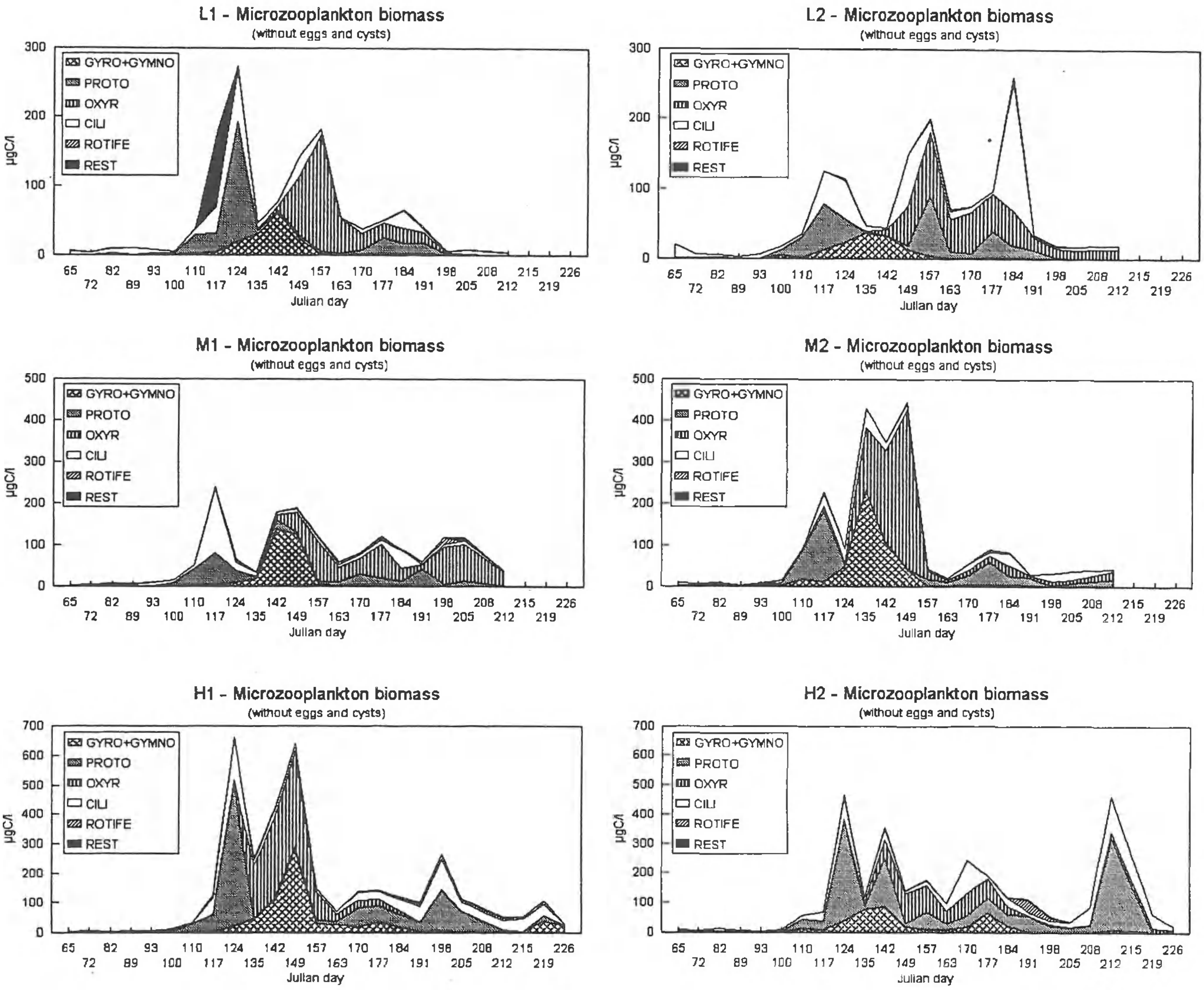


Table 4.1
Mean microzooplankton biomass (without eggs and cysts) in µgC/l in spring (Julian days 65-149), summer (Julian days 157-212/226) and spring + summer (Julian days 65-212/226) for the treatments L1-H2.

Treatment	Spring	Summer	Spring + summer
L1	66.2	45.3	56.7
L2	47.8	81.4	63.1
M1	66.9	87.5	76.3
M2	140.0	42.3	97.8
H1	179.9	109.3	143.2
H2	103.4	147.3	126.2

Figure 4.4b
Mesozooplankton biomass (without eggs and fish larvae) in μgC/l during the 1995 experiment in all mesocosms. L1, L2, etc.: see text. CALANAU = calanoid nauplii, CALACOP = calanoid copepodites + adults, CYHARNAU = cyclopoid + harpacticoid nauplii, CYHARCOP = cyclopoid + harpacticoid copepodites + adults, NEMATODE = nematodes, CIRRIPED = cirriped nauplii and cypris larvae, POLYCHAE = polychaete larvae, APPEND = *Fritillaria borealis* + *Oikopleura dioica*, REST = rest.

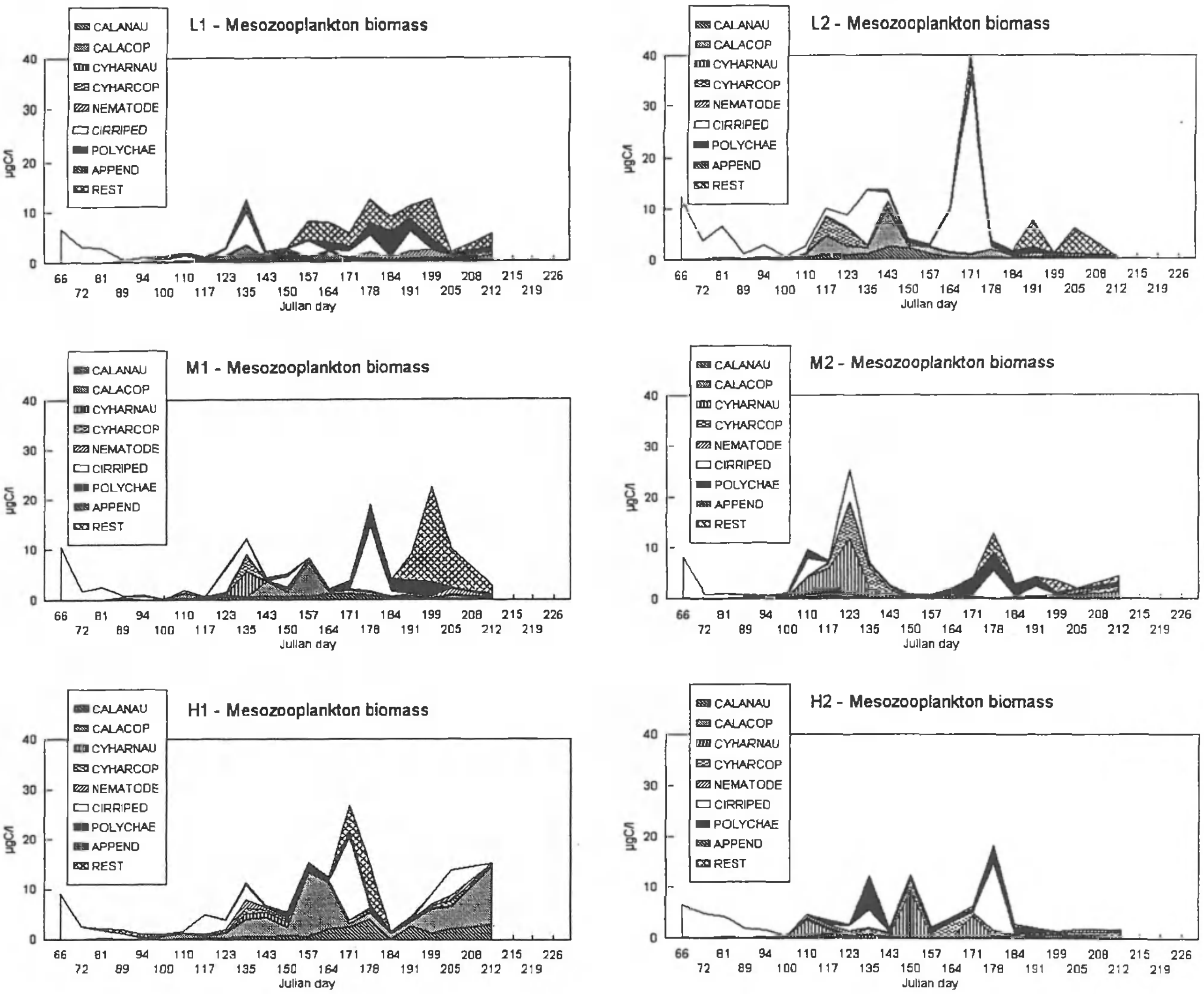


Table 4.2
Mean mesozooplankton biomass (without eggs and fish larvae) in μgC/l in spring (Julian days 66-150), summer (Julian days 150-212) and spring + summer (Julian days 66-212) for the treatments L1-H2.

Treatment	Spring	Summer	Spring + summer
L1	3.15	7.67	5.20
L2	6.66	7.67	7.12
M1	3.92	8.77	6.13
M2	5.39	3.99	4.76
H1	4.37	12.85	8.22
H2	4.65	4.17	4.43

4.4 DISCUSSION

4.4.1 Microzooplankton development

For a comparison of microzooplankton densities that developed in the mesocosms with field data, recent results from the Dutch biomonitoring network (31 stations; Tripos, 1994, 1995a, 1995b, 1996a, 1996b) were used.

In many samples it was difficult to distinguish between the genera *Gyrodinium* and *Gymnodinium* on the basis of morphological characteristics. Almost all *Gyrodinium* species are heterotrophs (Dodge, 1985). Within the genus *Gymnodinium*, however, also many autotrophic species occur. Because densities of *Gymnodinium* species were low, this will hardly influence our results. During the 1995 experiment the maximum densities of *Gyrodinium* spp. were between ca. 8000 and 50000 cells/l and these densities were also found in samples from the Dutch coastal biomonitoring stations. The highest densities of *Gymnodinium* spp. varied between ca. 2000 and 13000 cells/l, which are much lower values than found during the 1994 experiment (a maximum of ca. 100000 cells/l) and also much lower when compared with densities occurring at the Dutch coastal biomonitoring stations. *Protoperidinium* spp. reached maxima between ca. 55000 and more than 200000 cells/l and these values were also found during the 1994 experiment and also occurred under field conditions. *Oxyrrhis marina* occurred with very high densities between ca. 100000 and 400000 cells/l in the mesocosms. In samples from the Dutch biomonitoring network *Oxyrrhis marina* was found in a few samples from 3 stations: in the Eems-Dollard, in the Dutch Wadden Sea and in brackish Lake Veere. Only in Lake Veere densities > 1000 cells/l were found: ca. 16000 cells/l in March 1994 and ca. 39000 cells/l in June 1995. *Oxyrrhis marina* thrives in productive aquatic environments where prey species are present in large abundance, e.g. tidal pools and intensive algal cultures (references in Goldman et al., 1989) or polluted seawater aquaria (Drebes, 1974). From *Oxyrrhis marina* feeding on a wide assortment of phytoplankton species is known. Klein Breteler (1980) used cultured *Isochrysis galbana* and *Rhodomonas* sp. and Goldman et al. (1989) cultured *Phaeodactylum tricornutum*, *Isochrysis galbana* and *Dunaliella tertiolecta* as food items for *Oxyrrhis marina*. Klein Breteler et al. (1982) reported especially *Rhodomonas* being a favourite food item. Also nanoflagellates and diatoms are grazed by *Oxyrrhis* (references in Gaines & Elbrächter (1987) and Hansen (1991)) and the dinoflagellate even can resort to chemotrophy when food particles are unavailable (references in Goldman et al., 1989). Goldman et al. (1989) found maximum observed ingestion rates from about 50 cells dinoflagellate^{-1} day^{-1} when *Dunaliella tertiolecta* was the prey to 250-350 cells dinoflagellate^{-1} day^{-1} when *Isochrysis galbana* or *Phaeodactylum tricornutum* were eaten. The difference in maximum ingestion values reflected the difference in size between prey species, *Dunaliella tertiolecta* being ca. 4-5 times bigger by volume than the other two species. Furthermore, some measurements of phytoplankton growth and microzooplankton grazing rates were done by Van der Molen (1995). In two of these measurements (Julian days 151 and 155, mesocosm H1) a severe grazing pressure, measured as a rapid decrease in fluorescence (phytoplankton growth k - grazing g was -0.8, respectively -1.2 d^{-1}, see Appendix B in Van der Molen (1995)) was estimated. During these period microzooplankton biomass reached very high values, between 400 and 600 µgC/l, to which *Oxyrrhis marina* was the major contributor. Thus, *Oxyrrhis marina* can be considered an effective grazer on the smaller phytoplankton size fraction (see also later).

Ciliates were of less importance within the microzooplankton developing in the mesocosms. Loricate (tintinnid) ciliates did not reach high densities, between ca. 1000 and 35000 cells/l and these densities also occur under field situations in Dutch coastal and other (see references in Wetsteyn & Vink-Lievaart, 1995) waters. The most important species were *Tintinnopsis beroidea* and *Tintinnus pectinus*. Aloricate ciliates, mainly Ciliate sp.1, *Strobilidium* spp. and *Strombidium* spp. developed in all mesocosms to maximum densities between ca. 20000 and 35000 cells/l, with the exception of some high values (80000-95000 cells/l) measured in mesocosm H1. The 1995 mesocosm densities were higher than those measured at the biomonitoring stations in 1995, but comparable with the 1994 monitoring data from the Dutch coastal zone and other (see references in Wetsteyn & Vink-Lievaart, 1995) marine waters. *Trichocerca marina* was the most dominant rotifer with peak densities between ca. 1000 and 6000 animals/l; *Synchaeta* spp. occurred in much lower densities with maxima of ca. 40-250 animals/l in some of the mesocosms. The euryhaline rotifer *Trichocerca marina* is known from all brackish and marine waters in the Dutch Delta area: Lake Grevelingen (Bakker, 1978), the Oosterschelde (Bakker, 1994), Lake Veere (Bakker & de Pauw, 1975) and the Westerschelde (Bakker & de Pauw, 1975). Quantitatively, *Trichocerca marina* was not of importance in these waters when compared with *Synchaeta* spp., and no documented information is available of field densities of *Trichocerca marina* in the Dutch Delta area. During the 1993 summer experiment *Trichocerca marina* was found in one of the mesocosms with densities up to ca. 4100 animals/l and a severe decrease of the chlorophyll-*a* concentration in this mesocosm was explained by rotifer grazing (Escaravage et al., 1996). During the 1995 experiment *Trichocerca marina* biomass was not of importance in the mesocosms (see Figure 4.4a) and grazing pressure must have been small.

In all mesocosms a number of organisms of benthic or sessile origin occurred: peritrichous ciliates (like *Acineta tuberosa*, *Corynophrya* sp. and *Vorticella* sp.), *Amoeba* spp. (like *Amoeba* sp. and *Vahlkampfia guttula*) and nematodes. These organisms are also known from the Dutch coastal biomonitoring stations, but with much lower densities. Apparently, these species benefited from the favourable surface to volume ratio in the mesocosms.

An always re-occurring difficulty is the presence of, sometimes, large quantities of eggs. In some cases these eggs (75*40 µm) could be recognized as *Trichocerca* eggs. These eggs always were observed during peaks of *Trichocerca* and must be emanated from reproducing (amictic) females. With respect to Eggs sp. (diameter range 25-80 µm), however, it was not possible to discover from what organisms these eggs originated, or what organisms hatched from these eggs.

Comparison of microzooplankton biomass (Figure 4.4a) with the chlorophyll-*a* concentrations (Figure 3.8) in the mesocosms shows a direct response of the microzooplankton, especially in the M and H mesocosms. Table 4.3 shows that our estimated range of

Table 4.3
Microzooplankton biomass in µgC/l in different areas and periods. Microzooplankton groups indicated with 1 = heterotrophic dinoflagellates, 2 = mixotrophic dinoflagellates, 3 = heterotrophic nanoflagellates, 4 = ciliates, 5 = rotifers, 6 = copepod nauplii and 7 = rest.

Biomass	Area	Period	Groups	Source
<0.2->1	Dogger Bank area	May 1990	1	1)
<1->15	Dogger Bank area	May 1990	4	1)
5.4-17.2	Western North Atlantic Ocean	May 1993	1+4	2)
2-14	North-Eastern Atlantic	May-August 1989	1+2+4	3)
2-10	Irish Sea	April 1989	1+4	4)
2-22	Irish Sea	1992	1+4	4)
34	Irish Sea	May 1987	1+4	4)
41	Irish Sea	April 1988	1+4	4)
15-40	North Atlantic	Spring 1989 and 1990	1+3+4	5)
2.7-42	Southampton Water (Calshot)	June 1986-June 1987	4	6)
1.4-219	Southampton Water (N.W. Netley)	June 1986-June 1987	4	6)
10-230	Estuarine enclosures	June 1987	4+5+6	7)
10-407	Oosterschelde (western part)	1989	1+4	8)
5-220	Oosterschelde (western part)	1990	1+4	8)
60-300	Marsdiep	Spring-summer 1992	4	9)
3-176	Mesocosms(1-6) Jacobahaven	May-June 1994	1+4+5+7	10)
2-314	Mesocosms(1-3) Jacobahaven	June-July 1994	1+4+5+7	10)
3-(60)-273#	Mesocosms(L1+L2) Jacobahaven	March-August 1995	1+4+5+7	11)
1-(87)-444#	Mesocosms(M1+M2) Jacobahaven	March-August 1995	1+4+5+7	11)
1-(135)-663#	Mesocosms(H1+H2) Jacobahaven	March-August 1995	1+4+5+7	11)

Minimum-(mean)-maximum value for both mesocosms.
1) Nielsen et al. (1993), 2) Verity et al. (1996), 3) Sleigh et al. (1996), 4) Edwards & Burkill (1995), 5) Stoecker et al. (1994), 6) Leakey et al. (1992), 7) Riemann et al. (1990), 8) Bakker & Vink (unpubl.), 9) Brussaard et al. (1995), 10) Wetsteyn & Vink (unpubl.), 11) this chapter.

microzooplankton carbon biomass during the 1995 experiment is comparable with values from more productive systems.
In most cases the patterns of microzooplankton development in the replicate mesocosms were not comparable if looked at the densities reached by each distinguished group. On the other hand there was a great similarity in the onset of the development of the different groups. In terms of mean carbon biomass there was a significant effect of the treatment for the spring + summer period (ANOVA, P=0.016), but not for the spring or summer period separately. At the 5% level there were significant differences in mean biomass between treatments L and H (P=0.007) and between treatments M and H (P=0.025) and no significant difference between treatments L and M (P=0.099).
Obviously, non-thecate heterotrophic dinoflagellates contributed

most to microzooplankton biomass (see Figure 4.4a). Non-thecate dinoflagellates are phagotrophic and routinely contain visible ingested food (Gaines & Taylor, 1984). Most often small green flagellates are the apparent prey of phagotrophic dinoflagellates (references in Gaines & Elbrächter (1987)). Moreover, the clearance rates of heterotrophic dinoflagellates and ciliates are similar (Lessard & Swift, 1985). Thus, the high densities of microzooplankton, especially non-thecate heterotrophic dinoflagellates of which *Oxyrrhis marina* was most abundant, and the potential of these dinoflagellates having high ingestion rates of smaller phytoplankton species, must have resulted in a severe grazing pressure between days 120 and 210 on the smaller phytoplankton size fraction.

4.4.2 Mesozooplankton development

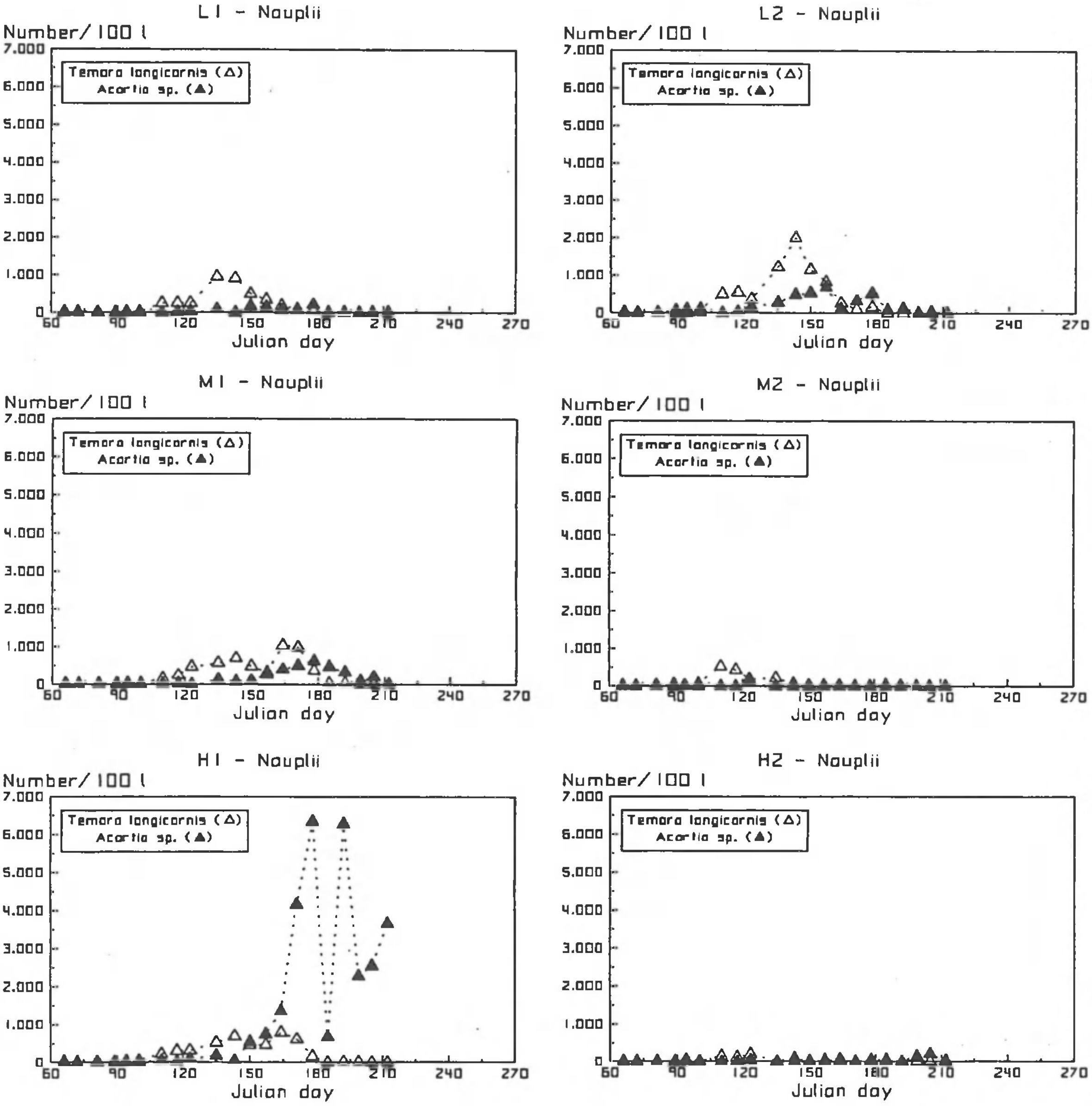
Directly after filling the mesocosms with Oosterschelde water densities of calanoid copepod (*Temora longicornis*) stages were low: eggs were lacking, naupliar stages ranged from 0.08 to 0.22 (mean 0.16) nauplii/l, the number of copepodites varied between 0 and 0.06 (mean 0.03) copepodites/l and adult stages were not seen. These low values are not surprising because of the low water temperature (6.6 °C) and the low food availability in the Oosterschelde (< 2 μg chlorophyll-*a*/l) at the beginning of March. Similar densities of *Temora* stages during this time of the year were also reported by Fransz et al. (1992; estimated from their Figure 4) for a tidal inlet to the Dutch Wadden Sea (Marsdiep) during the period 1973 to 1991 and by Bakker & van Rijswijk (1987; estimated from their Figure 4) for the mouth of the Oosterschelde in 1983. The development of nauplii of the two most important species, *Temora longicornis* and *Acartia* sp., is illustrated in Figure 4.5; all other nauplii belonged to *Centropages hamatus*.

Densities of nauplii increased after day 110, when food level and water temperature favoured growth of the *Temora* nauplii (see also Klein Breteler & Gonzalez, 1986). At first, only *Temora* nauplii became more abundant, and after day 150 also *Acartia* nauplii increased in numbers. In mesocosms L and M maximum densities of nauplii (all species) ranged from ca. 5 to 25 nauplii/l. In mesocosm H1, after day 160, *Acartia* nauplii rapidly increased to a maximum density of ca. 65 nauplii/l at day 178. Due to the leakage (see 4.3.1) nauplii numbers dropped then to ca. 7 nauplii/l. After refilling the mesocosm, with almost only Oosterschelde water, again ca. 65 nauplii/l were counted. Although during summer *Acartia* very often is the most abundant copepod species in the Oosterschelde (Bakker & van Rijswijk, 1994), the sampled inlet water contained hardly any eggs (0.4 eggs/l) and no *Acartia* nauplii. These nauplii very probably originated from sedimented eggs produced by the adult stages that were observed before the leakage. In mesocosm H2 calanoid nauplii did not develop at all.

Copepodites (mostly *Temora*) appeared in the samples from day 117 onwards, but densities were extremely low; most animals were copepodites I-II and only a few copepodites III-IV were seen. In mesocosms L and M between ca. 0.2 and 1.6 copepodites/l were counted, in mesocosm H1 ca. 0.2-5 copepodites/l and in mesocosm H2 ca. 0.2-0.6 copepodites/l. In mesocosm H1 these copepodites belonged to *Acartia* sp.

As could be expected from the copepodite data adult copepods hardly developed: ca. 0-0.6 adults/l (*Temora*) in mesocosms L and

Figure 4.5
Densities of *Temora longicornis* and *Acartia* sp. nauplii in the mesocosms during the 1995 experiment. L1, L2, etc.: see text.



M and ca. 0.02-1.9 (*Acartia* sp.) adults/l in mesocosm H1.
The observed densities of *Temora* nauplii in the mesocosms were similar to values reported for the Dutch coastal area during the period March-half April 1974 (ca. 3-16 nauplii/l; Fransz, 1976) and a Dutch tidal inlet (Marsdiep) to the Wadden Sea during the period half March-June (ca. 4-25 nauplii/l; Fransz et al., 1992). In the mouth of the Oosterschelde Bakker & van Rijswijk (1987) measured ca. 30-70 *Temora* nauplii/l during the period April-July in 1983 and 1984. However, nauplii in the mesocosms developed slower than in the field situations mentioned above.
The number of *Temora* copepodites, reported for the same periods by the same authors, were ca. 0.5-6 copepodites/l for the Dutch coastal area, ca. 1-12 copepodites/l for the Marsdiep and ca. 8-12

cited in Cloern, 1996). Thus, the densities found in the mesocosms also occur under field conditions. Meroplanktonic larvae of polychaetes (*Polydora ciliata*) from 3 up to 8 segments ingest nannoplankton and detritus particles < 20 μm, while larvae from 9 up to 16 segments are carnivorous feeding on particles from 50 up to 150 μm like copepod nauplii, first stages of *Balanus*, larvae of lamellibranchs, etc. (Daro & Polk, 1973). Sullivan & Banzon (1990) report feeding of larvae of polychaetes (mixed species) on eggs and nauplii of *Acartia* spp. Moreover, the last authors found that meroplankton (especially larval polychaetes) and attached benthos were effective competitors and predators of planktonic copepods in both nutrient-limited and nutrient-enriched systems (MERL enclosures). Population densities of the (calanoid) copepod *Acartia hudsonica* and other abundant copepod species were significantly higher in all tanks without sediments than in those with sediment.

Table 4.4
Mesozooplankton biomass in μgC/l in different areas and periods. Mesozooplankton groups indicated with 1 = copepods (nauplii), 2 = copepods (copepodites + adults), 3 = cladocerans, 4 = larvae of bivalves and polychaetes, 5 = appendicularians, 6 = cirripeds (nauplii) and 7 = rest.

Biomass	Area	Period	Groups	Source
1.1-3.2	Dogger Bank area	May 1990	1	1)
15,25,17#	Oyster Grounds	May,July,Sept. 1981	2	2)
20,30,25#	Southern Bight, offshore	May,July,Sept.1971-79	2	3)
5-60	Estuarine enclosures	June 1987	2+3+4	4)
2-70	Killary Harbour, Ireland	March-July 1981	2+3+5	5)
13-114	Western North Atlantic Ocean	May 1993	2	6)
20-225	Marsdiep	Spring-summer 1992	2	7)
100-300*	Oosterschelde (western part)	1987-1988	2	8)
55-75**	Oosterschelde (western part)	1987-1988	2+4+7	8)
267***	Friese Front	June 1986	2	9)
564***	Friese Front	June 1986	2+4+7	9)
30-80	Meetpost Noordwijk	July-August 1984	2	10)
25-165	Meetpost Noordwijk	July-August 1985	2	10)
0-(7.5)-70^	Mesocosms(1-6) Jacobahaven	May-June 1994	1+2+4+5+6+7	11)
0-(7.4)-49^	Mesocosms(1-3) Jacobahaven	June-July 1994	1+2+4+5+6+7	11)
1-(6.2)-41^	Mesocosms(L1 +L2) Jacobahaven	March-August 1995	1+2+4+5+6+7	12)
1-(5.4)-25^	Mesocosms(M1 +M2) Jacobahaven	March-August 1995	1+2+4+5+6+7	12)
1-(6.3)-27^	Mesocosms(H1 +H2) Jacobahaven	March-August 1995	1+2+4+5+6+7	12)

C estimated as 0.5*dry weight.
* Maximum values and C estimated as 0.5*dry weight.
** Annual averaged values and C estimated as 0.5*dry weight.
*** Monthly averaged value and C estimated as 0.5*dry weight.
^ Minimum-(mean)-maximum value for the indicated mesocosms.
1) Nielsen et al. (1993), 2) Fransz et al. (1984), 3) Fransz & Gieskes (1984), cited in Reichert & Daan (1992), 4) Riemann et al. (1990), 5) Rodhouse & Roden (1987), 6) Verity et al. (1996), 7) Brussaard et al. (1995), 8) Figures 2 and 7 in Bakker & van Rijswijk (1994), 9) Kuipers et al. (1991), 10) Daan (1989), 11) Wetsteyn & Vink (unpubl.), 12) this chapter.

Perhaps, the small biomass of larvae of polychaetes may have had effect on the copepod stages. During the 1994-2 experiment, when inert sand was used in the sediment chamber, larvae of polychaetes occurred only occasionally and with low densities. Thus, the use of aged sediment means that a lot of meroplanktonic larvae of polychaetes may occur in the mesocosms; the number of larval polychaetes entering the mesocosms with the flushing water (see 4.2.1), is much smaller. The conclusion is that using (natural) sediments instead of inert sand in the mesocosms may reduce the possibility of a large (pelagial) copepod standing stock.
Nauplii of cirripeds (*Semibalanus* sp.) occurred periodically in all mesocosms. *Balanus* spp. in the Oosterschelde mainly feed upon phytoplankton > 20 μm (Tackx et al., 1989). Because of the small cirriped biomass in the mesocosms, grazing impact on the phytoplankton is assumed to be of minor importance.
In many cases the densities of each distinguished mesozooplankton group in the replicate mesocosms differed substantially. On the other hand there was a great similarity in the onset of the development of the different groups. Mesozooplankton biomass remained low in all mesocosms (Figure 4.4b). Comparison with literature data (see Table 4.4) shows that the measured values were much lower than those reported for Dutch coastal North Sea waters. This is caused by the low abundance of late copepodite and adult stages of calanoid copepods, which, if present, may contribute much to mesozooplankton biomass. An ANOVA analysis showed no significant effect of the treatments on mean biomass for the spring, summer or spring + summer period.

4.5 CONCLUSIONS

-During this long-term experiment a better development of mesozooplankton was expected, but not realized. The most plausible explanation is that overgrazing of the smaller phytoplankton size fraction by the large microzooplankton standing stock, resulted in a poor food situation (a dominance of greater diatom species) for the nauplii and early copepodite stages and further development ceased.
-The use of (natural) sediment instead of inert sand in the mesocosms may reduce the possibility of a large (pelagial) copepod standing stock.

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5 Microzooplankton grazing pressure in mesocosms

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ABSTRACT

The sea water dilution method was used to estimate grazing rates of natural microzooplankton assemblages (<100 μm) in land-based mesocosms. During July and August 1995, incubations were done in bottles under constant light and nutrient levels. Phytoplankton development was estimeted from *in vivo* fluorescence. Grazing rates were 0.6 - 1.50 d^{-1}. The grazing community consisted of loricate ciliates (*Eutintinnus tubulosus, Tintinnopsis beroidea*), aloricate ciliates (*Strombidium, Strobilidium , Ciliates sp.*), heterotrophic dinoflagellates (*Protoperidinium bipes, P. sp., P. minutum , Gymnodinium sp., Gyrodinium spp., Oxyrrhis marina*) and rotifers (*Trichocerca, Synchaeta*). Observed grazing rates were comparable to published values and were related to phytoplankton growth.

5.1 INTRODUCTION

Phytoplankton dynamics are determined by gain and loss factors. Three main loss factors are sedimentation, cell lysis and grazing (Brussaard et al., 1995). Among many grazers exploiting phytoplankton, microzooplankton (< 100 μm) is believed to play an important role in regulating phytoplankton standing stocks as well as species composition. Due to high growth rates (1-2 d^{-1} : Verity, 1985; Vertity, 1986; Gilron & Lynn, 1989), microzooplankton population development can rapidly follow phytoplankton blooms as described by Wetsteyn & Vink-Lievart (1995) in mesocosm experiments. Furthermore, microzooplankton grazing appears to exceed all other sources of nutrient regeneration in most environments.

In mesocosm experiments attention is paid to all factors affecting the phytoplankton dynamics. Until now, however, little was known about grazing pressures due to microzooplankton. It was however known from 1994 experiments (Wetsteyn & Vink-Lievart, 1995) that the microzooplankton community was numerically dominated by aloricate ciliates, which are delicate organisms and therefore sensitive to manipulations.

Grazing in natural communities remains a difficult process to assess. Gifford (1988) makes a distinction between direct and indirect methods, the latter having the advantage of being non-manipulative, but also being harder to extrapolate to *in situ* conditions.

One direct method, using gut content fluorescence, is a true *in situ* method which does not require manipulation of the microzooplankton and their prey. However, because of uncertainty

about the conversion efficiency of chlorophyll to phaeopigments, the true quantitative power of this approach remains to be demonstrated (Helling & Baars, 1985; Gifford, 1988). The method used in this study, the seawater dilution method (Landry & Hasselt, 1982; Landry et al.1995), uses natural microzooplankton assemblages and their food. The water is diluted rather than size fractionated or concentrated and therefore manipulation of delicate organisms is minimized. The dilution method is still used, with a few alterations, in a lot of research in all kinds of marine environments (Brussaard et al., 1995; Burkill et al., 1987; Evans & Paranjape, 1992; Gifford, 1988).
The goal of this study was to estimate grazing in mesocosms and to find a relationship with phytoplankton development.

5.2 MATERIAL AND METHODS

A description of the mesocosm conditions is given by Prins et al. (1995). The dilution method is descripted in Chapter 2 of this volume. The estimation of microzooplankton grazing in mesocosms was carried out from July 18 till August 7. During this period 16 experiments were done with samples from different mesocosms (Table 5.1). The temperature in the incubator was set to levels similar to mesocosm conditions (19 to 21 °C, see Figure 5.1).
A set of four strip-lights (Philips 33, 58W) hanging above the incubator was used to reproduce a mesocosm-like light climate within the incubator. The average irradiance measured within the incubation bottles during one revolution equalled 72 $\mu E.m^{-2}.s^{-1}$. For comparison, the light intensity within the mesocosm water column averaged over the 16 sampling dates, equalled 83 $\mu E.m^{-2}.s^{-1}$.
Incubations started at noon and were terminated after 24h. Phytoplankton biomass was estimated at t=0 and 24h by *in vivo* fluorescence of chlorophyll. The bottles were stored in the dark for 15 minutes. Subsequently a sample of 3 ml was poured into a cuvet (l= 1cm) and fluorescence was measured at 683 nm when exposed to an excitation beam at 434 nm (Fluorescence Spectrophotometer, Hitachi F-2000).
Each fluorescence measurement was made in triplicate. Every dilution was incubated in duplo, so two groups with equal sample

Table 5.1
Mesocosms used for incubation and sampling dates.

mesocosms	date	julian day
M1, M2	July 3-4	184
M1, M2	July 18-19	199
L1, H2	July 20-21	201
M1, H2	July 24-25	205
M1, H2	July 27-28	208
M1, M2	July 31-Aug.1	212
H1, H2	Aug. 3-4	215
H1, H2	Aug. 7-8	219

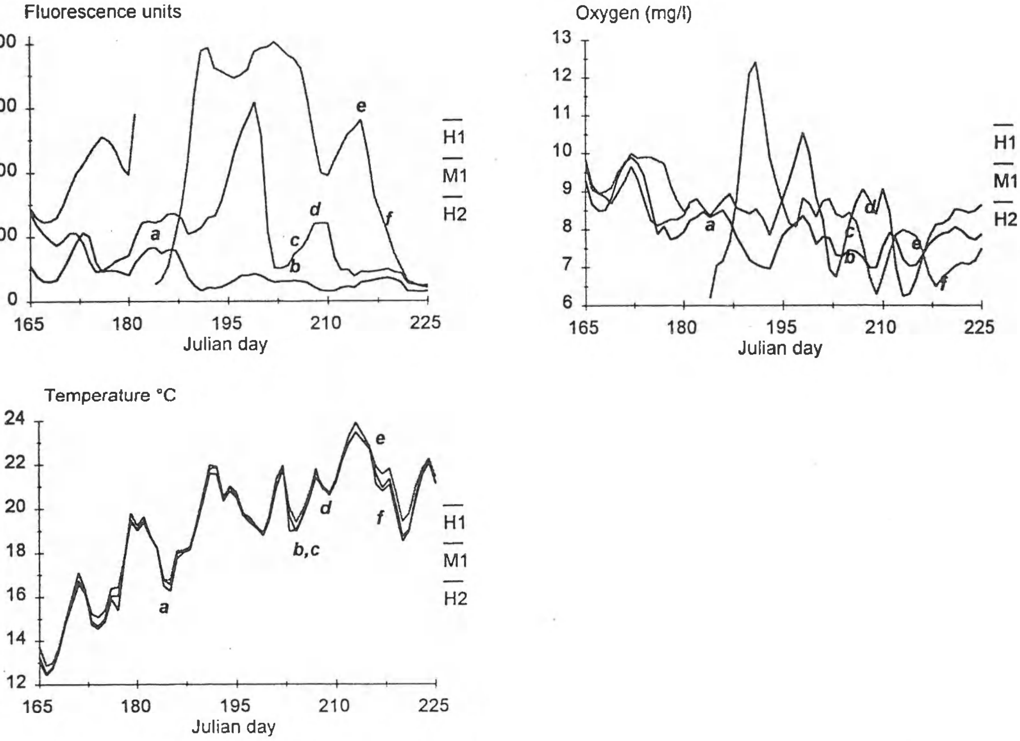
sizes were generated. A test of significance for differences between means of the two groups was done using a t-test (Systat for Windows, version 5.02).
The algal growth coefficient (*k*), grazing coefficient (*g*) and their standard errors were determined from linear regression analysis of the relationship between the rate of change of fluorescence (apparent growth rate, calculated with means of fluorescence) and the dilution factor (Systat for Windows, version 5.02).
At the start and at the end of the incubation 1 litre of undiluted mesocosm water was preserved with Lugol's iodine fixative (ca. 0.2 %) to estimate the abundance of microzooplankton. After concentration (x10) by passive sedimentation, microzooplankton numbers, and species compositions were determined using an inverted microscope.
Microzooplankton was identified in functional groups: heterotrophic dinoflagellates, loricate ciliates, aloricate ciliates, rotifera.

5.3 RESULTS

5.3.1 Experimental conditions vs. mesocosm conditions

The phytoplankton fluorescence in the mesocosms during July and August is illustrated in Figure 5.1. At julian day 184 mesocosm H1 emptied because of a leakage in one of the benthos chambers. After the leakage, the mesocosm was filled again with water from the Oosterschelde and, during one week, with the overflow water (100 l d^{-1}) from mesocosm H2, and fluorescence restored quickly. At julian day 215 a decline of fluorescence started in mesocosm H1, resulting in an equal amount of fluorescence as in the other mesocosms at julian day 224.

Figure 5.1
Fluorescence, oxygen concentrations and temperature measured in mesocosms H1, M1, H2 where incubations were performed. The experiments leading to significant results (See §5.3.2) are indicated by letters **a** to **f**. (See also Table 5.2)



The temperature of the mesocosm water varied from 16 - 23 °C, comparable to the Oosterschelde. The oxygen concentration varied between 6mg.l^{-1} to 12.5 mg.l^{-1}. (Figure 5.1).
Phosphate concentrations were limiting (<0.5 μM) at all sampling days. DIN concentrations were limiting (<2 μM) at julian days 215 and 219 in mesocosm H1. Silicate concentrations were limiting (<2 μM) at julian day 208 in mesocosm H2 and also at julian days 215 and 219 in mesocosm 1 (See Chapter 3).

5.3.2 Grazing rates vs. phytoplankton growth rates

In 12 out of the 16 experiments no calculation of grazing was possible as a result of erratic responses of the different dilutions. Different factors could have been responsible for this lack of response. In most cases fluorescence was too low to make accurate measurements. In one case however (julian day 201, in mesocosm H2) fluorescence in all dilutions declined during the incubation in such a way that no significant differences could be shown.
Six experiments showed significant differences in growth rate between dilutions ($p<0.05$) (Figure 5.2).
The corresponding grazing coefficient(*g*), determined with linear regression (Figure 5.2), is given in Table 5.2, together with the net algal growth coefficient (*k*) and the resulting net phytoplankton growth estimated by the difference (**k-g**).

Figure 5.2
Phytoplankton net growth rate (Log of fluorescence ratio after/before incubation) along the mesocosm water dilution gradient in the six experiments producing significant results.

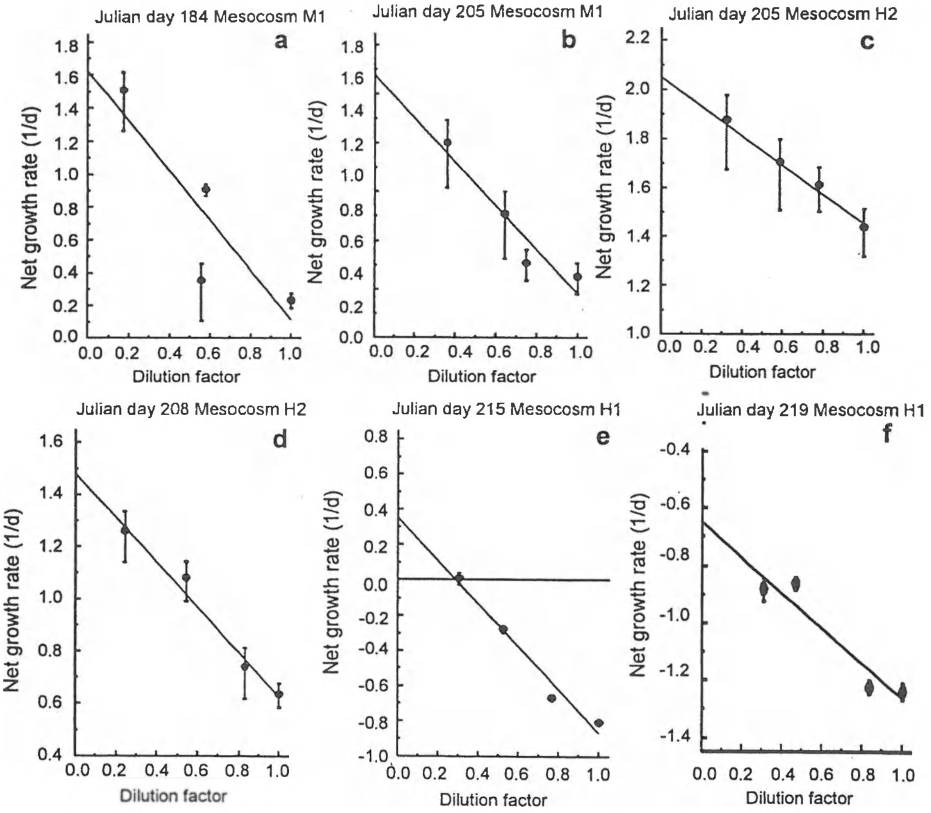


Table 5.2
Phytoplankton growth (k) and microzooplankton grazing (g) rates and standard errors during experiments. Phytoplankton net growth rate estimate (k-g).

Julian day (mesocosm)	Exp.	k (d^{-1}) (s.e.)	g (d^{-1}) (s.e.)	k-g (d^{-1})
184 (M1)	a	1.622 (0.222)	1.511 (0.343)	0.1
205 (M1)	b	1.079 (0.159)	0.895 (0.219)	0.2
205 (H2)	c	2.05 (0.167)	0.596 (0.232)	0.5
208 (H2)	d	1.48 (0.125)	0.852 (0.175)	0.6
215 (H1)	e	0.354 (0.062)	1.219 (0.089)	-0.8
219 (H1)	f	-0.649 (0.065)	0.616 (0.092)	-1.2

Table 5.3
Summary of growth and grazing results (Chl-a growth $d^{-1} = k/\ln 2$; Potential production, $P_p = (P_0 e^k) - P_0$; Actual production, $P_a = (P_0 e^{(k-g)}) - P_0$; Initial Chl-a grazed $d^{-1} = (P_0 e^{-g} - P_0)/P_0$; Potential production grazed $d^{-1} = (P_p - P_a)/P_p$).

julian day (mesocosm)	Initial Chl-a ($\mu g\ l^{-1}$)	Chl-a growth (d^{-1})	Potential production Chl-a ($\mu g\ l^{-1}$)	Actual production Chl-al ($\mu g\ l^{-1}$)	Initial Chl-a grazed d^{-1} (%)	Potential production grazed d^{-1} (%)
184 (M1)	9.8	2.3	39.8	1.2	77.9	97.1
205 (M1)	2.7	1.6	5.2	0.6	59.1	89.6
205 (H2)	5.5	3.0	37.2	18.0	44.9	51.53
208 (H2)	15.5	2.1	52.6	13.5	57.3	74.24
215 (H1)	33.9	0.5	14.4	-19.6	70.5	236.3
219 (H1)	11.2	-0.9	-5.3	-8.0	46.0	50.34

In Table 5.3 a summary of the results is given. At julian day 184 *k* and *g* were of the same magnitude, suggesting that phytoplankton growth and microzooplankton grazing were in balance (potential production grazed per day was 97.1%).

5.3.3 Microzooplankton and phytoplankton composition

At the start of each experiment samples were taken to determine the initial abundance of microzooplankton. Samples mainly contained loricate ciliates (*Eutintinnus tubulosus, Tintinnopsis beroidea*), aloricate ciliates (*Strombidium* , *Strobilidium* , *Ciliates sp.*), heterotrophic dinoflagellates (*Protoperidinium bipes, P. sp., P. minutum* , *Gymnodinium sp., Gyrodinium sp., Oxyrrhis marina*) and rotifers (*Trichocerca, Synchaeta*). The abundances of these groups

Table 5.4
Incubation temperatures, initial Chl-a concentrations and characteristics of the microzooplankton/phytoplankton communities (♦ = diatom; ♠ = autotrophic flagellate; NA = not available).

Julian day (mesocosm)	Temperature (°C)	Chl-a (µg/l)	Loricate ciliates (n l^{-1})	Aloricate ciliates (n l^{-1})	Heterotrophic Dinoflagellates (n l^{-1})	Trichocerca (n l^{-1})	Dominant phytoplankton (mean size)
184 (M1)	16.8	9.8	6000	2600	41900	1600	♦ *Thalassiosira rotula* (30-60 µm) ♠ *Cryptophyceae* (<5 µm)
205 (M1)	19.6	2.7	500	10000	105000	200	♦ *Nitzschia closterium* (10 µm) ♠ *Cryptophyceae* (<5 µm)
205 (H2)	19.7	5.5	2000	3100	28700	0	♦ *Skeletonema costatum* (3.5-6 µm) ♠ *Cyanobacteria* (<=1.5 µm)
208 (H2)	20.7	15.5	13100	9700	19500	100	♦ *Thalassiosira rotula* (30-60 µm) ♠ NA
215 (H1)	22.8	33.9	200	80400	6200	600	♦ *Leptocylindrus danicus* (5-16 µm) ♠ NA
219 (H1)	20.7	11.2	2000	68700	97400	1840	♦ *Chaetoceros socialis* (4-11 µm) ♠ *Cryptophyceae* (<5µm)

are given in Table 5.4. In Table 5.4 also the dominant phytoplankton species are given. *Thalassiosira rotula* (200-1300 cells/ml) was present in all but the last two experiments (julian day 215, 219), but other species were sometimes more dominant. Populations of autotrophic flagellates were mainly containing Cryptophyceae (<5 µm).

5.4 DISCUSSION

The dilution method to estimate the grazing impact of microzooplankton on phytoplankton assumes that phytoplankton growth is exponential and unaffected by dilution, and that the grazers do not respond to decreased food levels (i.e. dilutions) (Landry & Hassett, 1982). In order to estimate grazing as it appears in the mesocosms it is necessary that microzooplankton is not affected by manipulations in the preparation of dilution mixtures, and that environmental conditions during incubation are comparable to those in the mesocosms. Gifford (1988) and Paranjape (1987) have reported losses of oligotrich grazers due to collection, handling and nutrient addition. Because of the reverse filtration used during this experiment, damage to these aloricate ciliates are expected to be minimal. Observations made before and after sample preparation

suggested that ciliates underwent no substantial losses.
To prepare filtered seawater, GF/C filters (1 μm) were used. *In vivo* fluorescence of the filtered seawater was found to be approximately 10% of the fluorescence in the most diluted samples and therefore its impact on the estimation of the grazing rate is minor.
Gifford (1988) used different kinds of phytoplankton as food 'items' for oligotrich ciliates cultures and found that dinoflagellates (20 μm) were preferred. This does not imply that oligotrich ciliates will only consume particles of this size, grazing upon picoplankton is possible. Tintinnids prefer grazing upon nanoplankton (2 - 10 μm) (Verity, 1985).

5.4.1 Growth rates in mesocosm vs. experiments

In most cases (13 of the 16 experiments), growth during the incubation was equal or higher than the growth observed in the mesocosm (Figure 5.3). The stimulation of the phytoplankton growth during the incubation could be ascribed to the experimental enrichment with nutrients. Qualitative comparisons can also be made between the growth rates measured during the incubation and in the mesocosm: in most cases (11 experiments out of 16) net growth during the incubation had the same sign as growth in the mesocosm indicating the low level of disturbance introduced by the dilution method.

5.4.2 Changes in microzooplankton abundance during incubations

It was found that during the dilution experiment microzooplankton abundance could change. Table 5.5 shows that aloricate and loricate ciliates can have 1 - 2 doublings in 24 hours. These results do not correspond to the assumptions of the dilution method but Evans & Paranjape (1992) concluded that microzooplankton grazers have generation times not much different from the cells they are eating, and therefore their population can also be expected to change during the incubation. Gallegos (1989) studied the impact of the growth of microzooplankton on grazing results. He found little alteration of grazing rates when grazing was below 1.0 d^{-1}. At higher grazing rates (e.g. julian day 184 (**a**), **g** = 1.5 d^{-1})

Figure 5.3
Comparison between growth rates measured during the incubations and in the mesocosms (lines are plotted through the origins and along the 1:1 axis).

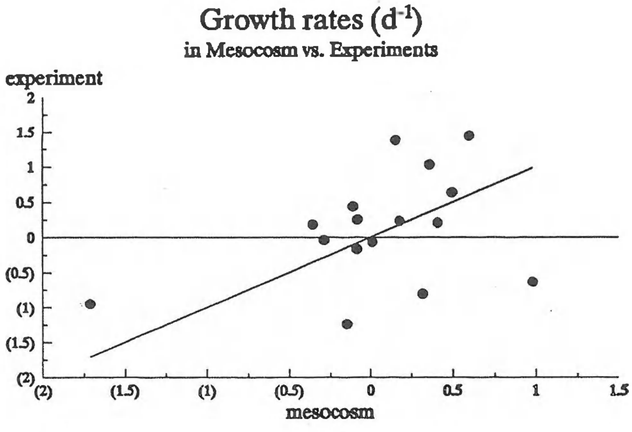


Table 5.5.
Microzooplankton growth (d^{-1})

Julian day (mesocosm)	Loricate ciliates	Aloricate ciliates	Heterotrophic dinoflagellates
184 (M1)	0.8	1.5	0.4
205 (M1)	1.1	0.3	-0.2
205 (H2)	1.0	0.4	-0.3
208 (H2)	0.8	0.2	0.1

microzooplankton growth can become important. As a result, the grazing rate estimated from experiment a could have been an overestimate of the actual grazing rate in the mesocosm.

5.4.3 Grazing rates vs. phytoplankton growth rates

In Table 5.2 k is the growth rate of phytoplankton under the theoretical circumstances that grazing is absent. Following equation $P_t = P_o e^{(k-g)t}$ (see Table 5.3) k-g could give quantitative information about phytoplankton development. When $k > g$ one can expect growth of phytoplankton, whereas $k < g$ a decrease of phytoplankton is expected. These expectations were confirmed by comparing the values of (k-g) in Table 5.2 with the fluorescence evolution in Figure 5.1. At julian days 184 and 205 in mesocosm M1, k and g did not differ much, suggesting a stable phytoplankton community. Indeed the fluorescence around those days was constant. Grazing was smaller than growth at julian days 205 and 208 in mesocosm H2, implying a growing phytoplankton community (see Figure 5.1). At julian days 215 and 219 in mesocosm H1, the phytoplankton community was declining rapidly. During this period grazing exceeded growth (Table 5.2).

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6 Mussel growth rates and secondary production of the benthos in response to external nutrient loading

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ABSTRACT

In a 7 month experiment, carried out in 1995, 3 different nutrient loading scenarios were applied to 6 land-based marine mesocosms, representing 1) treatment H: the present condition in the Dutch coastal zone (N and P loadings in 1995 equal to respectively 90% and 50% of the loadings in the 1980's), 2) treatment M: the situation after a 50% reduction in anthropogenic N- and P-loading, and 3) treatment L: the situation with a 75% reduction in anthropogenic N- and P-loading. Effects of these differences in nutrient loading on secondary production of the macrobenthos were monitored by measuring mussel growth during the experiment, and total macrobenthic biomass at the end of the experiment.

Mussel growth showed no significant differences during the spring period, despite significantly lower phytoplankton production in treatment L. During summer, mussel growth was reduced in treatment L resulting in significantly lower ash-free dry weights of the mussels at the end of the experiment.

Overall effects of the treatments on macrobenthic production were limited. A 75% reduction in primary production resulted in only a 35% reduction in secondary production. The net increase in macrobenthos biomass during the entire experiment showed a significant correlation with phytoplankton primary production. However, differences between the three nutrient loading treatments were not significant.

6.1 INTRODUCTION

Nutrient loading affects primary production and phytoplankton biomass, and differences in nutrient loading may result in changes in secondary production. Changes in macrobenthos biomass and production in the Western Wadden Sea have been attributed to eutrophication (Beukema & Cadée, 1986). Increased mussel production in the Western Wadden Sea has also been related to increases in nutrient loading and primary production (Van der Veer, 1989), but this is not supported by a more extensive recent analysis (Van Stralen, 1996). It has been suggested that fish production at the North Sea is related to anthropogenic nutrient loading (e.g. Boddeke & Hagel, 1991). However, generally effects of eutrophication on fish production are difficult to demonstrate because several other factors may have changed in time, like fishery effort. In addition, eutrophication may have adverse effects on secondary production through increased anoxia or effects on

species diversity (Kerr & Ryder, 1992; Heip, 1995).
One of the objectives of this research was to establish the relation between a change in nutrient loading, through primary production, on secondary production of macrobenthos and zooplankton. In previous experiments, the research focused on monitoring the growth of the blue mussel *Mytilus edulis*. The mussel was used because it is considered to be a generalized filter feeder, feeding on the phytoplankton. Therefore, mussel growth will reflect changes in phytoplankton primary production more directly than growth of benthic organisms that depend mainly on the supply of food from the water column through sedimentation. Mussels have an advantage over zooplankton, because they are easier to manipulate, and growth is easier to measure. In the experiment reported here, a small amount of mussels was added to each of the mesocosms, and the growth of mussels was measured during the course of the experiment. At the end of May, the number of mussels in the mesocosms was reduced by taking a random subsample of the mussels, in order to prevent high grazing rates by the mussels from overshadowing nutrient loading effects on the phytoplankton. This also provided the opportunity to measure individual ash-free dry weights in June and in September. In addition, production of the macrobenthos that had colonized the sediment in the mesocosms was measured at the end of the experiment. A more extensive description of the methods is given in chapter 2.

6.2 RESULTS

6.2.1. Mussel growth rates

The mussels increased in shell length from 19 mm in March to 40-45 mm at the end of July (Julian day 205). In the period August-September shell lengths showed no increase at all (Figure 6.1). Although significant differences in shell lengths between mesocosms were observed at every sampling date, differences between treatments were not significant on any of these dates (nested ANOVA, $p>0.05$).
The ash-free dry weights of the mussels increased from 0.04 g (fish+shell) in March to weights between 0.4 and 0.8 g at the end of the experiment in September (Figure 6.2). At the end of May, average ash-free dry weights were the same in all treatments (nested ANOVA, $P>0.05$); as with shell lengths, significant differences were observed between mesocosms within the treatment. In September, ash-free dry weights of the mussels in the H and M treatments were significantly higher than weights of the mussels in the L treatment (nested ANOVA, $P<0.001$). Some mortality was observed, both in the period March-June as in the period June-September. The highest mortality observed was 15%. However, no significant treatment effect on the mortality rates was observed.

6.2.2 Total macrobenthos production

Despite the differences in individual mussel weights in September, total mussel production during the entire experiment was not significantly different between treatments. This was mainly due to the large differences between the two mesocosms in treatment H (Table 0.1). Mesocosm 1 in treatment H had a relatively low total

Figure 6.1
Average length of mussels in the mesocosms, measured at 4-5 week intervals. Circles: treatment H; squares: treatment M; triangles: treatment L.

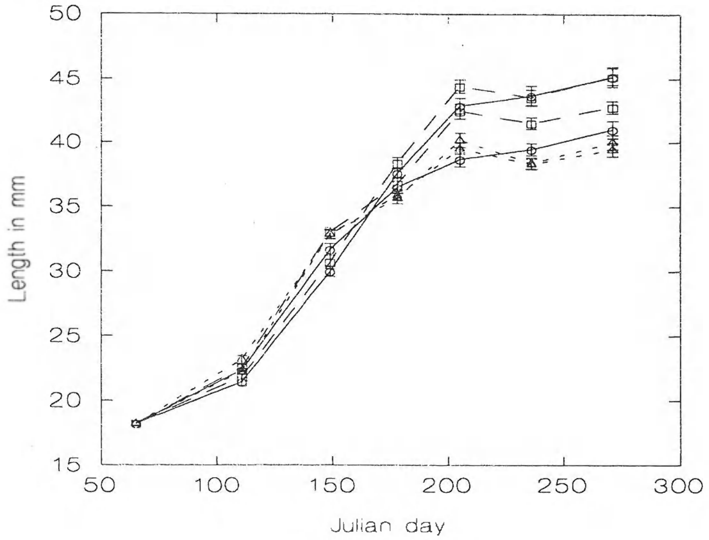
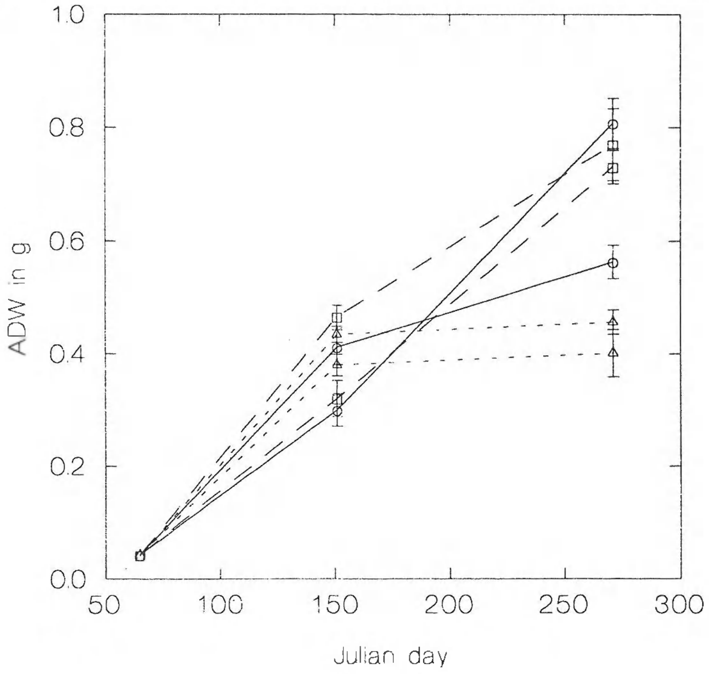


Figure 6.2.
Average ash-free dry weights (fish + shell) of the mussels in the six mesocosms. Symbols as Figure 6.1



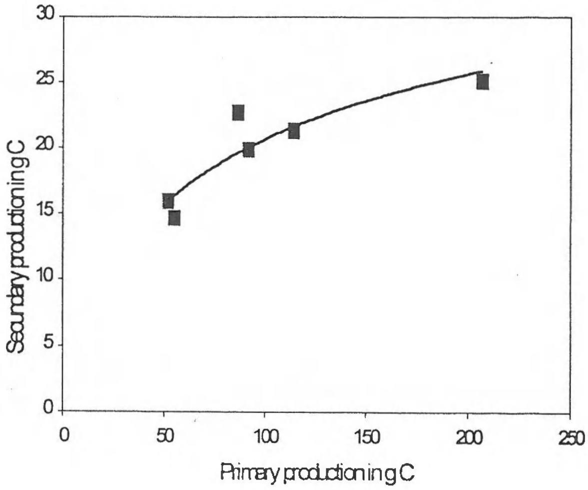
mussel production, which was the combined effect of slightly lower individual weights and slighly higher mortality than in the replicate mesocosm.

Whereas mesocosm 1 had a relatively low mussel production, the biomass of *Nereis succinea* at the end of the experiment in this mesocosm was much higher than in the other mesocosms. Other species that were abundant in the macrobenthos of the mesocosms were the tunicate *Molgula* sp. (mainly growing on the inner wall and the mixer), and the amphipod *Jassa* sp. (mainly observed on the inner wall). Total secondary production of the macrobenthos was calculated by adding the total biomass of the macrobenthos, other than mussels, at the end of the experiment, to the total production of mussels (Table 6.1). Total mussel production included the production of the mussels that were removed from the mesocosms in June. It was assumed that the initial (March) biomass of other macrobenthos was negligible. Secondary production in treatment L was lowest of all treatments, and treatment H had the highest secondary production. However, ANOVA showed that the differences between treatments H and L were not significantly different ($p = 0.058$). Secondary production showed a positive correlation with primary production (Fig. 6.3).

Table 6.1
Total mussel production in the period March-September, and total biomass of *Nereis succinea* and other macrobenthos at the end of the experiment in all mesocosms. Total secondary production was estimated as the sum of mussel production and total biomass of all other macrobenthos.

Treatment	Tank	Mussel production (g ADW) (1)	*Nereis* biomass (g ADW) (2)	other species (gADW) (3)	Total secondary production (g ADW) (1+2+3)
H	1	14.0	10.83	0.24	25.07
	6	18.8	0.29	2.21	21.30
M	2	21.0	0.45	1.22	22.67
	5	18.0	0.93	0.89	19.82
L	3	12.8	1.70	0.09	14.59
	4	15.2	0.57	0.17	15.94

Figure 6.3
Secundary production of the macrobenthos as a function of primary production in the period March-September.
$SP = -12.4 + 7.19 * \ln(PP)$; $r^2 = 0.84$, $p < 0.05$.



6.3 DISCUSSION

Differences in nutrient loading in the 7-month experiment that is reported here, have been shown to have clear effects on phytoplankton biomass and primary production. In the spring period, P became one of the limiting nutrients and differences between treatments with respect to phytoplankton biomass and production could be related to the external P-loading (see Chapter 3).
Mussel growth was measured as one of the parameters reflecting the translation of effects of differences in nutrient loading, through primary production, on secondary producers. The results showed that individual mussel weights were not different between treatments at the end of May, just after the end of the spring bloom. This was probably caused by the fact that phytoplankton biomass during the spring bloom was high, and food concentrations for the mussels were not limiting.
In the summer period, phytoplankton biomass and primary production showed differences between treatments that could be related to differences in external N-loading (Chapter 3). Phytoplankton biomass was also much lower during this period. At the end of the experiment, a significant difference in individual mussel weight was observed between treatments H and M on the one hand, and treatment L on the other hand. In treatment L, the mussels showed hardly any growth in weight during the summer period (Fig. 6.2). This indicated that mussel production in treatment L was affected by the reduced N-loading in this treatment.
Overall, phytoplankton biomass and production showed a correlation with external N-loading (Chapter 3). A significant correlation was observed between phytoplankton primary production and total secondary production (defined as the net increase in macrobenthos biomass over the experiment). However, within-treatment variability in secondary production was relatively large, and as a consequence differences in secondary production between treatments were not significant.
It can be concluded that differences in the intensity of the phytoplankton spring bloom will probably have only minor effects on secondary production of benthic filter feeders, as food concentrations for these animals are high during the spring bloom, and probably exceed the concentrations where food becomes limiting. A reduction in external P-loading, within the range used in this experiment, will probably only affect the spring bloom and may therefore be unimportant with respect to secondary production. Moreover, the colony-forming *Phaeocystis* sp. contributes to a large extent to the spring primary production in the field (Cadée & Hegeman, 1986). The colonies of this species probably have a poor food value for suspension feeders (Pieters et al., 1980; Weisse, 1983; Kamermans, 1994; Prins et al., 1994, Smaal & Twisk, 1997).
However, it should be emphasized that an excess primary production during spring, may still be available as food for benthic organisms during summer after sedimentation of the spring bloom, and might indirectly have some effects on secondary production. It is more likely however, that a major fraction of this material is respired during microbial degradation.
During summer, phytoplankton concentrations are generally much lower, and changes in phytoplankton production during summer as a result of changes in nutrient loading, might have effects on

secondary production. This is illustrated by the low mussel growth in treatment L during summer.
However, overall effects of differences in nutrient loading on secondary production were limited, and a 75% reduction in primary production in treatment L, compared to treatment H, resulted in only a 35% reduction in secondary production.

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7 Nitrogen and phosphorus cycling in relation to season and external nutrient loading

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ABSTRACT

In a 7 month experiment, carried out in 1995, 3 different nutrient laoding scenarios were applied to 6 land-based mesocosms. For each of the mesocosms, retention of nitrogen and phosphorus was calculated by comparing the rates of import and export.

Retention of N was low during the first month of the experiment, but increased after the start of the phytoplankton bloom. N retention was caused by storage in sediment detritus and macrobenthos biomass. During summer, retention remained high, and N balances indicated that denitrification and storage in 'wall' biomass probably were important sinks for N. Retention of P showed little seasonal variation, and was at a high level during the entire experiment. Storage of P in the sediment was a likely cause for the high P retention.

A comparison was made of the rates of N and P uptake by primary producers, with the rates of external nutrient supply and internal nutrient regeneration. Estimates showed that P-uptake exceeded P-supply during spring, whereas uptake and supply approximately balanced during summer. N uptake rates exceeded supply rates during summer. Internal regeneration was the major source of inorganic nutrients during the summer period, and pelagic regeneration was more important than benthic regeneration.

It is argued that the shift to N-limitation of primary production in summer was related to differences in the cycling of N and P. N:P ratios in the external nutrient supply were higher than the Redfield ratio, but deficits in the N and P balances were also higher than the Redfield ratio, leading to a preferential loss of N from the systems, probably due to denitrification.

7.1 INTRODUCTION

The construction of nutrient mass balances for the mesocosms can provide information on the distribution of nutrients between dissolved and particulate forms, among the sediment and the water column. These budgets are a useful tool in describing the fate of the nutrients in the mesocosms, and give indication of the relative importance of loss processes. In previous experiments, deficits in the N and P budgets showed that some of these loss processes may be quantitatively important. Especially the adsorption of phosphate on sediment was a likely cause of relatively large deficits in the phosphorus balances (Prins et al., 1994; 1995). This process was also assumed to be responsible for the relatively high retention of P by the systems. The nitrogen budgets suggested that there was a relation between the loss of N and the external nitrogen loading. The budgets also showed an increased N retention with an increase in external loading, and it was suggested that denitrification was the main cause for the nitrogen deficit in the balances

(Prins et al., 1994; 1995).
Like in the previous experiments, the results from the present experiment were used to calculate nutrient retention and construct nutrient mass balances. In addition, an attempt was made to compare the rates of nutrient uptake, by phytoplankton and epipelic algae growing on the wall of the mesocosms, with the rates of internal and external nutrient supply. Internal nutrient supply as a result of regeneration in the water column was estimated from data on metabolic rates (Chapter 8) and the stoichiometry of the seston particles (cf. Doering et al., 1995). Benthic regeneration rates were determined by comparing concentration changes in the benthic chambers and by incubations of sediment cores. This comparison should give an indication of the importance of external and internal nutrient sources in determining changes in nutrient limitation.

7.2 MATERIAL AND METHODS

Total inputs of N and P to the mesocosms were deduced from the concentrations of dissolved and particulate N and P in the seawater added and the experimental addition of nitrate and phosphate to the systems. As the systems were well mixed, the output of N and P through the overflow was assumed to be equal to the concentrations in the water column times the water flux through the mesocosms. The retention was calculated as:

$$\%retention = \frac{input - output}{input} \cdot 100\%$$

By definition, retention included losses of nutrients to the sediment, benthic biomass or wall biota, and losses to the atmosphere (denitrification).
The amounts of nutrients stored in water column, sediment and benthic fauna were determined by sampling porewaters, sedimented detritus and macrobenthos, as described in chapter 2. Cumulative amounts of nutrient import and export, and inventories of the stocks of inorganic and organic nutrients within the mesocosms were used to construct mass balances.
Nutrient demand by the phytoplankton was estimated from ^{14}C measurements of phytoplankton primary production rates. Primary production rates (^{14}C measurements) were recalculated to rates of N and P by dividing the production rates by the observed average elemental composition of the seston:

$$N\text{-}demand = {}^{14}C_{production} \;/\; C{:}N_{seston}$$

$$P\text{-}demand = {}^{14}C_{production} \;/\; C{:}P_{seston}$$

Running means of the elemental composition were used to make the results less sensitive to inaccuracies in the determination of C:N and C:P ratios of the seston at low seston concentrations.
Total rates of nutrient demand by primary producers in the mesocosms (including epipelic algae) were based on estimates of net daytime production, following methods described by Doering et al. (1995). Net daytime production in the water column (including wall production) was estimated from changes in O_2 concentrations between dawn and dusk, after correction for diffusion and respiration in the benthos chambers (Prins et al., 1995). Rates of system production were converted to

carbon units assuming a production quotient (PQ) of 1 mol O_2 mol^{-1} CO_2. Nitrogen and phosphorus demand was calculated by dividing production estimates by the stoichiometric ratio of the seston.

Water flowing through the benthos chambers was sampled twice a week before and after passage through the chambers. From these data fluxes of inorganic nutrients between the benthos and the water column could be estimated.

Water column regeneration of nutrients was estimated from measurements of water column respiration, following methods described by Doering et al. (1995). Water column respiration was estimated from changes in O_2 concentrations between dusk and the following dawn, after correction for diffusion and respiration in the benthos chambers (Prins et al., 1995). This value included respiration by the biota on the wall of the mesocosm. Rates of respiration were converted to carbon units assuming a respiratory quotient (RQ) of 1 mol CO_2 mol^{-1} O_2. Nitrogen and phosphorus regeneration rates were calculated by dividing respiration estimates by the stoichiometric ratio of the seston.

7.3 RESULTS

7.3.1. Import and export of N and P

Total inputs of nitrogen and phosphorus were a function of the treatments. In addition to the input of nitrate and phosphate, there was some input of ammonium, dissolved organic N and P, and particulate N and P resulting from the addition of seawater to the mesocosms at a rate of 100 l day^{-1}. The loading of the mesocosms with (dissolved and particulate) organic nitrogen was on average 3 mmol day^{-1}. The loading with (dissolved and particulate) organic phosphorus was approximately 0.11 mmol day^{-1}.

The input of nitrogen to the mesocosms was high in spring, and decreased during the experiment. A major fraction of the total N import to the mesocosms was exported again. The retention of N by the mesocosms increased from spring to summer (Figure 7.1). Cumulated over the 7 months of the experiment, retention was 26-38 % of the input (Table 7.1).

The input of P to the mesocosms was high in spring, and decreased to minimum values in the period between day 140 and day 220. Relative to N, the export of P formed a smaller fraction of P import, but still accounted for most of the losses of P from the water column (Figure 7.2). The retention ranged from 46-57 % of the input (Table 7.2). P retention was high in all treatments from the start of the experiment. In contrast to N, there was no difference in retention during the spring bloom (March-June) and the retention during the summer (July-September). Differences in the percentage of retention between treatments were not significant for either N or P.

The mesocosms retained only a fraction of the imported nutrients, but the processes within the mesocosms were important for the transformation of inorganic nutrients into organic nutrients. As a consequence, the mesocosms were a sink for inorganic nutrients. There was an export of DON in all mesocosms, an export of PN in the H treatment and an import of PN in the L treatment. A small export of DOP was observed in the M and H treatments. As with PN, the H treatment exported PP and the L treatment imported PP.

Figure 7.1
Daily total N-import and -export in the mesocosms (left-hand figures) and retention of N by the mesocosms (right-hand figures), in treatments H (Fig. A), M (Fig. B) and treatment L (Fig. C). Retention curves were fitted by distance weighted least squares regression (Wilkinson, 1992); the two lines show the estimates for the two replicate mesocosms.

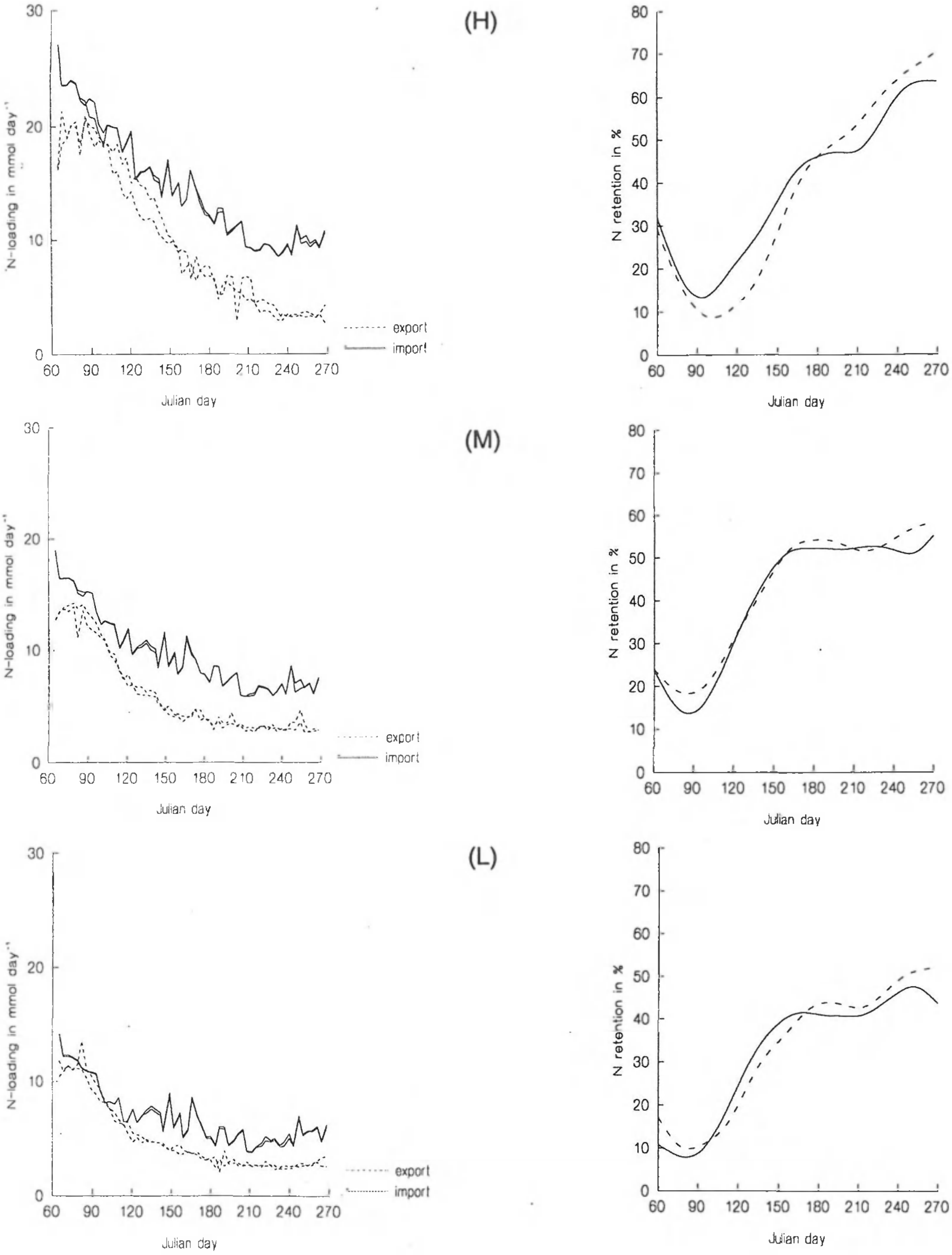


Table 7.1
Total input and output of nitrogen to the mesocosms and corresponding percentage of retention (average for the entire experimental period).

Treatment	Tank	Total input (mol)				Total output (mol)				Retention (% of input)
		DIN	DON	PN	Total-N	DIN	DON	PN	Total-N	
H	1	2.40	0.46	0.15	3.01	1.12	0.70	0.39	2.21	26.5
	6	2.40	0.47	0.15	3.01	1.22	0.65	0.22	2.09	30.4
M	2	1.38	0.47	0.15	2.00	0.55	0.59	0.15	1.28	35.7
	5	1.40	0.47	0.15	2.01	0.48	0.61	0.16	1.25	37.9
L	3	0.80	0.47	0.15	1.42	0.35	0.57	0.10	1.03	27.4
	4	0.80	0.47	0.15	1.42	0.33	0.59	0.10	1.01	29.1

Table 7.2
Total input and output of phosphorus to the mesocosms and corresponding percentage of retention (average for the entire experimental period).

Treatment	Tank	Total input (mol)				Total output (mol)				Retention (% of input)
		PO_4^{3-}	DOP	PP	Total-P	PO_4^{3-}	DOP	PP	Total-P	
H	1	0.066	0.013	0.009	0.088	0.014	0.013	0.020	0.047	46.6
	6	0.066	0.013	0.009	0.088	0.015	0.014	0.013	0.042	52.2
M	2	0.066	0.013	0.009	0.088	0.016	0.014	0.009	0.040	54.8
	5	0.067	0.013	0.009	0.089	0.014	0.014	0.011	0.039	56.1
L	3	0.047	0.013	0.009	0.070	0.012	0.013	0.006	0.031	55.8
	4	0.047	0.013	0.009	0.070	0.011	0.013	0.006	0.030	57.3

Figure 7.2
Daily total P-import and -export in the mesocosms (left-hand figure) and retention of P by the mesocosms (right-hand figure), in treatments H (Fig. A), M (Fig. B) and treatment L (Fig. C). Retention curves were fitted by distance weighted least squares regression (Wilkinson, 1992); the two lines show the estimates for the two replicate mesocosms..

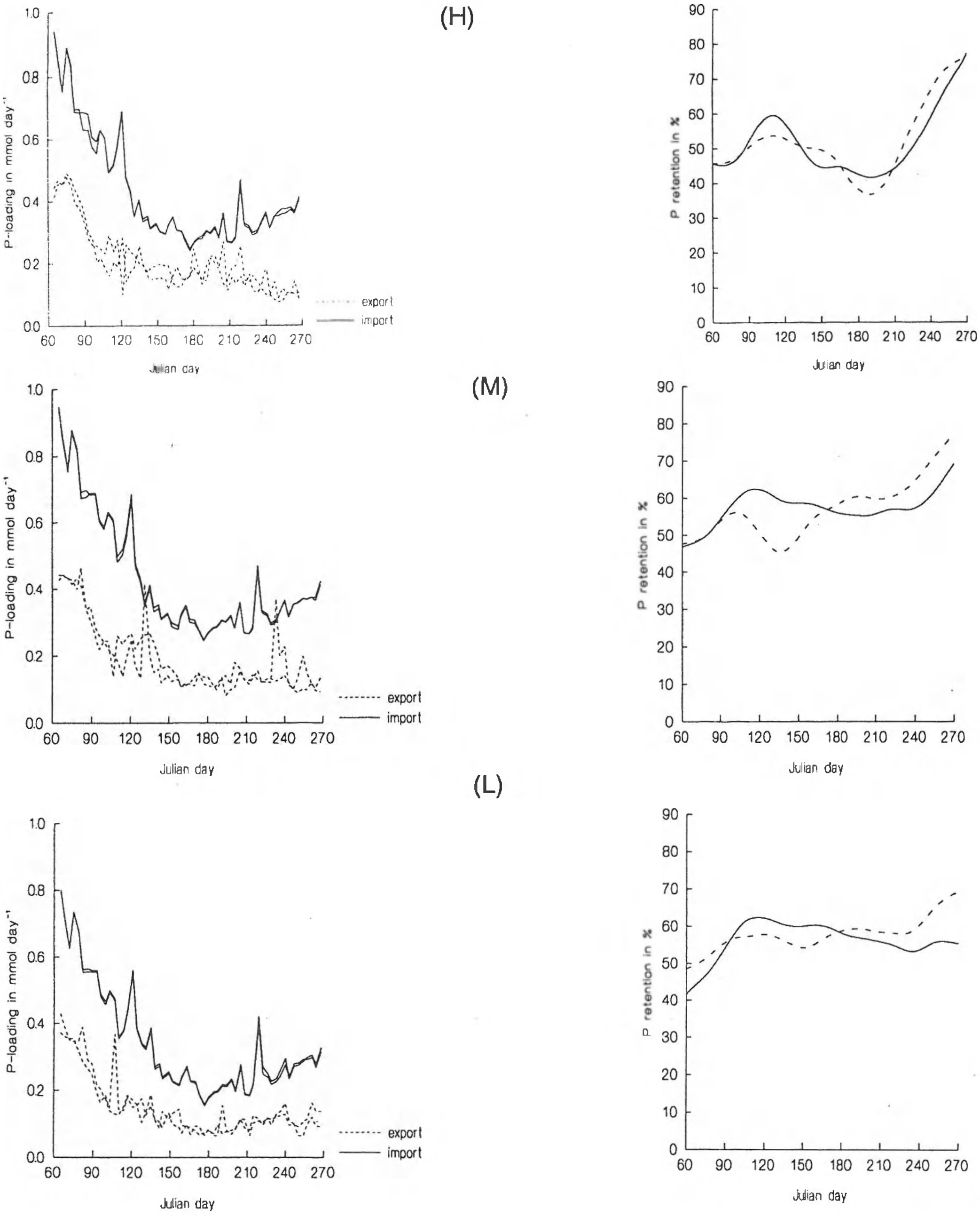


Figure 7.3
Total amounts of particulate N and P accumulated on the sediments of the mesocosms.
Circles: treatment H; squares: treatment M; triangles: treatment L.

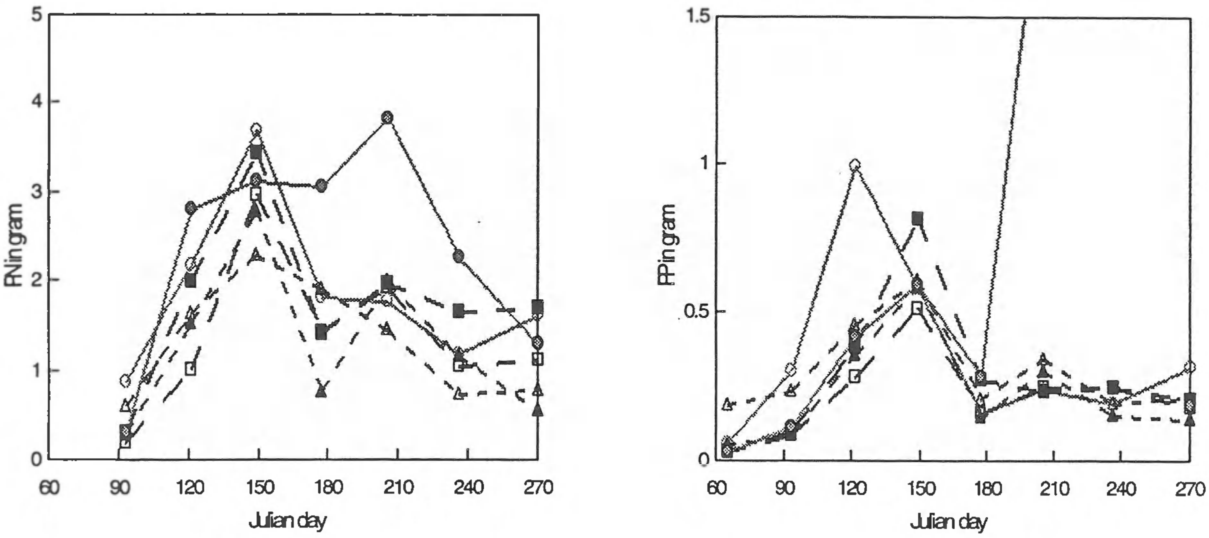
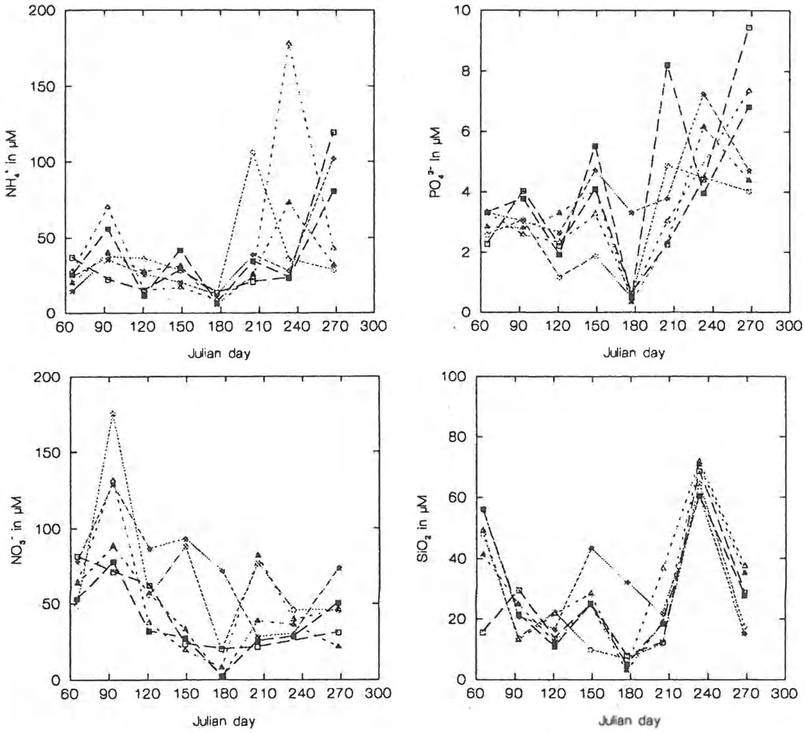


Figure 7.4
Average concentrations of ammonium, phosphate, silicate and nitrate in the porewaters of the sediment of the pelagic systems.
Symbols as in Figure 7.3.



7.3.2 Accumulation of N and P in sediment

Inventories of the amount of N and P accumulated in the sediment as particulate matter or inorganic nutrients were made at 4-week intervals. Results showed an initial increase in the amount of PN and PP in the sediments of all mesocosms (Figure 7.3). Maximum amounts were observed in all mesocosms at day 150. This was after the end of the spring phytoplankton bloom. After day 150, the amounts of PN and PP decreased in all mesocosms, with the exception of mesocosm 6 (treatment H), where PN and PP amounts in the sediment remained high. Very high values of PP were observed in this mesocosm at day 205. This value was caused by anomalously high PP values in the sediment of the mussel chamber. N:P and C:P ratios in this sample were 6-7 times lower than in other observations, and suggest an overestimation of the amount of PP.

Porewater concentrations of most inorganic nutrients showed changes with time, but no differences between treatments (Figure 7.4). Ammonium concentrations showed an increase in all mesocosms, especially in the period after day 150. Similar patterns were observed for phosphate and silicate, with minimum values at day 180, and an increase afterwards. The increases in ammonium, phosphate and silicate concentrations reflected the deposition and mineralization of organic matter on the sediment. Nitrate concentrations in treatment H were significantly higher than in treatment M ($p<0.05$), but there were no differences with treatment L. Nitrate concentrations in the porewater gradually decreased in all mesocosms.

7.3.3 Nutrient export and storage

As was shown in Table 7.1 and Table 7.2, a relatively large part of the imported nutrients was exported again from the mesocosms. An inventory of the nutrients present in water column, sediment and biota was made at day 150 and at the end of the experiment, in order to establish the fate of nutrients imported to the mesocosms. Results showed that 55-67 % of the N imported to the systems had been exported again at day 150. Relatively large fractions of N were stored in detritus on the sediment and in mussel biomass (Figure 7.5). In this inventory,less then 10% of the nitrogen was not accounted for by export or storage in one of the sampled compartments (sediment, water column or mussel biomass).

The results of the inventory at the end of the experiment again showed the predominance of export in the N balance (Figure 7.6). Relatively small amounts of N were recovered in the samples of sediment and the water column. Mussel biomass contained 5-10 % of the nitrogen. A sampling of the biota on the wall and mixer of the mesocosm had shown that the total biomass of the fouling biota was 20-40 g ADW. Assuming Redfield ratios for this biomass, the total amount of N stored in wall biomass was 1.8-3.5 g N, which was 4-14% of the total amount of N imported. Macrobenthos biomass of other species than mussels was relatively small (10-25 % of mussel biomass) except for mesocosm 1, where *Nereis* biomass was comparable to mussel biomass. The inventories, including total biomass of fouling biota and macrobenthos, still showed a deficit in the N balance. An amount of 11-26 % of the imported N was not accounted for by the storage in water column, sediment and wall biota.

Figure 7.5
Percentage of N input lost from the mesocosms by export, or retained by storage in sediment , water column or biomass at day 150.

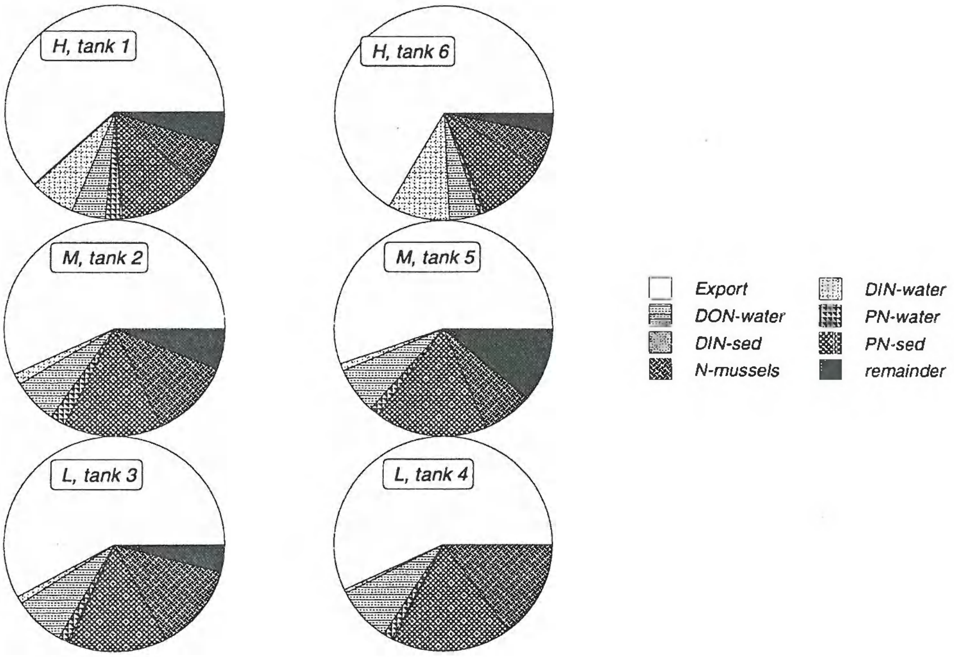
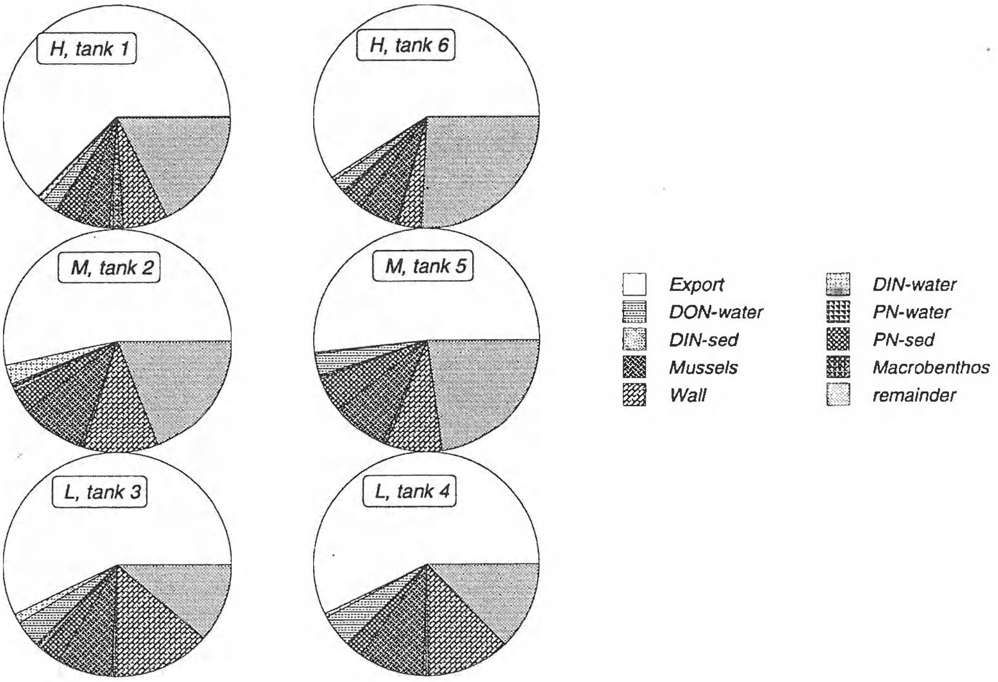


Figure 7.6
Percentage of N input lost from the mesocosms by export, or retained by storage in sediment , water column or biomass at the end of the experiment.



The P inventory at day 150 also showed a predominance of export, although the fraction of P exported (30-43 %) was smaller than in the N balance. A relatively large fraction of P was stored in sediment detritus, and 10-26% of the P imported to the systems was not accounted for by export or storage (Fig. 7.7).
At the end of the experiment, the pattern was comparable to the N inventory, with a large contribution of export, and a significant contribution of wall biota to storage of P in the mesocosms. Of the total amount of imported P 21-39% was not accounted for by storage in sediment, water column or wall biomass (Fig.7.8).
The N:P ratios of the material 'missing' in the inventories at the end of the experiment were high in treatment H (29 and 22), somewhat lower in treatment M (20 and 14) and lowest in treatment L (7 and 8). The total amount of P missing in the budgets was approximately equal in all treatments, whereas the amount of N missing was lowest in treatment L (0.2 mol) and highest in treatment H (0.6-0.9 mol).

7.3.4 External and internal nutrient supply rates and phytoplankton nutrient uptake

We made a comparison between the nitrogen and phosphorus demand by primary producers in the mesocosms, and the external and internal nutrient supply rates. Nutrient demand was estimated from measurements of phytoplankton primary production and system daytime production.
Results showed that in the last two months of the experiment phytoplankton primary production was low compared to total system production in all mesocosms (Figure 7.9). This was due to the development of a high biomass of epipelic algae on the walls of the mesocosms, combined with low levels of phytoplankton biomass in the water column. Nutrient demand by the algae exceeded the nutrient supply rates in the period of the spring bloom, and this resulted in a depletion of the DIN pool in the water column (see Chapter 3). During the summer, nutrient supply through regeneration in the water column was a substantial source of inorganic nitrogen. The contribution of regeneration of ammonium by the benthos was relatively limited. Nitrogen regeneration accounted for approximately 50% of the N supply in the systems and the data suggest that system production was largely sustained by remineralization of N in the pelagic system
Similar observations were made for the uptake and supply of phosphorus in the mesocosms (Fig. 7.10). P demand by the phytoplankton exceeded P supply rates in spring. In summer regeneration of P in the water column was a major source of P and supplied 30-50% of the algal P demand. P regeneration by the benthos was relatively small. Again, there was an approximate balance between P demand and P supply through external loading and internal regeneration.

Figure 7.7
Percentage of P input lost from the mesocosms by export, or retained by storage in sediment , water column or biomass at day 150.

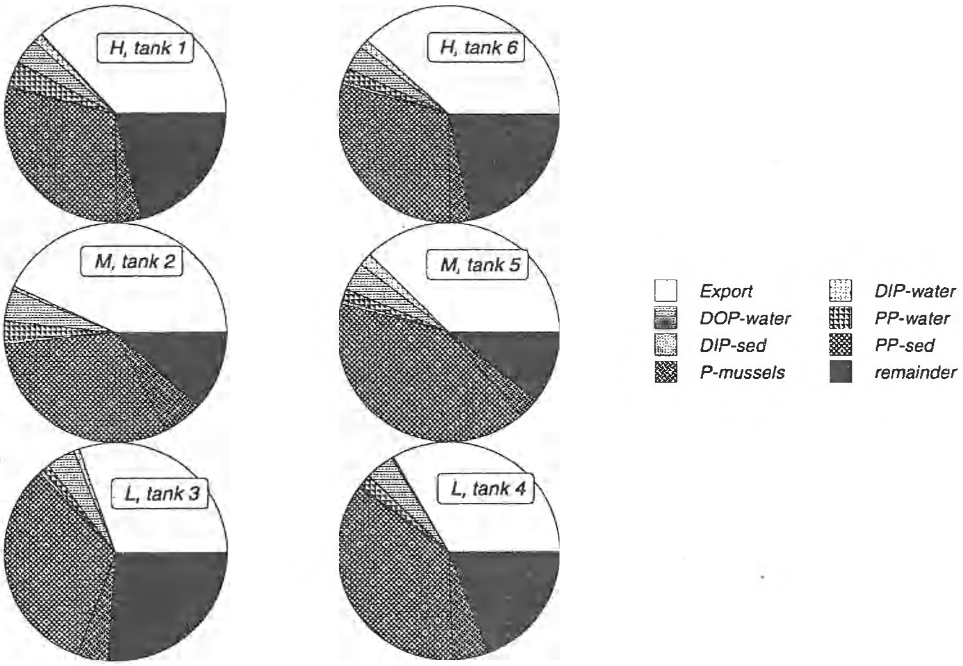


Figure 7.8
Percentage of P input lost from the mesocosms by export, or retained by storage in sediment , water column or biomass at the end of the experiment.

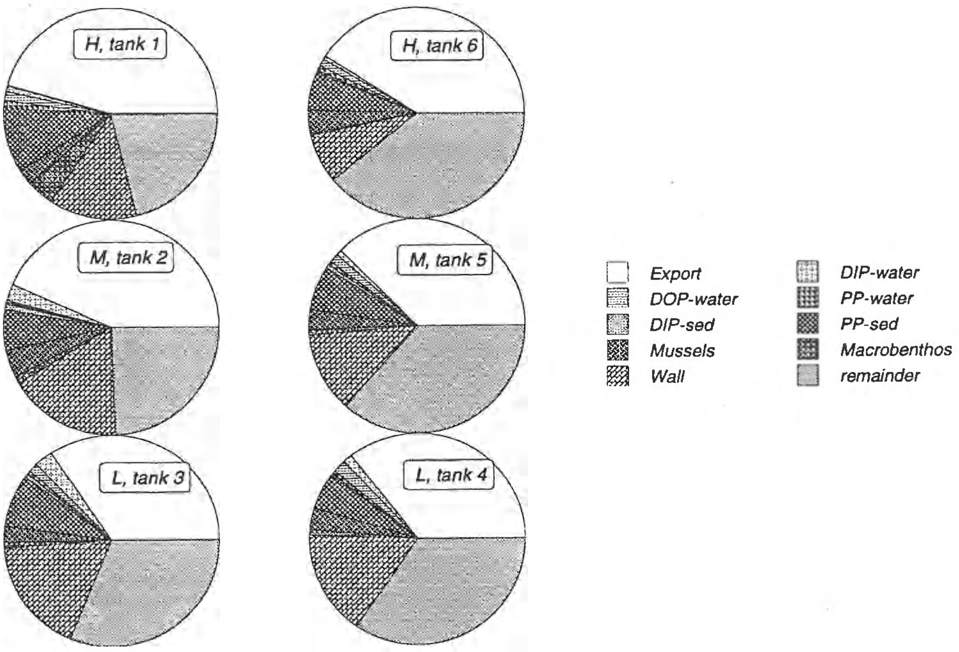


Figure 7.9
Rates of N-demand, external N-loading and N-regeneration. The gray line represents N-demand by all primary producers, estimated from daytime system production. The bars show N-demand by the phytoplankton, estimated from ^{14}C-incubations. The dotted line shows the rate of external N-loading, the broken line shows the sum of external loading and water column regeneration, and the full line shows the sum of external loading, water column regeneration and benthic NH_4^+ regeneration. During the first 4 weeks of the experiments no O_2 measurements were carried out.

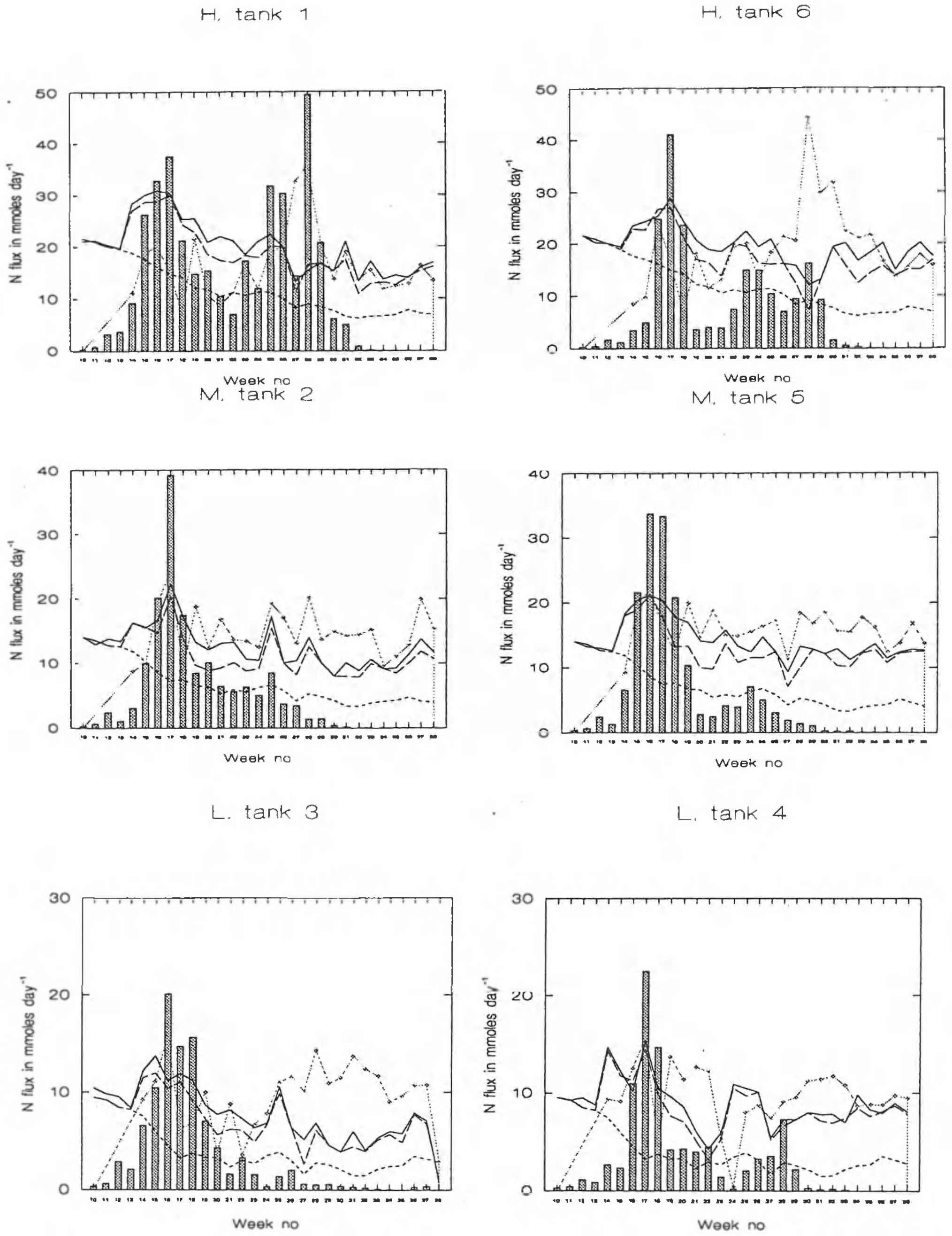
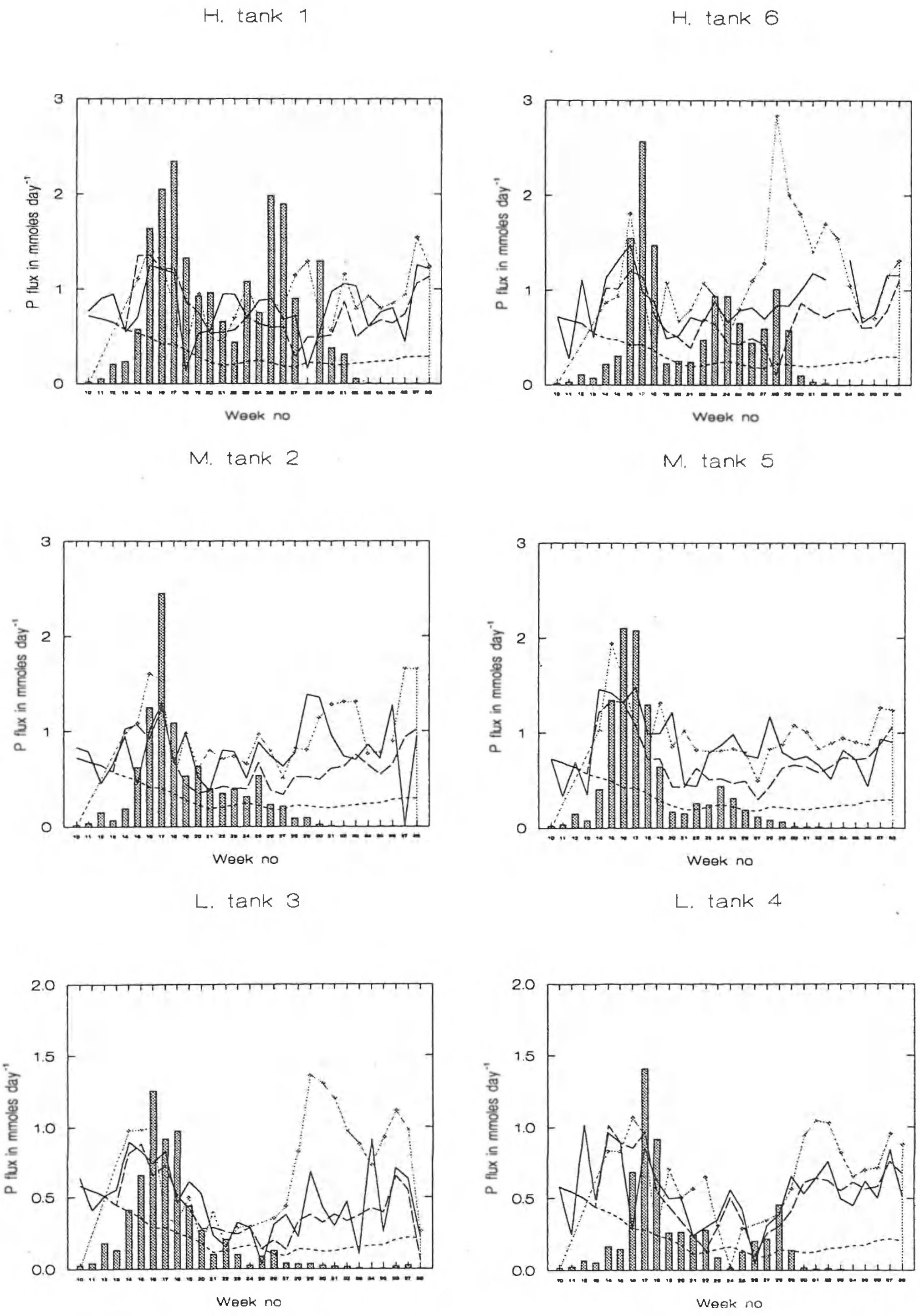


Figure 7.10
Rates of P-demand, external P-loading and P-regeneration in treatment H. The gray line represents P-demand by all primary producers, estimated from daytime system production. The bars show P-demand by the phytoplankton, estimated from ^{14}C-incubations. The dotted line shows the rate of external P-loading, the broken line shows the sum of external loading and water column regeneration, and the full line shows the sum of external loading, water column regeneration and benthic PO_4^{3-} regeneration. During the first 4 weeks of the experiments no O_2 measurements were carried out.


H. tank 1

H. tank 6

M. tank 2

M. tank 5

L. tank 3

L. tank 4

7.4 Discussion

7.4.1 Nitrogen balances

Comparison of import and export fluxes of nitrogen indicated that the retention of N was low in the first month of the experiment. With the onset of the phytoplankton bloom (after Julian day 90) nitrogen retention started to increase, suggesting a link with biological processes. Nitrogen balances at day 150 showed that the major fraction of the nitrogen, imported to the system, was exported again. Of the fraction retained in the mesocosms, a major fraction was stored in sediment detritus or macrobenthos. At this time, storage in the sediment was high as a result of sedimentation of the spring phytoplankton bloom. The fraction that was retained by the mesocosms but was not accounted for in the inventory, was relatively small, showing that all major N pools were sampled. During summer, N retention remained high or even increased more. The inventory of the N pools at the end of the experiment showed that the amount of N in sediment detritus had decreased as a consequence of mineralization processes (Fig. 7.3), resulting partly in an increase of porewater ammonium (Fig. 7.4). Wall biota formed a significant N pool by the end of the experiment. This pool was not sampled at day 150, but the small deficits in the N balances at day 150 show that 'wall' biomass was unimportant during the first months of the experiment.

A significant amount of N was missing in the balances at the end of the experiment. The deficits in the N balances showed a positive relation with external nutrient loading. Although the retention of nitrogen, averaged over the experiment, did not show a clear relation with external loading, a comparison of the retention by the mesocosms during summer, showed that retention tended to increase to values above 60% in the H treatment, whereas retention remained at a constant level in the other treatments. Similar patterns of increasing retention with increasing N-loading were observed in the short term experiments of 1993 and 1994 (Prins et al., 1994; 1995). Denitrification is a likely cause for the disappearance of some of the N in the mesocosms. This is supported by the observation that the deficits occurred in summer, after the collapse of the spring bloom and the accumulation of organic material in the sediment. Denitrification increases with temperature, and has shown a positive relation with N loading in the MERL experiments (Seitzinger & Nixon, 1985). The deficits in the H treatment were 0.6-0.9 mol, which would equal a loss rate of ± 5-7.5 mmol day^{-1} assuming that the losses occurred during the summer period. Observed benthic denitrification rates under comparable external nutrient loadings (up to 250 μmol m^{-2} h^{-1}; Seitzinger, 1988; Kamp-Nielsen, 1992) could explain the calculated N loss. In addition, there may have been some denitrification in the water column or on the wall.

7.4.2 Phosphorus balances

Retention of P by the mesocosms showed much less seasonal variation than N retention. There was a slight increase in P retention with the start of the phytoplankton bloom. Also, an increase was apparent during the last month of the experiment, but overall P retention was at a relatively high level during the entire experiment. Phosphorus balances at day 150 showed that a major fraction of P was stored in sediment detritus. This fraction of P was much larger than the analogous fraction in the N balances. In addition, the balances showed a large deficit. At the end of the experiment, the deficits in the P balances were even larger, with 21-39% of the P unaccounted for by the inventories of the various P pools. Relatively large deficits in the P balances were observed in the previous experiments too (Prins et al., 1994; 1995). It was assumed that storage of P in the sediment, as a consequence of sorption of phosphate on sediment particles under aerobic conditions (Froelich, 1988; Sundby et al., 1992), was a major cause of these deficits. This fraction of the total P pool was probably not determined by the analyses of particulate P accumulated on the surface of the sediment and phosphate in the porewater.

7.4.3 Nutrient recycling

The comparison of external and internal nutrient supply rates with nutrient demand, estimated from productivity measurements, largely corresponds with the observed trends in inorganic nutrient concentrations in the water column. In spring, with the onset of the phytoplankton bloom, a sharp decrease in phosphate concentrations was observed (Chapter 3). This corresponds with the observations (Fig. 7.10) of a high P-uptake, exceeding P-supply. In summer, P-supply and P-demand approximately seemed to be equal, which suggests that phosphate concentrations in the water column remained low as a consequence of the balance between sources and sinks. In spring, the N-demand in the H and M treatments was approximately equal to the N-supply (Fig. 7.9), which is in accordance with the observation of a slower decrease in DIN concentrations in the water column, compared to the trends for phosphate. During the summer period, N-demand was consistently higher than N-supply, resulting in a depletion of DIN in all mesocosms (Chaptyer 3).

The comparisons also show, that during the course of the season, internal regeneration of inorganic nutrients becomes increasingly important as the nutrient source to sustain primary production. A large part of the nutrient regeneration occurs in the water column, as is also apparent from the comparison of benthic and pelagic respiration rates (Chapter 8). Finally, it is remarkable that, in spite of an excess supply of inorganic N (N:P ratio in external loading in all treatments > 16), N gets depleted in all treatments during summer, resulting in a shift from P-limitation in spring to N-limitation in summer. There are 3 major factors which control whether N or P will be limiting (Howarth, 1988): 1) the ratio of N:P in the external inputs; 2) the preferential loss of N or P from the water column by processes like sedimentation, denitrifcation or adsorption; 3) the extent to which N fixation makes up for denitrification losses.

The ratio of N:P in the external supply are higher than the Redfield ratio of 16, both when calculated for DIN:DIP or for Total N: Total P. The ratios even increase during summer, indicating that the shift from P to N limitation in summer is not caused by relatively low N in the external supply. The potentially significant loss of inorganic P to the sediment as a result of sorption processes (see above) could theoretically result in an even enhanced P-depletion, but apparantly is of minor importance compared to sinks in the N-budgets. As was pointed out above, deficits in the N balances occurred mainly during summer, and it was suggested that this was caused by denitrification. The N:P ratios of the deficits in the nutrient balances were higher than 16 in the H and M treatment, showing a preferential loss of N. In the L treatment the N:P ratio of the deficit was less than 16, suggesting a preferential loss of P. However, when the deficits were caused by denitrification and sorption of phosphate, as discussed above, N was really lost from the system, whereas P was just not determined in the inventory but may still have been (at least partly) biologically available.

Concluding, the shift from P limitation in spring to N limitation in summer may have been determined by preferential N loss as a consequence of denitrifcation, counteracting the effect of N excess in the external supply. In addition, in Chesapeake Bay it has been shown by Malone et al. (1996) that N limitation occurs at N:P ratios <40, which is much higher than the commonly used Redfield ratio, and it has been suggested that additional P pools may be available as a result of exchanges between internal pools (particulate and dissolved, organic and inorganic).

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8 System metabolism and productivity as a function of nutrient loading and benthic activity in an experimental marine ecosystem

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ABSTRACT

To adress the question of potential changes in productivity of the Dutch coastal zone of the North Sea, as a consequence of reductions in anthropogenic nutrient loadings, a number of experiments were carried in land-based mesocosms. The mesocosms have a volume of 3 m^3, a pelagic and a benthic compartment, and mimick the physical conditions in the Dutch coastal zone off Noordwijk. Production and respiration processes in the pelagic and the benthic system were measured by following diel changes in the O_2 concentrations in the water column.
In a number of short term experiments, pelagic respiration rates showed a close coupling to pelagic primary production rates. Benthic respiration was relatively low, as a consequence of the fact that the experiments started with an azoic sediment and the time span of the experiments was only 4 weeks. Addition of mussels mainly resulted in a reduction of phytoplankton biomass and pelagic production and respiration rates, without effect on benthic respiration.
In a 7 month experiment, carried out in 1995, 3 different nutrient loading scenarios were applied, representing the present condition in the Dutch coastal zone (N and P loadings in 1995 being equal to respectively 90% and 50% of the loadings in 1985), the situation after implementation of a 50% reduction in anthropogenic N- and P-loading, and a more extreme situation with a 75% reduction in N- and P-loading. Again, pelagic production and respiration were closely coupled, and showed a positive relation with external N-loading. It was estimated that 44% of net pelagic daytime production was respired in the water column during the night. Estimates suggested that algal respiration and respiration by the microbial food web dominated pelagic respiration. Benthic respiration was not affected by differences in external nutrient loading. Although net system production showed a positive relation with external nutrient loading, the effect of reductions in nutrient loading to the Dutch coastal zone on net system production are much smaller than the effects on primary production.

8.1 INTRODUCTION

Measurements of oxygen production and respiration in well-defined bodies of water can provide information on the balance between system production and respiration. Eutrophication in coastal marine ecosystems has led to increased phytoplankton growth, and measurements of system metabolism have shown this increased pelagic production may be counteracted only partly by increases in pelagic and benthic respiration (Oviatt et al., 1986). Studies have shown a strong coupling between production and respiration in the pelagic system (Jensen et al. 1990; Kemp et al. 1992; Smith and Kemp 1995), whereas benthic respiration often shows no, or a delayed response to increased production in the water column (Kemp et al. 1992; Oviatt et al. 1986; Rudnick and Oviatt 1986). The final balance between system production and respiration, being net system production, depends on factors affecting the level of primary production (a.o. external nutrient loading), and on factors determining the total rate of respiration. The extent to which pelagic respiration increases with increasing phytoplankton production, and the effect of the benthos on the pelagic system, will therefore be of major importance in determining if a system is net autotrophic or net heterotrophic (Oviatt et al. 1993; Heip et al. 1995). Net system production is a relevant parameter as changes in net system production due to environmental changes, like changes in nutrient loading, may reflect the potential effects on secondary production.

In the framework of a research project studying the effects of reductions in nutrient loading to the North Sea on primary production and the productivity at the secondary trophic level (Peeters et al. 1993), we have carried out a number of studies in experimental marine ecosystems. Experiments were carried out to study the relationships between changes in phosphate and nitrate loading on phytoplankton production and composition (Escaravage et al. 1995a; b; 1996) and the interactions between grazing and phytoplankton production (Prins et al. 1995b; Prins et al. 1995a). A number of short term (4 weeks) experiments were carried out in 1993 and 1994, followed by a long-term (7 months) experiment in 1995. In most of these experiments measurements of system metabolism were carried out by measurements of diel changes in O_2. The benthic community was manipulated in some of the short-term experiments by addition of mussels, and the development of the benthic community was also influenced by the duration of the experiments, thus providing the opportunity to compare systems with a low to systems with a high benthic activity. The long term experiment was designed to give information on the relations between external nutrient loading, phytoplankton production and pelagic and benthic respiration, and to establish the relation between nutrient loading and net system production. Similar types of experiments have been carried out in experimental marine ecosystems, applying a range of nutrient loadings varying by a factor of 32 (Oviatt et al. 1986). In our long term study we applied a much smaller range of nutrient loadings, that represented an environmentally realistic simulation of the conditions in the Dutch coastal zone of the North Sea. The experiment was designed to simulate three different conditions: the actual nutrient loading to the

Dutch coastal zone in 1995 (N and P loadings in 1995 being equal to respectively 90% and 50% of the loadings in 1985), the situation after implementation of nutrient reduction programmes that should lead to a reduction of the anthropogenic N and P loading to 50% of the loadings in 1985, and a case study of a more extreme sanitation programme with a reduction of anthropogenic N and P loading to 25% of the rates in 1985.

8.2 MATERIAL AND METHODS

8.2.1 Experimental design

In 1993 and 1994 a series of short-term experiments was carried out with manipulation of the external nitrate or phosphate loading as experimental treatment, combined with low densities of the bivalve suspension feeder *Mytilus edulis* in each mesocosm. In another series of experiments external nutrient loadings were kept at a high level, and bivalve density was varied between treatments. Sediments in the mesocosms consisted of clean, azoic sand with a median grain size of 210 μm and an initial organic content of 0.16 %. Table 8.1 shows the main characteristics of the experiments.
In all these experiments, the mesocosms were filled simultaneously on the evening before measurements started, with water pumped directly from the Oosterschelde estuary. The next morning the water column was sampled to determine the initial concentrations of particulate and dissolved substances and phytoplankton. A continuous addition of inorganic nutrients was started ($NaNO_3$, NaH_2PO_4, Na_2SiO_3). The mesocosms were continuously flushed with seawater at a rate of 100 l day^{-1}, resulting in a residence time of the water of 30 days. The mussels were collected in the field one week before the experiment, near the low-water level at a site close to the field station. The mussels were added to one of the benthos chambers of each mesocosm, the second benthos chamber of each mesocosm served as a control.
Experimental design used in 1995 and analytical methods are extensively described in chapter 2. For comparison with previous experiments, average N,P -loadings and mussel biomass used in 1995 are given in Table 8.1. Treatments in 1995 experiment were encoded with letters L, M and H corresponding to low, medium and high nitrogen load.
At the end of the experiments, the particulate organic material accumulated at the bottom of the pelagic tank and in the benthos chambers was sampled and analysed for POC. Mussel biomass at the start and the end of the experiments was measured as ash-free dry weight.

Table 8.1
Description of the main characteristics of the experiments. In 1995 nutrient loading was varied seasonally. Shown here is the average loading.

Period, duration	Treatment		Nitrate loading mmol m^{-2} d^{-1}	Phosphate loading mmol m^{-2} d^{-1}	Mussel biomass g ADW m^{-3}
June-July 1993 4 weeks	P-loading		12.3	0.09 to 0.69	0.7
August-September 1993 4 weeks	mussel biomass		11.0	0.69	0.6 to 4.4
May-June 1994 4 weeks	N-loading		5.9 to 11.7	0.63	0.6
June-July 1994 4 weeks	N-loading		9.3 to 16.4	0.81	0.5
August-September 1994 4 weeks	mussel biomass		8.6	1.11	0.7 to 5.9
March-September 1995 7 months	N and P loading	L:	4.0	0.22	0.6
		M:	6.9	0.22	0.6
		H:	11.7	0.31	0.6

8.2.2 System metabolism and production

Continuously measured oxygen data were used to estimate system metabolic rates and productivity. The O_2 diffusion flux from or to the atmosphere was calculated from an empirical model (Equation 1) derived from previous measurements in our mesocosms (Prins et al., 1995c). In the latter measurements the mesocosm water column was made anaerobic by addition of Na_2SO_3 and the increase in O_2 was monitored. These measurements were performed for different water temperatures.

$$D = 0.03 * 1.127^{(T-20)} * [O_2]_{dev}^{1.296} \qquad (1)$$

with D = O_2 diffusion in g O_2 m^{-2} h^{-1}
T = Water temperature in °C
$[O_2]_{dev}$ = Deviation of actual O_2 concentration from O_2 concentration at 100% saturation in g O_2 m^{-3}

For undersaturated water D was negative (directed into the water column), for supersaturated water D was positive.

Net daytime production of the pelagic system (NDP) was calculated from the change in oxygen concentration from dawn to dusk, with a correction for benthic respiration and exchange with the atmosphere during the day:

$$NDP = ([O_2]_{dusk}-[O_2]_{dawn}) * V + D + BR_{day} \quad \text{in g } O_2 \text{ } m^{-2} \text{ } d^{-1} \qquad (2)$$

with V = volume mesocosm in m^3
Br_{day} = benthic respiration during daytime in g O_2 m^{-2} d^{-1}

From this calculation follows that net daytime production represents net community production (Williams 1993) for the water column during daytime, and is equal to gross primary production minus algal respiration and respiration by heterotrophs in the water column. NDP also includes production and respiration by wall biota.
Oxygen uptake by the benthos in the separate benthos chambers was calculated from the differences in O_2 concentrations between the inflow and the outflow of the chambers. Oxygen uptake by the sediment in the pelagic tanks was measured by core incubations. Two cores of the sediment of each tank were collected by SCUBA-diving in May, July and September 1995. Cores (ϕ19.6 cm^2) containing approximately 15 cm sediment and 15 cm filtered seawater were incubated for 2-3 hours in a flow-through system, and oxygen decrease was measured during this period.
The results from the core incubations were used to establish a relation between the sediment respiration rate (R), the sediment carbon content (POC) and the temperature (T):

$$R = 0.564 * e^{0.11*(T-20)} * [POC]^{0.289} \quad (r^2=0.564, n=30, p<0.001) \quad \mathbf{(3)}$$

This model was used for all experiments to produce estimates of mesocosm sediment respiration based on sediment POC concentration and water temperature. Estimates of benthic respiration rates over the entire experimental period were obtained by interpolation.

Night time pelagic respiration (NPR) was calculated from the decrease in oxygen concentration from dusk to the following dawn, after correction for benthic respiration and exchange with the atmosphere:

$$NPR = ([O_2]_{dawn}-[O_2]_{dusk}) * V - D - BR_{night} \quad \text{in g } O_2\ m^{-2}\ d^{-1} \quad \mathbf{(4)}$$

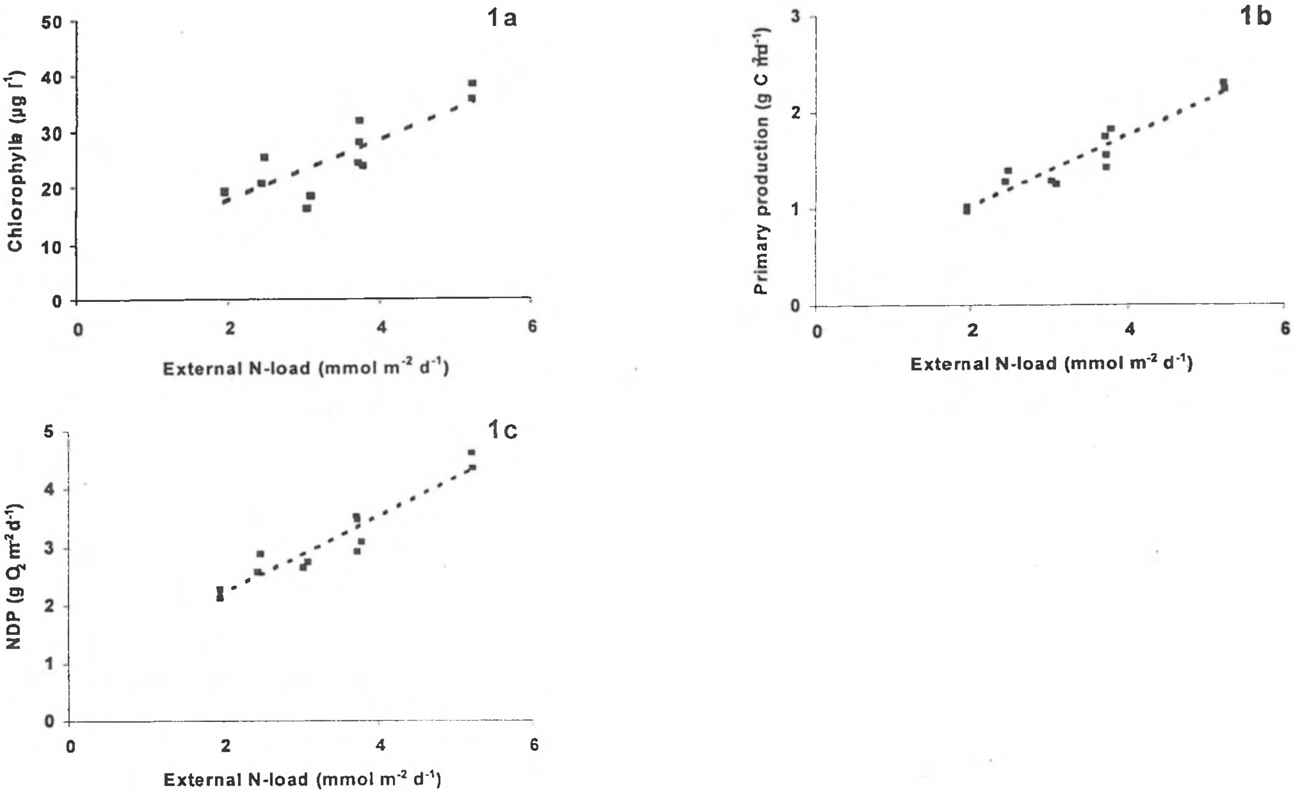
with Br_{night} = benthic respiration during night time in g O_2 m^{-2} d^{-1}

Net system production NSP was equivalent to the net increase in O_2 over 24 hours after correction for exchange with the atmosphere, and calculated as:

$$NSP = NDP-BR - NPR \quad \text{in g } O_2\ m^{-2}\ d^{-1} \quad \mathbf{(5)}$$

In the long-term experiment of 1995, net system production was also determined by assessing the net production of organic carbon over the 7 month experimental period. The change in concentrations of particulate organic carbon (POC) and dissolved organic carbon (DOC) in the water column between the start and the end of the experiment was calculated from water column samples. Inventories of the amount of POC in the sediment at the start and end of the experiment (triplicate samples) were used to asses the accumulation of organic carbon at the bottom of the pelagic system and in the benthos chambers. The amount of organic carbon stored in mussel biomass was calculated from the increase in ash-free dry weight (ADW) during the experiment, assuming a carbon content equal to 50% of ADW (Hawkins & Bayne, 1985). Import of POC and DOC into the systems was estimated from samples (2 $week^{-1}$) of the seawater pumped to the mesocosms. Export

Figure 8.1
Mean chlorophyll-*a* (1a), phytoplankton primary production (^{14}C) (1b) and net daytime production (1c) as a function of external N-loading, in the experiments of May-June 1994 and June-July 1994.



was calculated from water column concentrations of POC and DOC and the water renewal rate.

8.3 RESULTS

8.3.1 Experiments 1993 and 1994

The development of phytoplankton biomass in the 4-week experiments is a function of nutrient loading, irradiance and mussel grazing (Escaravage et al. 1995b; 1996; Prins et al. 1995b). As an example, the results of the two N-loading experiments, carried out between May and July 1994, are shown (Figure 8.1).

Average chlorophyll-a concentrations, primary production measured by ^{14}C incubations and net daytime production all increased with increased external N-loading (Figure 8.1-a,-b,-c). Furthermore net daytime production showed a strong correlation with primary production estimates from ^{14}C incubations ($r^2=0.91$, $n=26$) and night time pelagic respiration rates (NPR) correlated with chlorophyll-*a* and with NDP values. On a day-to-day basis the correlation for all experiments was significant, but showed a large unexplained variance. However, with average values per mesocosm and per experiment, NPR as well as

Table 8.2
Pearson correlation matrix of chlorophyll-*a*, net pelagic daytime production (NDP), night time pelagic respiration (NPR) and net bacterial production (BP), based on mean values per mesocosm for the experiments of 1993 and 1994. All correlation coefficients were significant at $p<0.001$ ($n=26$).

	Chl-a	NDP	NPR
NDP	0.913		
NPR	0.897	0.948	
BP	0.709	0.877	0.89

chlorophyll-a levels and NDP showed a highly significant correlation (Table 8.2).
Figure 8.2 shows the relation between chlorophyll-*a*, pelagic production and respiration rates.The geometric mean estimate for the slope between NPR and NDP was 0.42 ± 0.06 (mean ± 95% confidence limits; Ricker 1973). Bacterial production also correlated with chlorophyll-*a*, NDP and NPR (Table 8.2).
A conservative estimate of bacterial respiration rates was calculated from bacterial production, assuming a net growth efficiency of 50% and a respiratory quotient (RQ) of 1.0 (Figure 8.3). A high estimate was made using a lower net growth efficiency (33%) and a lower RQ (0.8). Compared to measured pelagic respiration rates, bacterial respiration rates were relatively small. The estimated contribution of bacterial respiration to total pelagic respiration was between 5 and 14%.

Figure 8.2
Rates of night time pelagic respiration in relation to chlorophyll-*a* (2a) and net daytime production (2b) in the experiments of 1993 and 1994. Triangles: mesocosms with mussel biomass < 1 g ADW m^{-3}; squares: mesocosms with mussel biomass > 1 g ADW m^{-3}. Dotted line in Fig.b shows $y=x$.

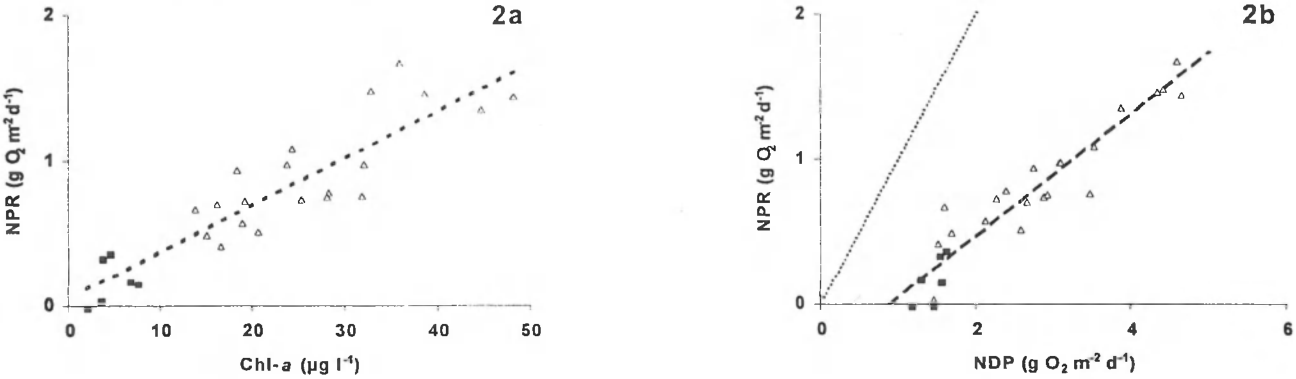
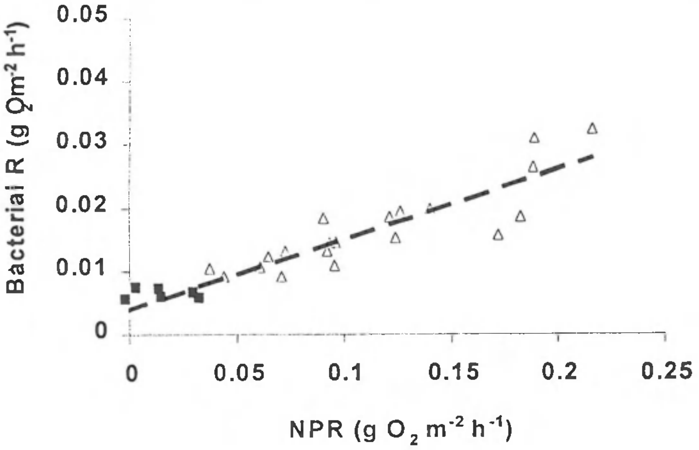
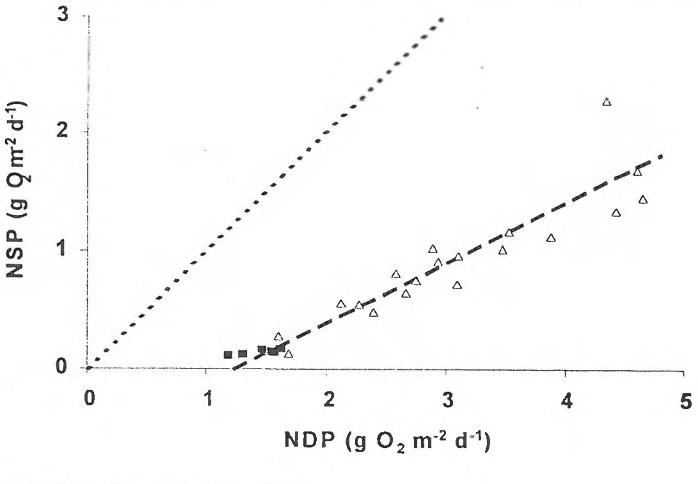


Figure 8.3
Estimated contribution of bacterial respiration to total pelagic respiration rates in the experiments of 1993 and 1994. Bacterial production was converted to oxygen uptake rates assuming a net growth efficiency of 50% and a respiratory quotient (RQ) of 1. Triangles: mesocosms with mussel biomass < 1 g ADW m^{-3}; squares: mesocosms with mussel biomass > 1 g ADW m^{-3}.



Benthic respiration was much smaller than pelagic respiration in the experiments with low mussel biomass, and there was only a weak correlation between benthic respiration and water column production. ($r^2=0.32$, $n=20$, $p<0.01$). In the experiments with increased mussel biomass, benthic respiration formed a major fraction of total respiration rates in the mesocosms (circa 70%) in contrast to the mesocosms with low mussel biomass (circa 30%). Benthic respiration was not correlated with NDP ($r^2=0.07$, $n=6$, $p>0.05$). Respiration rates of the mussels were calculated from an allometric relation describing respiration rate as a function of body weight (Smaal et al. 1996). The contribution of mussel respiration to total benthic respiration increased from less than 10% in the mesocosms with low mussel biomass (<1 g ADW m^{-3}) to approximately 35% in the mesocosms with higher mussel biomass (>1 g ADW m^{-3}).

Figure 8.4
Net system production in relation to NDP for experiments carried out in 1993 and 1994. Triangles: mesocosms with mussel biomass < 1 g ADW m^{-3}; squares: mesocosms with mussel biomass > 1 g ADW m^{-3}.



Net system production increased with increasing pelagic production. In the experiments with increased mussel biomass, pelagic production was relatively low as a consequence of reductions in phytoplankton biomass caused by mussel grazing. Net system production was also reduced (Figure 8.4).

8.3.2 Experiment 1995

In the 1995 experiment, average chlorophyll-a concentrations, primary production measured by ^{14}C incubations and net daytime production all increased with increased external N-loading (Figure 8.5). ANOVA showed that chlorophyll-*a*, ^{14}C-production and NDP were significantly higher in treatments H and M than in treatment L.
Pelagic respiration rates again showed a strong correlation with NDP (Figure 8.6) ($r^2=0.69$, $n=6$, $p<0.05$). The slope of the regression of NPR on NDP was not significantly different from the results of the short term experiments shown above. The common slope of NPR on NDP for all years together was 0.44 ± 0.057. The results showed a seasonal variation in the proportion of pelagic production that was respired, with relatively high pelagic respiration in April and September and no significant differences between treatments (Figure 8.7).

Figure 8.5
Mean chlorophyll-*a* (5a), ^{14}C primary production (5b) and net pelagic daytime production (5c) as a function of external N-loading in the 1995 experiment.

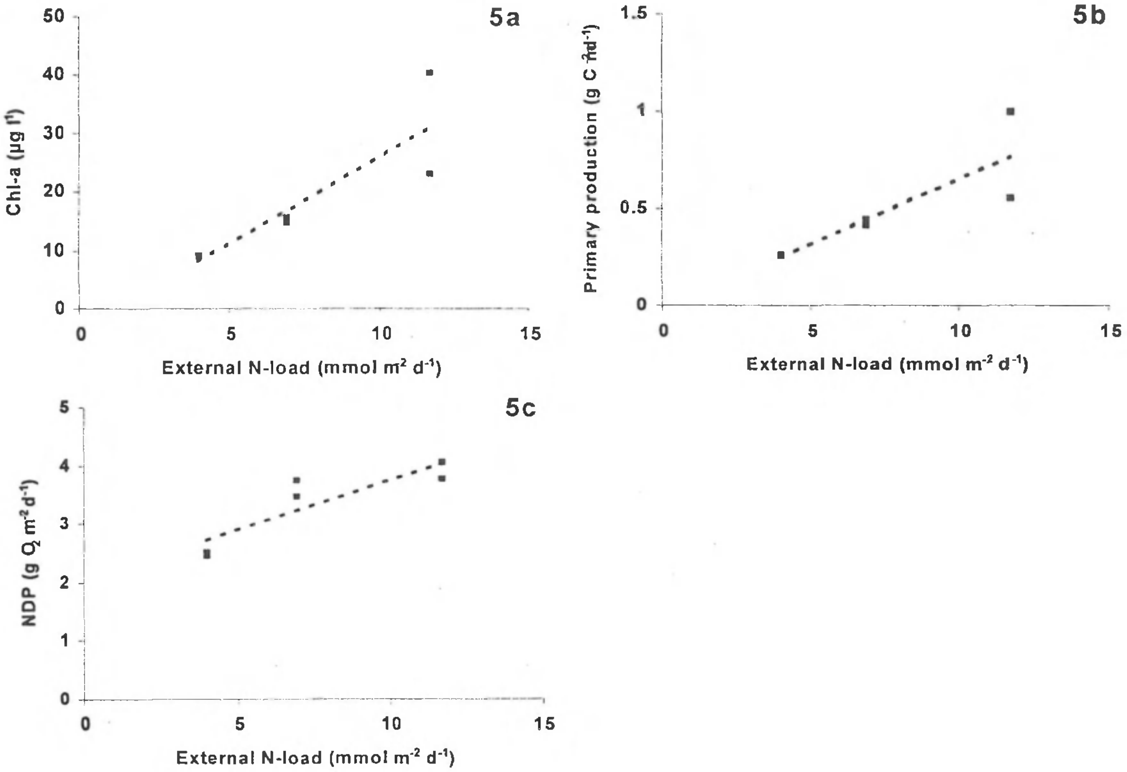


Figure 8.6
Rates of night time pelagic respiration in relation to net daytime production. Black circles: experiment of 1995; grey triangles: experiments of 1993 and 1994.

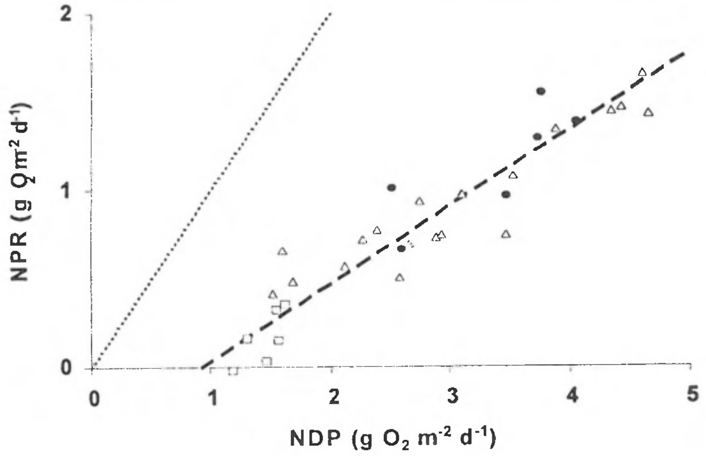
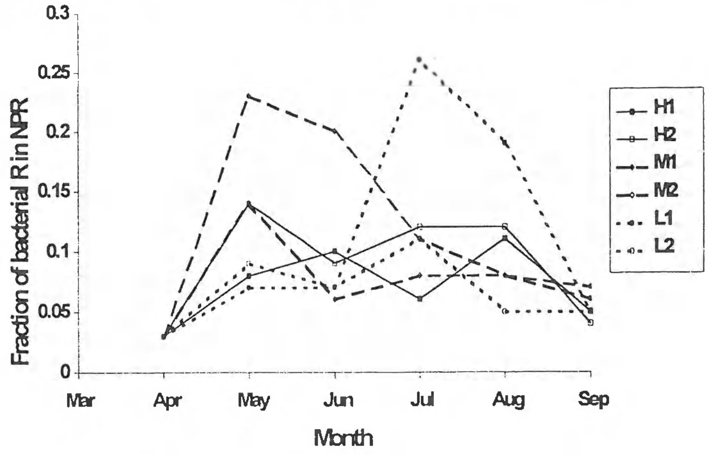


Figure 8.7
Seasonal variation in contribution of bacterial respiration to pelagic respiration rates in 1995. Bacterial production was converted to oxygen uptake rates assuming a net growth efficiency of 50% and a Respiratory Quotient (RQ) of 1.



Phytoplankton respiration is the sum of maintenance respiration and a variable fraction related to gross photosynthesis (Langdon 1993). Minimum and maximum estimates of the contribution of phytoplankton to pelagic respiration were made, using values of 0.003 and 0.018 μmol O_2 (mg chl)$^{-1}$ h^{-1} for maintenance respiration and a fraction of 0.06 for production related dark respiration (Langdon 1993; Kromkamp and Peene 1995). It was assumed that phytoplankton respiration rates in the light were equal to dark respiration rates. As a substantial growth of algae on the walls of the mesocosms was observed in August and September, respiration by wall biota may have formed a significant (but not easily quantifiable) fraction of the pelagic respiration in this period. Therefore, the contribution of phytoplankton respiration to pelagic respiration was estimated for the period April-July. According to these estimates, phytoplankton respiration was a substantial part of pelagic respiration (Table 8.3).

Table 8.3
Estimated contribution of phytoplankton respiration and bacterial respiration to total pelagic respiration in 1995 (average for entire experiment). Minimum and maximum estimates of phytoplankton respiration were made using different coeffcient for the relation between respiration and phytoplankton biomass. Minimum and maximum estimates of bacterial respiration were made using different values for net growth efficiency and RQ (see text).

Mesocosm	Phytoplankton R in % of total pelagic R	Bacterial R in % of total pelagic R
H1	29-77	7-17
H2	20-50	14-35
M1	14-37	11-27
M2	11-28	8-19
L1	14-36	8-19
L2	20-52	10-24

Averaged over the entire experiment, bacterial production increased with increasing NDP ($r^2=0.96$, $n=6$, $p<0.001$).

An estimate of the contribution of bacterial respiration to total pelagic respiration showed that bacterial respiration was a relatively constant fraction of NPR during most of the months, but occasionally higher values were observed (Figure 8.7). Again, estimates of the contribution of bacterial respiration were made using extreme values for net growth efficiency and RQ. The calculations showed that bacterial respiration could account for approximately 10 to 30% of pelagic respiration (Table 8.3).

Respiration in the benthic chambers accounted for the major part of benthic respiration (70-85%). An estimate of mussel respiration rates, using coefficients from Smaal et al. (1996) showed that mussel respiration was less than 5% of total benthic respiration. Benthic respiration was low in spring, and increased during summer to values equal to approximately 50% of total system respiration (Figure 8.8). Averaged over the entire experiment, benthic respiration showed no correlation with pelagic production.

Figure 8.8
Seasonal variation in contribution of benthic respiration to system respiration rates in 1995.

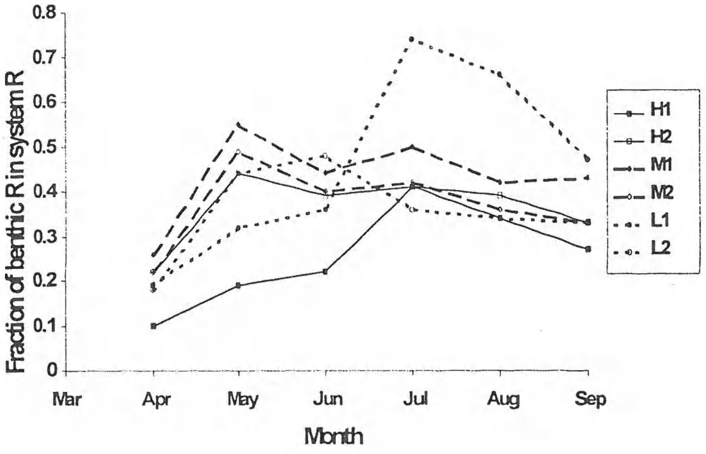
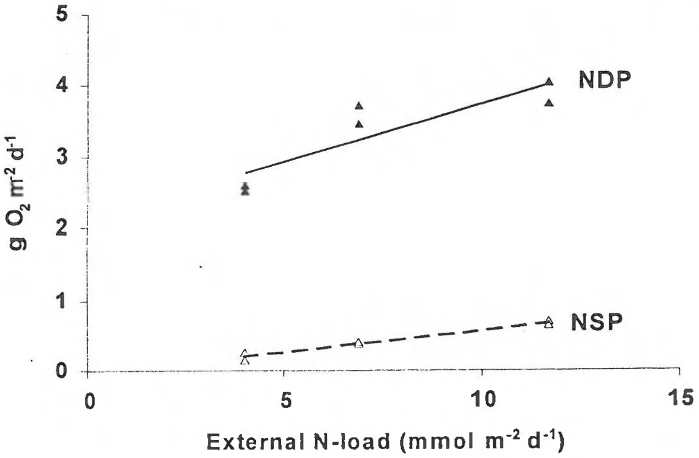
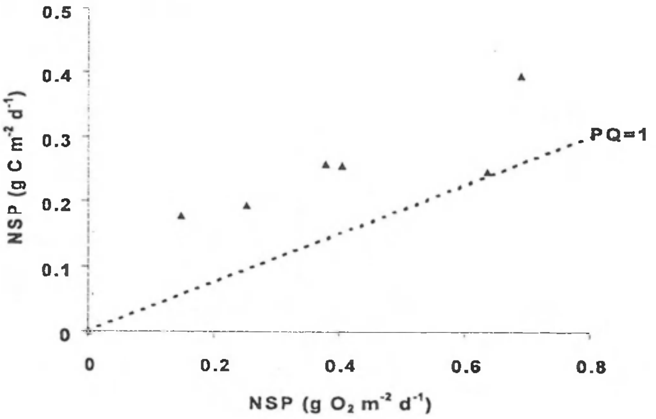


Figure 8.9
Net daytime production and net system production as a function of external N-loading in the 1995 experiment.



Net system production showed an increase with increasing NDP, as in the short-term experiments of 1993 and 1994. However, rates of respiration in the 1995 experiment were higher than in the short-term experiments of 1993 and 1994, mainly due to increased benthic respiration, and this resulted in a proportionally lower net system production in 1995 (Figure 8.9). NSP was highest in treatment H, and lowest in treatment L (ANOVA, $p<0.010$).

Figure 8.10
Net system production estimates, based on net production of organic C, compared to net system production estimates based on O_2. The dotted line indicates a (system) PQ value of 1.



In the 1995 experiment net system production was also measured by inventorying the net increase in organic carbon in the mesocosms, corrected for import and export of DOC and POC. A comparison of the NSP estimates based on oxygen measurements, and NSP estimates based on organic carbon, showed a significant correlation ($r^2 = 0.68$, $n = 6$, $p < 0.050$). The results from one of the mesocosms deviated from the other results. When this observation was removed, the estimates of net system production showed a linear relation with a geometric mean estimate for the slope between net oxygen production and organic carbon increase (PQ) of 0.92 ± 0.18 (Figure 8.10). The intercept suggested that net carbon production was overestimated.

8.4 DISCUSSION

The mesocosms were developed to simulate conditions in the Dutch coastal zone off Noordwijk. For a number of parameters, the behavior of the mesocosms was comparable to the situation in the field (Chapter 2). A comparison of phytoplankton biomass and primary production, observed in the model ecosystems, to field observations from the years 1988-1990 (Peeters, unpubl. results) showed a good agreement, both for total phytoplankton biomass and production, as well as for the timing of the spring and summer phytoplankton blooms. Inorganic nutrient concentrations showed a seasonal pattern similar to the seasonal changes in the field, with a rapid depletion of Si and P in spring, resulting in a limitation of the spring bloom by P. This was followed by a decrease of the inorganic N concentrations in summer. The time scale of an experiment in a model ecosystem forms a major constraint on the development of organisms with generation times exceeding the time span of the experiment. Consequently, in our 4-week experiments the biomass of the pelagic heterotrophic community was dominated by microzooplankton, that showed concentrations typical for estuarine and shallow coastal systems. Calanoid copepods were virtually absent as a consequence of the short duration of the experiments and low concentrations in the Oosterschelde at the time the mesocosms were filled (Wetsteyn and Vink-Lievaart, 1995). In the long term experiment of 1995, the longer duration of the experiment allowed for a more extensive development of mesozooplankton. Results showed a development of higher numbers of different naupliar and copepodite stages and adult copepods, especially in late spring and summer (Chapter 5).

Despite these differences between the 4-week experiments and the 7-month experiment, the relation between pelagic production and pelagic respiration was remarkably robust, showing that approximately 44% of net daytime production was respired by the pelagic system during the night. This fraction was almost similar to observations in an eutrophication experiment in the MERL mesocosms, where the increase of night water column respiration with increasing nutrient loading was equal to 45 % of the increase in water column production (Oviatt et al. 1986). The results showed that there is a strong coupling between pelagic production and pelagic respiration on the time scale of weeks to months, as has been observed for various natural or experimental estuarine or marine ecosystems (Kemp and Boynton 1980; Oviatt et al. 1986; Jensen et al. 1990; Kemp et al. 1992; Smith and Kemp 1995). The strong coupling indicates a dependence of heterotrophic processes in the water column on the production of organic material, or a dependence of autotrophic production on pelagic nutrient regeneration (Smith & Kemp, 1995). The small variation in the ratio of NPR:NDP is partly due to the physical scale of the mesocosms. As phytoplankton production only occurs in the euphotic zone, whereas phytoplankton respiration takes place in the entire water column, the depth of the water column partly determines the P/R ratio of the pelagic system (Kemp et al., 1992). Moreover, our observations in both the short term experiments and the long term experiment were limited to the spring-summer period. Low temperatures during winter have a strong impact on the pelagic system and can lead to much lower rates of respiration and higher P/R ratios (Kenney et al. 1988; Smith and Kemp 1995). Pelagic respiration correlated with both phytoplankton biomass and phytoplankton production. Regression of respiration rates with phytoplankton biomass, pelagic production and bacterial production has been used to estimate the relative contribution of phytoplankton and bacteria to total pelagic respiration (Jensen et al. 1990). This method may overestimate the respiratory activity of phytoplankton and bacteria as other heterotrophs are not included in the regression analysis but may covary with phytoplankton or bacterial production. In our experiment of 1995, we estimated phytoplankton respiration, using algal respiration rates from laboratory culture studies. The estimates showed that respiration by phytoplankton was a major fraction of total pelagic respiration. In addition, bacterial respiration was estimated to be about 10 to 30% of pelagic respiration. A study of the microbial carbon budget in marine enclosures showed that the carbon flux through the microzooplankton approximately equals net bacterial production rates (Riemann et al. 1990), making it probable that respiration by microzooplankton is of the same magnitude as bacterial respiration. These estimates indicate that a major part of pelagic respiration was related to respiration by algal cells and the microbial foodweb. This is in accordance with other enclosure and field studies showing the quantitative importance of the microbial food web in pelagic respiration (Williams 1981; Joiris et al. 1982; Hopkinson et al. 1989; Griffith et al. 1990). The relative importance of phytoplankton respiration and the microbial food web, and the short time scale at which the microbial food web can respond to changes in algal production, explains the close coupling between pelagic production and pelagic respiration. Benthic respiration often constitutes a major part of the heterotrophic processes in estuarine and shallow coastal ecosystems (Kemp et al. 1992; Heip et al. 1995). This is a function of the spatial scale, as the limited depth increases the effect of benthic processes on the pelagic system. In our short term experiments, the benthic compartment was relatively unimportant in the experiments with low mussel biomass. This was a consequence of the experimental design, starting with an azoic sediment and with an experimental duration that was too short to allow for a development of a benthic community. In the experiments with high mussel biomass, benthic respiration by far dominated system respiration. This was mainly due to a strong reduction in pelagic production and respiration. Estimates of mussel respiration rates showed that bivalve respiration formed only a fraction of total benthic respiration, even in the treatments with high mussel biomass. The main effect of the bivalves was to induce a large flux of organic matter from the water column to the benthic system by filtration, thus reducing pelagic biomass and pelagic processes, and increasing the coupling between the pelagic and benthic system.

In the long term experiment, benthic respiration increased in importance during summer. Benthic respiration shows a strong temperature dependence (Rudnick and Oviatt 1986; Doering et al. 1987). The observed increase in benthic respiration occurred after the end of the phytoplankton spring bloom, which partly sedimented and resulted in a strong increase of organic matter in the sediment by the end of May, when water temperatures were above 15 °C. Mineralization processes during summer led to a reduction in the amount of detritus in the sediment (Prins et al. 1996). Our results illustrated the dependence of benthic respiration on organic matter supply from the pelagic system and on temperature. Benthic respiration showed no clear response to the differences in pelagic production related to differences in nutrient loading between mesocosms. Similar results were obtained in a 2-year eutrophication study in experimental marine ecosystems, where an increase in nutrient loading with a factor 32 resulted in an increase in pelagic production with a factor 3.5, and an increase in benthic respiration with only a factor 1.5 (Oviatt et al. 1986). In general, the relatively limited response of benthic metabolism is assumed to be due to spatial limitation for respiration at the sediment-water interface.

The experimental marine ecosystems used in this study were designed to mimick the Dutch coastal zone off Noordwijk. Our results show that a reduction in antropogenic nutrient loading to the Dutch coastal zone may be expected to result in a reduction in pelagic production and respiration. Pelagic respiration formed a more or less fixed fraction of pelagic production, while benthic respiration did not show clear differences between treatments. Consequently, net production in the pelagic system increased with increasing primary production. The differences in nitrogen loading with a factor 3 resulted in a 1.6 fold difference in pelagic daytime production between the treatments. Due to the concommitant changes in pelagic respiration, net system production showed a slight decrease from 0.66 to 0.19 g O_2 d^{-1}. Estimates of net system production, based on net organic carbon increase, confirmed the trends observed with the oxygen estimates. Concluding, reductions in nutrient loading to the Dutch coastal zone of the North Sea will mainly affect water column metabolism, leading to improved oxygen conditions, without extensively affecting net system productivity.

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9 A model structure for the analysis and extrapolation of the mesocosm results

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ABSTRACT

Adaptations of existing model formulations were introduced in order to fit the model to the dynamics of the mesocosm processes. Therefore a model structure was developed where different modules could operate independently from each other. The time scale was adapted to the time scale of the experiments, i.e. from days to hours, which means that within-day variability could be simulated. Different groups of phytoplankton are distinguished, and formulations are being developed to include a multispecies module into the mesocosm model. Improvements were made in administration of the fluxes in a uniform way for the various modules. The present model requires further testing and calibration before useful simulations can be presented. Main problems are the within-day dynamics of primary production and differences between measured and calculated phytoplankton growth, the unexplained loss of nitrogen as shown in the nitrogen budget calculation, and the incorporation of the BLOOM algorithm.

9.1 INTRODUCTION

Mathematical modelling of the mesocosm results makes a contribution to (i) the formal representation of the processes taking place in the actual mesocosm experiments, (ii) deriving general formulations capable of representing the most important processes under different conditions and consequently (iii) extrapolating the findings of the mesocom experiments to field conditions. The latter objective is the final aim of the experimental approaches followed in the mesocosm experiments.

The modelling of the mesocosm results was started with the model of van der Tol (1994). This model was largely based on models available for natural systems, although a number of changes in the model formulations were introduced, compared to, e.g. MOSES (Soetaert & Herman, 1993) or SMOES (Klepper et al., 1994). During the model exercise by van der Tol (1994) a number of problems and opportunities became clear, which shaped the content and objectives of the present modelling sub-project.

One advantage of mesocosm experiments is that they provide a set of data in manipulated conditions, ideal for the comparative testing of different model formulations. It was therefore decided that the model should compare different alternative formulations for different processes. In the first place, the modelling framework should make this systematic comparison of model formulations possible. It was decided that this should be accomplished by the construction of an object-oriented modelling approach.

Secondly, it was also apparent that the time scale of the mesocosm experiments was too small for the application of model formulations aimed at representing seasonal time scales in nature. Adaptation of the model time scale to represent within-day variations was therefore decided.

Thirdly, the mesocosm data set offers a number of variables rarely observed at this time scale in nature, but useful to constrain model behaviour. In particular, continuous observations of light, oxygen concentration and pH were available. These will be exploited to constrain primary production and mineralization in the mesocosm tanks.

The present report mainly focuses on the restructuring of the model framework (Section 9.2). Changes in the model formulations, compared with van der Tol (1994) are touched upon in section 9.3. This section also gives some preliminary output of the model in its present form, i.e. before calibration on the mesocosm data set has taken place. Finally, the report gives details of the present state of affairs in incorporating the Delft Hydraulics BLOOM module (Los, 1991) into the modelling framework for the mesocosms (Section 9.4). This task has necessitated the thorough analysis of this code, which has led to a (partially completed) recoding of the algorithm.

9.2 THE NEW MODELLING STRUCTURE FOR THE MESOCOSM MODEL

9.2.1 Introduction

The restructuring of the model implementation was started with the following objectives in mind:

- The functional modules of the model should be implemented as independently as possible. This must allow for the implementation of alternative formulations for a module without the necessity to change the implementation of other modules.

- Provisions had to be made to allow for the implementation of more than two phytoplankton groups (diatoms and non-diatoms), in order to be able to implement different formulations for this functional module.

- The administration of the fluxes between the components of the model should be uniform throughout the model, and independent of the formulation of the modules. Moreover, the fluxes should allow the investigation of the different future versions of the model, and facilitate the investigation of the relative importance of the different functional groups in the model.

- The time scale of the model should be adapted to the time scale of the experiments. The original time scale of days has been changed into hours. This allows for the resolution of processes that vary within a day (e.g. primary production). This change of time scale is also a pre-adaptation to the consideration of pH and O_2 changes within a day.

9.2.2 Overview of modules

In its present version, the mesocosm model consists of a number of modules. These modules more or less correspond with the functional groups in figure 9.1. The dynamics of the processes modeled in the current version are detailed in Section 9.3

Model modules:

- **General**

  The General module does not contain a functional group of the model. It contains some general bookkeeping and model support routines.

- **Import/Export**

  The Import module distributes the incoming flows among the different functional groups. This is an active module depending on the functional structure of the model, since seawater components have to be distributed over the relevant state variables. It also registers the outgoing flows, which is passively dependent on the state variables in the modules.

- **Sinks**

  The Sinks module registers the fluxes due to sedimentation. This is a passive module, since sedimentation fluxes are calculated within the relevant functional modules.

- **Chemo**

  The Chemo module models the dissolved matter (nutrients, oxygen, etc.) in the water column of the mesocosm.

- **Suspended matter**

  The Suspended matter module models the suspended matter in the mesocosm. It includes the adsorption/desorption processes of phosphate.

- **Detritus**

  The Detritus module models the dynamics of dead organic matter (detritus) in the water column of the mesocosm.

- **Phytoplankton**

  The Phytoplankton module models the behaviour of the phytoplankton in the water column of the mesocosm.

- **Zooplankton**

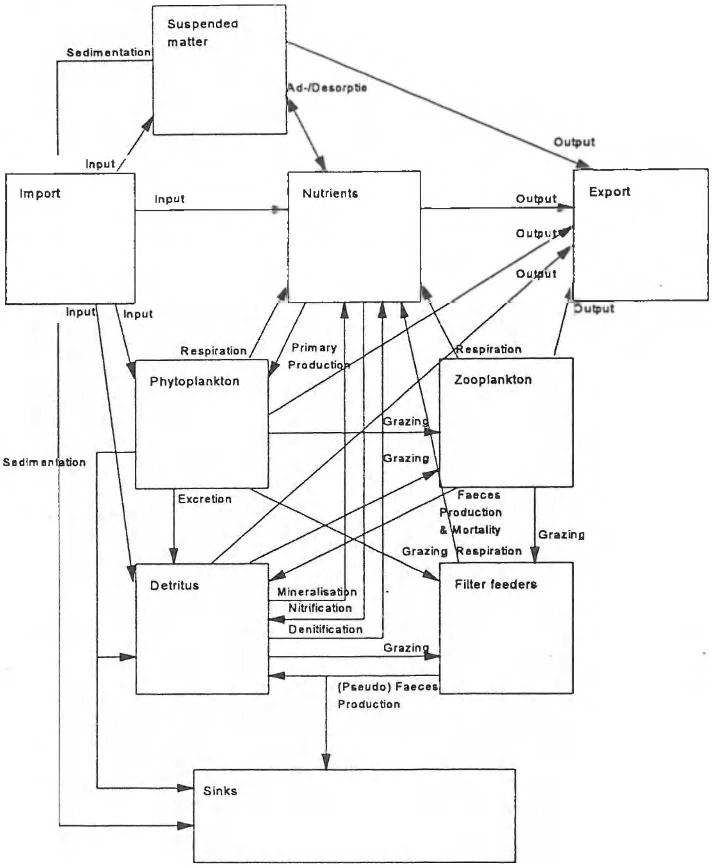
  The Zooplankton module models grazing by zooplankton on the plankton in the mesocosm.

- **Filter feeders**

  The Filter feeders module models grazing by mussels on the plankton in the mesocosm.

Figure 9.1

Functional groups and relations in the model



9.2.3 Flow of control

The following pseudo FORTRAN code shows the flow of control between the modules. The calling of the module routines is performed by the process routine, but the modeller keeps control of the calling sequence. This can, in some instances, materially influence model behaviour.

```
C Simplified flow of control
      TIME = 0
C Call model initialisation routine
      CALL XSTART(TIME)
      DO WHILE TIME <= ENDTIME
C Call module routines
        CALL General(TIME)
        CALL Import(TIME)
        CALL Chemo(TIME)
```

```
                   CALL Suspend(TIME)
                   CALL Detritus(TIME)
                   CALL PhytoPl(TIME)
                   CALL ZooPl(TIME)
                   CALL FilterF(TIME)
      C Integrate state variables and increase time
                   TIME = INTEGRATE(TIME)
                 END DO
      C Call model termination routine
                 CALL XEND(TIME)
```

9.2.4. Bookkeeping of fluxes

The model functional groups are charachterized by their concentrations of carbon, nitrogen, phosphorus and silicon. Flows of these constituents between the groups is administered by the general bookkeeping routines. Although, in the current version, the functional groups still have (group-specific) time-independent stoichiometric ratios, this can be changed without affecting the overall model structure. Model formulations for (time) variable stoichiometry are proprietary to the different modules. The introduction of this feature will be one of the module variations to be investigated in the model.
To implement the correct administration of these fluxes every functional group provides two interface routines, with the generic names AddFlux and SubFlux. These routines are used to specify a flux to resp. from that functional group. To ensure a closed mass balance every call to an AddFlux routine is matched with a call to a SubFlux routine (most likely from another functional group). E.g. the SubFlux routine of a phytoplankton group expects as input a carbon flux (in g $C.m^{-3}.h^{-1}$). It wil set the appropriate derivatives and will calculate and return the concomitant fluxes of the other modeled nutrients (nitrogen, phosporous and silicon). These four fluxes (carbon, nitrogen, phosporous and silicon) should then be passed to an AddFlux routine (e.g. in case of grazing by zooplankton to the AddFlux routine of the zooplankton group). This routine will also set the appropriate derivatives and depending on the internal stoichiometry compensate for deviating nutrient ratio's. E.g. if the N to C ratio of the incoming flux is too high it will compensate this by releasing some of the nitrogen to the dissolved nutrients pool (in the inverse case it will take some nitrogen from the dissolved nutrients pool).

9.2.5. An example

To illustrate the new structure of the model and the use of the flux administration routines the processes of the filter feeder module will be discussed.

The filter feeder module contains three processes:

- The grazing process models the grazing by the mussels on the available food groups, which includes the assimilation of carbon and the production of pseudo-faeces.
- The egestion process models the production of faeces by the mussels.
- The respiration process models the losses in biomass due to

respiration by the mussels.

The pseudo FORTRAN code for the filter feeder module is:

```
      C calculate filter feeder processes for every
   mesocosm tank
               DO iT = 1, cMxTank
                   CALL FfGrazing(iT, fxAssimC)
                   CALL FfEgestion(iT, fxAssimC)
                   CALL FfRespiration(iT)
               END DO
```

As an example of how processes are implemented the grazing process will be discussed. The grazing process has the following steps:

- The clearance rate of the mussels is calculated in function FfClearRate. The clearance rate is modelled as a function of the mussel biomass and the temperature.
- The concentration of the available food is determined (detritus, phytoplankton and zooplankton concentrations, returned by routine GrazeFood).
- The filtration flux (fxGrazeC) for every food group is calculated from the clearance rate and available food concentrations.
- After calculating the the assimilation fraction (AssimFrac) the assimilated carbon flux (fxMusC) and the pseudo faeces flux (fxDetC) can be calculated for every food group.

Now the fluxes are known the flux administration routines have to be called.

- The assimilated carbon flux (fxMusC) is substracted from the appropriate food group through the call to SubGrazeFlux. SubGrazeFlux expects as input a carbon flux (fxMusC) and returns the concomitant fluxes for nitrogen, phosphorous and silicate (fxN, fxP, fxSi).
- These fluxes are passed to the AddMusFlux routine which sets the derivatives of the mussel state variables. The two calls (to SubGrazeFlux and AddMusFlux) effectively realize a mass flow from the food groups to the mussels.
- The next two calls (SubGrazeFlux and AddDetFlux) denote a mass flow from the food groups to the fast detritus pool, which is the destination of pseudo faces in the model.
- The grazing process returns the total assimilated carbon flux (fxAssimC), which is a parameter for the egestion process.

The following FORTRAN code gives the implementation of the grazing process.

```
               SUBROUTINE FfGrazing(iT, fxAssimC)

      C reset total assimilation flux
```

```
     fxAssimC = 0
  C
  C calculate clearance rate
     ClearRate = FfClearRate(iT)
  C
  C get concentrations of available food
     CALL GrazeFood(iT, FoodC)
  C
  C calculate filtration fluxes for every food
group
     fxTotGraC = 0
     DO iG = 1, cMxGraze
      fxGrazeC(iG) = ClearRate *
pfEdible(iG) * FoodC(iG)
      fxTotGraC = fxTotGraC + fxGrazeC(iG)
     END DO
  C
  C calculate assimilation fraction
     AssimFrac = MIN(1.0D0,
pfTOCAssim/fxTotGraC)
  C
  C for every food groups
     DO iG = 1, cMxGraze

  C calculate assimilation flux
      fxMusC = AssimFrac * fxGrazeC(iG)
  C and pseudo faeces flux
      fxDetC = fxGrazeC(iG) - fxMusC
  C
  C set derivatives
      CALL SubGrazeFlux(iT, iG, fxMusC, fxN,
fxP, fxSi)
      CALL AddMusFlux(iT, fxMusC, fxN, fxP,
fxSi)
  C pseudo faeces are added to the fast detritus
pool
      CALL SubGrazeFlux(iT, iG, fxDetC, fxN,
fxP, fxSi)
      CALL AddDetFlux(iT, cDF, fxDetC, fxN,
fxP, fxSi)
  C
  C add food group assimilation flux to total
assimilation flux
      fxAssimC = fxAssimC + fxMusC
     END DO
  C
     END
```

The flux administration routines of the filter feeder module are shown here as an example of how the flux administration routines are implemented. The mussel state variable is called SMusC and is expressed in g C.m^{-3}. The derivative of mussel state variable is subsequently called DSMusC (g C.m^{-3}.h^{-1}).

- The SubMusFlux routine first adjust the derivative by substracting the carbon flux (FXC).
- After that it calculates the concomitant fluxes (FXN, FXP,

FXSI) by multiplying the carbon flux with resp. the N/C, P/C and Si/C ratio of the mussels.

- These ratios are returned by the interface routine MUSECRATIO.

```
             SUBROUTINE SubMusFlux(iT, fxC, fxN, fxP,
fxSi)
      C
      C substract carbon flux
             dsMusC(iT) = dsMusC(iT) - fxC
      C
      C calculate concomitant fluxes
             fxN = MusECRatio(iT, cN) * fxC
             fxP = MusECRatio(iT, cP) * fxC
             fxSi = MusECRatio(iT, cSi) * fxC
      C
             END
```

The ADDMUSFLUX routine first adjust the derivative by adding the carbon flux (FXC). Any silicate flux to the mussels (grazing of diatoms) is directly released to the biogenic silicon pool (ADDBIOSIFLUX). Finally the differences between concomittant nutrient fluxes given a fixed stochiometry (MUSECRATIO * FXC) and the actual given fluxes (FXN, FXP) are calculated. These differences (FXNH4, FXPO4) are added/substracted (depending on the sign) from the dissolved nutrients pool by the CHNUTFLUX routine (which is a flux administration routine from the Chemo module).

```
             SUBROUTINE AddMusFlux(iT, fxC, fxN, fxP,
fxSi)
      C
      C add carbon flux
             dsMusC(iT) = dsMusC(iT) + fxC
      C
      C silicate is released to the biogenic silicon
pool
             CALL AddBioSiFlux(iT, fxSi)
      C
      C fixed stochiometry
             fxCO2 = 0.0
             fxNH4 = fxN - MusECRatio(iT, cN) * fxC
             fxPO4 = fxP - MusECRatio(iT, cP) * fxC
             fxSiO = 0.0
      C
             CALL ChNutFlux(iT, fxCO2, fxNH4, fxPO4,
fxSiO)
      C
             END
```

9.2.6. Model flexibility

The first version of the model (van der Tol, 1994) has been rewritten in a way that should make it more flexible to implement future changes. The key to this flexibility is an object oriented-like structure. This allows for localized changes to the model without consequences for other parts (modules) of the model. This section will give some examples of possible modifications and how they can be implemented.

- ***Changing the functionality of a functional group***, e.g. changing how zooplankton mortality is modeled, can be easily accomplished because only one routine (or a few routines) has to be changed which can be easily localised and because the interface of the group will not change so other functional groups will not be affected. Of course parameters and/or variables have to be added/removed, but that is also straightforward.
- ***Adding additional functionality** to a **group***, e.g. adding sedimentation of zooplankton, is just as simple as changing existing functionality. One only has to add an additional call to a process routine in the module routine (e.g. Call ZplSedimentation in ZooPl), and write the new model routine.
- ***Disabling functionality of a group***. To investigate the effect of disabling one function of a group (e.g. disabling sedimentation of phytoplankton) one can add a conditional call to the relevant process routine in the (loop in the) module routine. The call can then be enabled/disabled by a parameter switch. Dependencies between processes are modelled through subroutine parameters. So disabling a process on which other processes depend will effectively disable all depending processes.
- ***Changing the import of substances in the model.*** In the present version of the model the import of substances is logically divided between what is imported with the sea water and what is experimentally added to the tanks (only nutrients in this setup). Both imports can easily be extended to other substances. This will only affect the appropriate import routine of the relevant functional group and the general module, because the latter acts as an intermediate between the import routines of the functional groups and the 'outside world' (forcing functions).
- ***Increasing/Decreasing the number of subgroups in a functional group.*** In the simple case of increasing the number of distinguished subgroups (e.g. another zooplankton group) without any new functionality (that is the existing formulations can be used) it comes down to increasing the appropriate parameter (e.g. MxZpl) and adjusting the size of the relevant parameter and variable arrays. If however the interface of the group is affected (e.g. the number of detritus pools are changed) then the change will also effect the functional groups that use the changed group. In general one can say that the lower the functional group is in the hierarchy of the model the greater the impact of a change in the interface on the model.
- ***Simulating a subset of the tanks.*** This feature has already been implemented. The MESOCOSM.PAR file contains the parameter switches PMCALCTANK for every tank which can

be set to zero if a specific tank should not be simulated. These parameter switches can also be used to disable tanks for those experiments that are not conducted in six tanks. All modules check in their module routine whether the simulation code for a specific tank should be executed by calling the CALCTANK function (defined in general.for).

- ***Disabling a functional (sub)group.*** A functional (sub)group can be disabled in the same way as tanks (see above). This is no problem for high level functional groups (such as filter feeders), but lower level functional groups (that are used by other groups) raise a problem. The interface routines from the disabled group will return a zero concentration, so all fluxes in the model that depend on the concentration of the disabled group are effectively disabled. But fluxes to and from the disabled group that do not depend on the concentration will just vanish and leave the model in an inconsistent state. This problem can be solved by redirecting the fluxes of a disabled group to some other functional group in the model (e.g. redirecting the detritus fluxes to the nutrient pools). This can be done in the flux bookkeeping routines (FXADD... and FXSUB...).
- ***Adding a new functional group.*** This is straightforward. Just add a new module, define an interface, create state variables and parameters, and add functionality in some process routines. Of course adding a new functional group can have a significant impact on the implementation of other groups. E.g. if bacteria are added to the model this will have an effect on the implementation of detritus, and also on functional groups that can graze the bacteria. So the implemantation of the functional groups have to be adjusted too.
- ***Adding a new nutrient.*** Adding a new nutrient is more or less the same as adding a new subgroup to a functional group. Adding a new nutrient will change the interface of the chemo group. This will have an impact on most functional groups in the model because the nutrients are at a low level in the model hiërachy. Many formulations have to be adjusted. Parts of the interfaces of functional groups have to be extended, because provisions have to be made for the new nutrient.

- ***Extracting additional model results.*** Results that are directly or indirectly calculated by the model can be easily extracted by declaring some new variable and assigning it the appropriate value. This will not effect the model because it does not change the state of the model. Due to the structured nature of the model, previously unavaliable information can now be extracted from the model. E.g. the turnover of the nutrients can easily be calculated by adding some additional statements to the flux bookkeeping routines.
- ***Adding a BLOOM module.*** A Bloom module which optimizes phytoplankton production by means of a linear programming

model, can now be added to the mesocosm model. See Ch. 3 for more details

- *Calibrating multiple experiments simultaneously.* This is not possible in the present set-up because the actual dates and times of the experiments are used for the forcing functions and observed data. So every experiment is represented by a distinct period. Moreover, a simulation can only be performed over a continuous period. It is, at present, not considered a disadvantage that this function is unavailable. One would rather use the calibration results of one experiment, to predict the outcome of another and check this prediction against data.

In conclusion, the present model structure allows a variety of changes to investigate model behaviour and compare different formulations. Although, evidently, not all desired changes to the model can be anticipated in advance, as a whole the model structure should be flexible enough to allow easy modification for future applications.

9.3. MODEL FORMULATIONS - PRESENT VERSION

9.3.1 A short overview of the modeled processes in each module that affect the state of the system.

General module:

No processes that actively change the state of the system.

Import module:

Import nutrients.
Import suspended matter.
Import organic matter.

Sinks module:

No processes that actively change the state of the system.

Chemo module:

Reareation.
Biogenic silica dissolution.
Export nutrients.
Export biogenic silica.

Suspended matter module:

Sedimentation.
Adsoption/desorption phosphate.
Export suspended matter.

Detritus module:

Nitrification
Denitrification
Mineralization
Export detritus.

Phytoplankton module:

Primary production.
Respiration.
Mortality.
Sedimentation.
Export phytoplankton.

Zooplankton module:

Grazing.
Egestion.
Respiration.
Mortality.

Filter feeders module:

Grazing.
Egestion.
Respiration.

9.3.2 Adaptation of the model formulations.

The present version of the model is based on the model developed by van der Tol (1994). The main effort has been devoted to create a flexible structure in which different process formulations can be investigated. But changing the model time scale from days to hours obliged us to make some changes. One obvious change was that all parameters that where expressed in days had to be converted to the new time scale.

A more radical change was needed for the calculation of phytoplankton primary production. With respect to light limitation, the first version used a function that calculated the time and depth integrated column production through a parameterization. This function could not be used in the new version because it calculated the average production over a day, given an average light intensity. In the present model the depth integrated production is calculated given the actual light intensity at the given moment.

With respect to nutrient limitation, a fundamental difference also exists between daily averaged and instantaneous formulations. In a first approximation and for a daily averaged formulation, production can be assumed to be limited by the actual nutrient concentrations. In an instantaneous formulation, however, decoupling between carbon production and (nutrient requiring) growth must be incorporated into the model. Observations in the mesocosms clearly show that primary production in terms of carbon fixation can attain instantaneous rates that are incompatible with the actual nutrient concentrations at that moment, since more than the total available amount of nutrients, plus the flux deriving from mineralization and input, should be incorporated into the biomass in less than two hours. Therefore a new formulation has been developed, which distinguishes between two pools in the phytoplankton: a metabolically inactive carbon ('sugar') pool, and a metabolically active ('protein') pool. By lack of more detailed measurements, chlorophyll a is taken as an indicator for the latter pool. Primary production in a first instance adds to the sugar pool, which is transformed into the protein pool if sufficient nutrients are available. Primary production itself is only light-limited. The sugar pool is preferentially used for respiration, and excess sugar is excreted when insufficient nutrients for growth are available. The formulation naturally leads to a variable stoichiometry of the phytoplankton, and to a variable chl a/ C ratio, both of which can be compared with observations.

Figure 9.2
Internal stocks of non-diatom phytoplankton ("alg") at short time scales during the build-up of a bloom. Due to primary production (Carbon fixation) the 'sugar pool' fluctuates with daylight, while the structural ('protein') pool is much more buffered within a day.

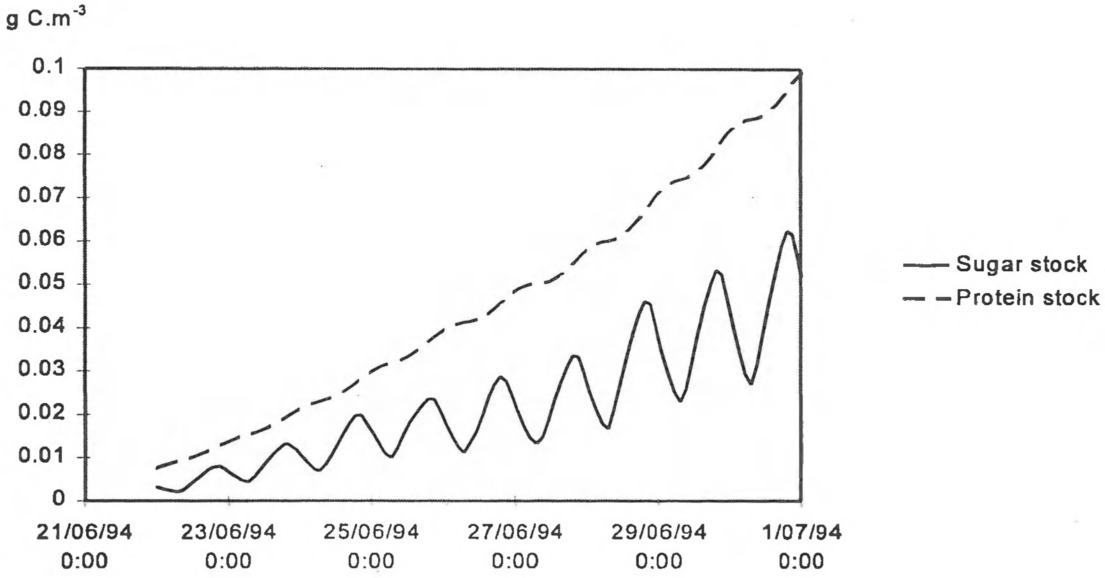


Figure 9.3
Carbon fluxes from the 'sugar pool' of non-diatom phytoplankton to different sinks. In the presence of sufficient nutrients (early increasing phase) most of the sugar is allocated to growth. Under nutrient limitation increasing proportions are devoted to excretion and (with increasing biomass standing stocks) respiration.

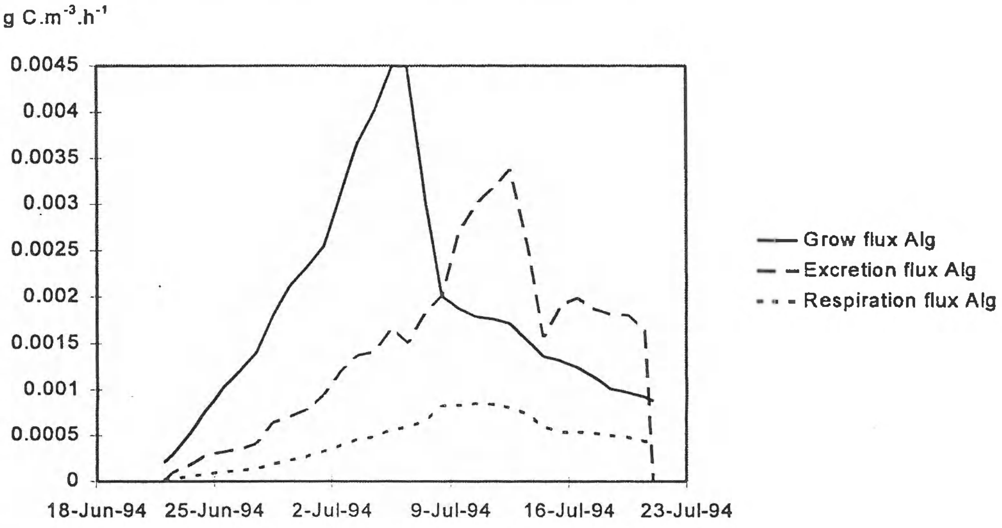
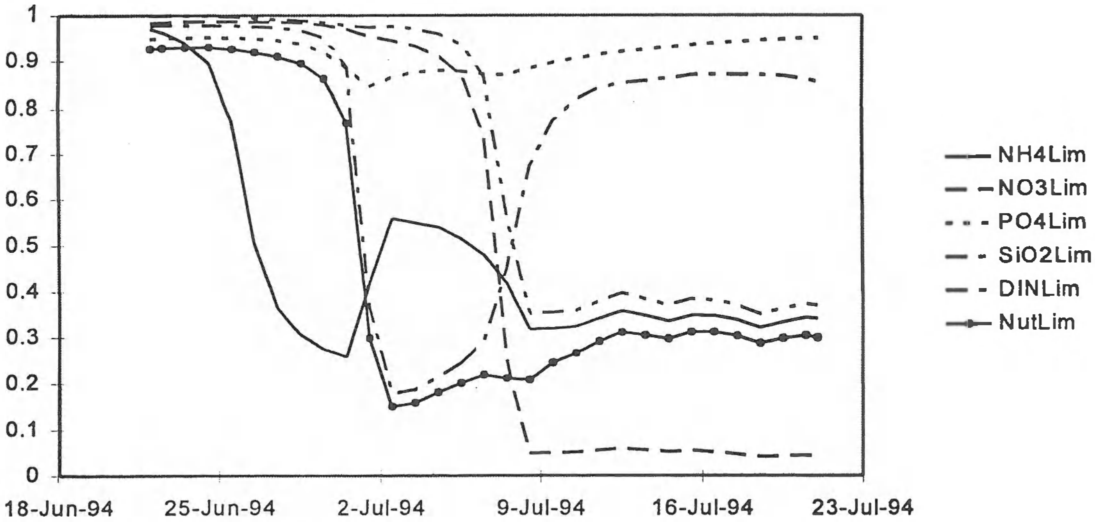


Figure 9.4
Nutrient limitation of diatoms during a mesocosm experiment. Total nutrient limitation ('NutLim') is a combination of limitation by inorganic nitrogen ('DINLim'), phosphat ('PO4Lim') and silicate ('SiO2Lim'). 'DINLim' itself is a complex function of ammonium and nitrate limitation.



In order to avoid 'stoichiometric correction nutrient fluxes' as in van der Tol (1994) to become the dominant processes in the model, the formulations for detritus breakdown have been changed to make them compatible with the variable stoichiometry formulations for phytoplankton. A detritus pool with variable stoichiometry has been defined. First-order decay rates are dependent on C/N ratios; the rates are higher for nutrients than for carbon. Sediment detritus has lower decay rates than suspended detritus.

Figure 9.5a
Molar C/N ratio of the POC in the water column compared with the phytoplankton molar C/N ratio. Except for the very beginning of the experiment, the POC is almost completely made up of phytoplankton. This feature of the model corresponds very well with observations

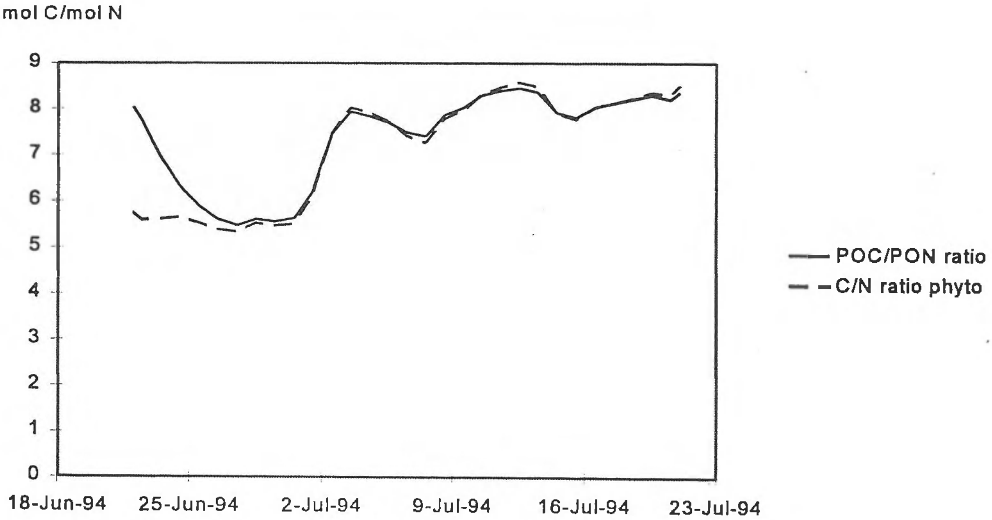
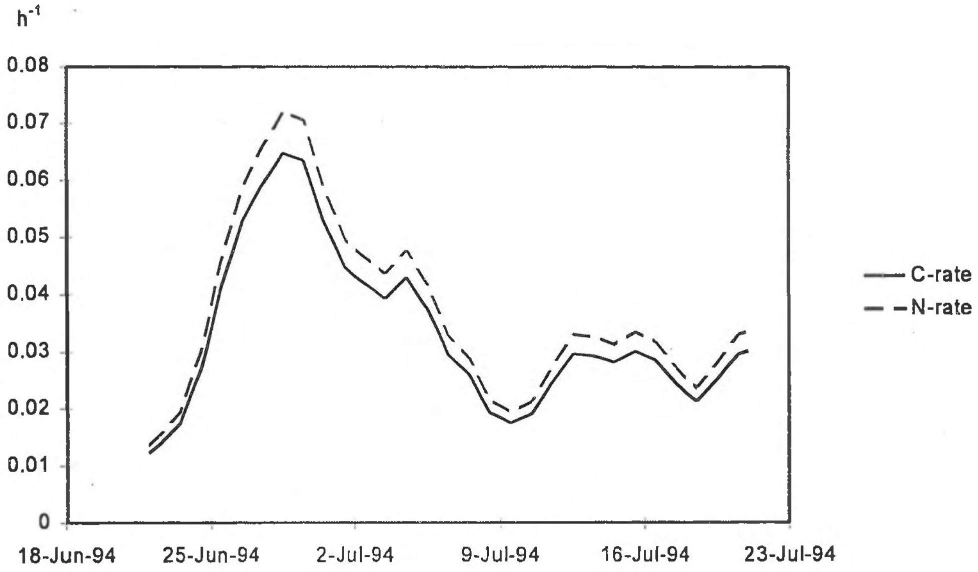


Figure 9.5b
Variation of the first-order decay rate of organic carbon and nitrogen in the same model run. Variations in C/N ratio are reflected in variations in the decay rates. Rates for N are always higher than for C, resulting in a relative impoverishment of organic matter in N.



Data analysis has demonstrated that the use of a single (time-invariant) conversion factor for the calculation of phytoplankton biomass from species countings yields inconsistent results, since the calculated biomass can exceed POC by a factor up to 10. New observations on time variable conversion factors have consequently been made, yielding much better consistency between the data series. It has been shown that POC in the water is mainly composed of live phytoplankton. Sinking rate of detritus in the mesocosm water column is high; a formulation for the process has been added to the model.

Routines have been developed to automatically check the mass conservation properties of the model. From the results of these calculations, a problem has been identified with the original formulations for ammonium preference (over nitrate) of phytoplankton. Numerical problems caused the inconsistencies in the nutrient budget. The formulations have been changed to avoid this problem, and the model nutrient budgets are now neatly closed. A problem arises, however, with the nutrient budgets in the observations. From first-order model calculations, it has been demonstrated that the gap in the nitrogen budgets reported earlier cannot be explained by denitrification. For phosphorus, an explanation by adsorption to the sediment cannot be ruled out, but the similarity between the gaps for nitrogen and phosphorus is suggestive for other mechanisms. Approximately 50 % of the cumulative nutrient input for both N and P cannot be found back in the cumulative outflow and the measured stocks at the end of the experiment. Practical investigations on the efficiency of the nutrient addition and water renewal processes will be performed. In the meantime, a consistency problem is posed between the mass-conserving model formulations, and the open nutrient budget from the observations. It is inevitable, in such a situation, that the model always fails to reproduce at least one time series of nutrient content in a state variable. Preliminary model runs use reduced nutrient input rates that assure a nutrient budget in accordance with the observations.

9.4 INCORPORATION OF BLOOM ALGAL DYNAMICS MODULE

9.4.1 Introduction

One of the driving reasons for restucturing the first version of the model was the objective to incorporate the BLOOM algorithm (Los, 1991) for optimization of the phytoplankton production. To this extent the BLOOM module, as obtained from Delft Hydraulics, has been extensively analyzed.

The BLOOM module as implemented by Delft Hydraulics can logically be divided in three parts (see Text Box for more details).

- The first part consists of the input and output functions: user interface functions, file input and output functions and interface functions to other coupled models. Multiple versions of BLOOM exist, one stand-alone steady-state version and some coupled dynamic versions (with CHARON, DELWAQ, ECOLUMN). In order to accomodate for these different versions, the first (I/O) part comprises a major part of the BLOOM module.
- The second parts consist of the functions for solving linear programming models.
- The last part is the actual BLOOM model, which derives a set of linear equations and a number of rules for handling exceptions. This latter part comprises only a small part of the BLOOM module code.

For our purposes only the two last parts are of interest. The input and output functions are handled by SENECA. Unfortunately these parts of BLOOM cannot be used seperately, because the code of BLOOM is very tightly coupled. No clear distinction has been made between the functional parts in the implementation of BLOOM. Because of this, the structure of the BLOOM module code is very complex. It is hard to understand the flow of control, which parts implement a specific feature and how the many switches affect the behaviour of the module. Our analysis led to the conclusion that when the BLOOM module, as it was delivered, would be used it would be very hard to ensure the correct working of the coupled models. It would be nearly implossible to maintain and to experiment with different model formulations. The coupled models would also be impossible to understand for persons without a thourough understanding of the BLOOM code. Therefore it was decided that the best way to proceed was to make a new implementation of the BLOOM module.
The implementation of the BLOOM model has almost been completed. For the linear programming algorithm the Simplex method as described in 'Numerical Recipes' (Press et al, 1986) is used. Most of the BLOOM model has also been implemented.

Some important problems have as yet to be solved. The main problem is how to incorporate in a consistent manner a BLOOM-like module, which uses an implicit (optimization-based) integration method, in the mesocosm model that otherwise uses explicit integration methods. The phytoplankton, when modelled in BLOOM, has multiple connections with the rest of the model: through nutrient uptake, grazing, mortality and detritus formation. Since all concentrations of other constituents are explicitly solved, a consistent way of back-transforming the implicitly calculated rates of change of phytoplankton into time-derivatives will have to be found. The theoretical investigation of this problem is one of the urgent needs for the final implementation.

Before the BLOOM module can be used some infomation has to be collected. First a decision has to be taken about which phytoplankton groups will be distinguished in the model. For each distinguished phytoplankton group the production-light curve has to be provided. The BLOOM module distinguishes three subtypes for every phytoplankton group, energy limited type (E-type), nitrogen limited type (N-type) and phosphorous limited type (P-type). For each subtype of the phytoplankton groups the stoichiometric ratios and the net growth rate (as a function of temperature) has to be provided.

9.4.2 Structure of the Bloom algorithm

The subject of linear programming is to maximize a given objective function in the form:

$$f(x_1,..,x_n) = a_1x_n + .. + a_nx_n$$

given the implicit constraints:

$$x_1 \geq 0, .. , x_n \geq 0$$

and a number of expicit constraints in the form:

$$b_1x_1 + .. + b_nx_n \leq c \text{ or}$$
$$b_1x_1 + .. + b_nx_n = c \text{ or}$$
$$b_1x_1 + .. + b_nx_n \geq c$$

In the BLOOM model the objective function is

$$F=\sum_j \sum_k Pn_{j,k} \cdot x_{j,k}$$

where:

$x_{j,k}$ = concentration phytoplankton species j type k
$Pn_{j,k}$ = net growth rate of species j type k

The net growth rate is defined as:

$$Pn_{j,k}=Pgmax_{j,k} \cdot EAvg_j+R_j$$

where:

$Pgmax_{j,k}$ = maximum gross growth rate for species j type k

$EAvg_j$ = time and depth averaged light efficiency for species j

R_j = respiraton rate for species j

Maximum gross growth rate:

$$Pgmax_{j,k} = Pnmax_{j,k} + R_j$$

where:

$Pnmax_{j,k}$ = maximum net growth rate for species j type k

R_j = respiraton rate for species j

Maximum net growth rate:

$Pnmax_{i,j}$ = 0.350 . 1.060^T, for diatoms type E

$Pnmax_{i,j}$ = 0.300 . 1.050^T, for diatoms type P

$Pnmax_{i,j}$ = 0.350 . 1.050^T, for flagellates type E

$Pnmax_{i,j}$ = 0.068 . (T - 0.0), for green algae type E

$Pnmax_{i,j}$ = 0.070 . (T - 5.0), for green algae type N

$Pnmax_{i,j}$ = 0.070 . (T - 5.0), for green algae type P

$Pnmax_{i,j}$ = 0.047 . (T - 3.0), for microcystes type E

$Pnmax_{i,j}$ = 0.045 . (T - 5.0), for microcystes type N

$Pnmax_{i,j}$ = 0.045 . (T - 5.0), for microcystes type P

$Pnmax_{i,j}$ = 0.045 . (T - 0.0), for oscilllatoria type E

$Pnmax_{i,j}$ = 0.034 . (T - 0.0), for oscilllatoria type N

$Pnmax_{i,j}$ = 0.034 . (T - 0.0), for oscilllatoria type P

where:

T = temperature

The Bloom model defines the following constraints.
Nutrient constraint:

$$\sum_j \sum_k a_{i,j,k} \cdot x_{j,k} + e_i = b_i$$

where:

$x_{j,k}$ = concentration phytoplankton species j type k

$a_{i,j,k}$ = amount of nutrient i per unit of species j type k

e_i = concentration of dissolved nutrient i

b_i = concentration of total available nutrient i

Energy constraint:

$$Kmin_{j,k} - Kd \leq \sum_j \sum_k K_{j,k} \cdot x_{j,k} \leq Kmax_{j,k} - Kd$$

where:

$Kmin_{j,k}$ = minimum extinction root for species j type k

$Kmax_{j,k}$ = maximum extinction root for species j type k

Kd = total extinction due to detritus

$K_{j,k}$ = specific extinction of living and dead material for species j type k

Growth constraint:

$$\sum_k x_{j,k} = xmax_j$$

where:

$x_{j,k}$ = concentration phytoplankton species j type k

$xmax_j$ = maximum attainable concentration level for species j in one time step

$$xmax_j = xs_j . e^{(Pgmax_j . EAvg_j - R_j) . \Delta t}$$

where:

xs_j = initial concentration of phytoplankton species j, which is defined as the maximum of the sum of the concentration of all types of species j and a specific base level concentration.

$Pgmax_j$ = maximum of the maximum gross growth rate of type k for species j, $max_k(Pgmax_{j,k})$

$EAvg_j$ = Time and depth averaged light efficiency for species j

R_j = respiraton rate for species j

Mortality constraint:

$$\sum_k x_{j,k} \geq xmin_j$$

where:

$x_{j,k}$ = concentration phytoplankton species j type k

$xmin_j$ = mimimum attainable concentration level for species j in one time step, which is defined as the sum of the biomass of species j at the end of the time step or if this sum is less than a specific base level concentration then it is zero.

$$xp_{j,k} = xs_{j,k} . e^{-M_{j,k} . \Delta t}$$

where:

$xp_{j,k}$ = concentration of species j type k at the end of the time step

$xs_{j,k}$ = concentration of species j type k at the begin of the time step

$M_{j,k}$ = mortality rate for species j type k

Respiration rate:

R_j = $0.031 \cdot 1.096^T$, for eukaryotic species
R_j = $0.012 \cdot 1.072^T$, for prokaryotic species
R_j = 0, for $T \leq 1.5$

where:
T = temperature

Mortality rate:

$M_{j,s}$ = $0.035 \cdot 1.080^T$, for energy types
$M_{j,k}$ = $0.045 \cdot 1.085^T$, for nutrient types
$M_{j,k}$ = 0.035, for $T \leq 1.5$

where:
T = temperature

This linear optimization problem can not be solved in one step, because the model might compute a bloom consisting of a number of types, all energy limited, but with different extinction roots (Kmin and KMax). This would be inconsistent. To solve this problem the extinction roots of all types are sorted and for each resulting extinction interval the model determines which types can sustain themselves in that interval and then the model is solved for those types. The final optimal solution is the maximum of the optimal solutions in the intervals. For a more detailed explanation of the chosen solution method see Los (1991).

The following pseudo code gives the structure of the Bloom solution algorithm. It does not address exceptions like inconsistent constraints and infeasible solutions. Neither does it adress how this solution algorithm should be incorporated in the mesocosm model.

```
Initialize model
DO WHILE Time < EndTime
  Get nutrient concentrations
  Get phytoplankton concentrations
  Calculate extinction roots
  FOR each extinction interval DO
    Determine sustainable types
    Set objective function
    Set nutrient constraints
    Set energy constraints
    Set growth constraints
    Set mortality constraints
    Solve linear program for Time + DeltaT
  END FOR
  Calculate maximum of maximum production per interval
  Time = Time + DeltaT
  Set nutrient concentrations
  Set phytoplankton concentrations
END DO
```

Most of this algorithm has been implemented, except the part that calculates the extinction roots and determines the extinction intervals.

9.4.3 CONCLUSIONS

The present model requires further testing and calibration before useful simulations can be presented. Main problems are the within-day dynamics of primary production and differences between measured and calculated phytoplankton growth, the unexplained loss of nitrogen as shown in the nitrogen budget calculation, and the incorporation of the BLOOM algorithm.

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10 Synthesis and conclusions

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10.1 SYNTHESIS OF THE 1995 EXPERIMENTS

The aims of the experiment, carried out in 1995, were to study the effects of changes in nutrient loading of the Dutch coastal zone of the North Sea on:
1. Phytoplankton composition and biomass
2. Phytoplankton production
3. Zooplankton development and biomass
4. Secondary production of the macrozoobenthos

The three different levels of nutrient loading, used in the experiment, were derived from the conditions in the Dutch coastal zone of the North Sea. One treatment ('H') represented the present situation in the North Sea, with a 50% reduction in anthropogenic phosphorus loading compared to the 1980's, and a small (10%) reduction in anthropogenic nitrogen loading. The second treatment ('M') represented the situation after implementation of the so-called North Sea Action Plan, with a 50% reduction in both anthropogenic phosphorus and nitrogen loading, compared to the situation in the 1980's . The third treatment represented a more extreme case with a 75% reduction of both N and P.

Replicate mesocosms were used within each experimental treatment. The experiment ran from March to September, in order to cover the entire growing season of phytoplankton and herbivores like zooplankton and zoobenthos.

10.1.1. Representativity of the model systems for the Dutch coastal zone

The mesocosms had been designed to mimic physical characteristics of the Dutch coastal zone in the outflow of the Rhine, and a transect off the coast at Noordwijk was chosen as a reference area. Light conditions in the mesocosms were comparable to irradiance values integrated over the water column in this area. Temperature development in the model systems was similar to the seasonal changes in the mouth of the Oosterschelde where the experiments were done.

The experimental nutrient loadings simulated the seasonal changes in riverine nutrient discharge. The seasonal changes in nutrient concentrations in the mesocosms showed a depletion of phosphorus during spring, followed by nitrogen depletion during summer. Silicate concentrations dropped to low levels during the spring phytoplankton bloom. Phosphate and silicate concentrations showed slight increases during summer as a result of benthic remineralization. This sequence in nutrient limitations in the model systems corresponded to the pattern in the North Sea.

A comparison of the mesocosm results from treatment 'H' with earlier observations from the reference points off Noordwijk showed that phytoplankton biomass was in the same range as field values during most of the experiment. Both the timing and the level of the spring phytoplankton bloom in the mesocosms were comparable to North Sea observations. During late summer, phytoplankton biomass in the mesocosms was relatively low, presumably due to microzooplankton grazing.

Primary production in the period March-September is approximately 90% of the yearly total primary production in the coastal zone of the North Sea. As with phytoplankton biomass, primary production in the mesocosms was at the same level as production in the North Sea, except for late summer values.

10.1.2. Phytoplankton composition

In short term experiments carried out in 1992-1994 changes in phytoplankton composition, especially shifts from diatoms to flagellates and increased abundance of *Phaeocystis sp.*, occurred in response to high N or P loading and increased N:Si or P:Si ratios. In the long term experiment in 1995, differences in phytoplankton composition between the three treatments were small. To a certain extent, this could be a consequence of the relatively small differences in nutrient loadings and nutrient ratios, as compared to the earlier short term experiments. In addition, there was a more extensive development of zooplankton in 1995, mainly due to the longer duration of the experiment. The phytoplankton in 1995 was dominated by larger diatom species, and it is postulated that increased grazing rates by microzooplankton on the smaller phytoplankton species caused the shift towards larger diatom species.

10.1.3 Phytoplankton biomass and production

The spring phytoplankton bloom (April-May) reached levels that were determined by the rate of phosphorus loading. Treatments H and M had a similar phosphorus loading and a comparable phytoplankton biomass and production. Treatment L had a lower P loading and lower phytoplankton biomass. This indicated that the level of the spring phytoplankton bloom was mainly determined by the rate of phosphorus loading.

The level of the summer blooms of phytoplankton differed between all three experiments, and was positively correlated with nitrogen loading. Differences between treatment H and treatment L were most apparent, with a reduction in N-loading of circa 70% resulting in a reduction in phytoplankton biomass and production with approximately 80-90%. Cumulated over the entire growing season phytoplankton biomass and production were correlated with nitrogen loading.

10.1.4 Zooplankton biomass and production

Microzooplankton showed a strong development in all mesocosms and was dominated by heterotrophic dinoflagellates and aloricate ciliates. Microzooplankton biomass showed a positive relation to phytoplankton biomass and hence to nitrogen loading.

Mesozooplankton showed a more limited development, and it was concluded that the small food web was of much higher importance than the metazoan food web. Especially the development of adult calanoid copepods was limited, which was ascribed to food limitation during the summer. Cyclopoid and harpacticoid copepods showed a better development, which reflects the conditions in the mesocosms, as the latter groups are more littoral-like in their behaviour, and probably benefited more from the high surface to volume ratio than the (truly pelagic) calanoid copepods. Mesozooplankton biomass did not show a relation with nutrient loading and phytoplankton biomass.

10.1.5 Macrozoobenthos production

Secondary production by mussels was used as one of the parameters to follow the bottom-up effects of nutrient loading on secondary production. In addition, total macrozoobenthos production was measured at the end of the experiment. Mussel production during the spring phytoplankton bloom was the same in all treatments. This was due to the fact that phytoplankton concentrations during the spring bloom were high and prevented food limitation of the mussels. During summer, phytoplankton concentrations were much lower, and this resulted in small differences in secondary production. However, variability between the replicates within one nutrient treatment was relatively large. Consequently, differences between treatments were not statistically significant.

Summarized over the entire growing season (March-September), secondary production was highest in treatment H and lowest in treatment L. Although the differences were not statistically significant, the results indicate that a reduction of the anthropogenic N loading with 75% could potentially affect secondary production.

10.1.6 Conclusions

The main conclusions of the study were:

1. In spring, a linear relationship between P input and primary production was observed, while in summer N was the main limiting factor for phytoplankton production. No effect of the nutrient input on the composition of the phytoplankton community was observed.
2. In spring, there was no relation between nutrient input and secondary production, while in summer a decrease in secondary production was only observed in scenario L, with the lowest nutrient input.
3. Reduction of N and P input to the mesocosms by 50 % does not affect the secondary production; only a reduction scenario of 75 % has shown a decrease in secondary production.

10.2 IMPLICATIONS FOR MANAGEMENT OF THE NORTH SEA COASTAL ZONE

The controversy between the management objective to diminish adverse eutrophication effects by reduction of inorganic nutrient loading to the North Sea on one hand, and on the other hand, the fear of negative effects on the fisheries due to reduced productivity of the North Sea, is probably smaller than it seems. Phosphate is limiting phytoplankton production during a relatively short time in spring. The present reduction in phosphate loading to the North Sea to approximately 50% of the anthropogenic loading in the 1980's, has not yet resulted in a decrease of phytoplankton biomass or production. The mesocosm results presented in this report and mathematical models suggest that a further reduction with 70-80% is necessary to affect the level of the spring phytoplankton bloom. A further reduction of the P-loading will probably result in less intense blooms of the harmful alga *Phaeocystis sp.* Additional arguments for reduced P-loading to the Nort Sea are the eutrophication problems in the freshwater systems.

It is not very likely that a reduction in the level of the spring phytoplankton bloom will result in a lowering of secondary production of fish or shellfish. At the time of the phytoplankton bloom, zooplankton biomass is still relatively small, and due to the long generation time zooplankton development will lag behind phytoplankton development. Consequently only a fraction of the primary production of the spring phytoplankton bloom will be transfered to higher trophic levels through the link from zooplankton to fish. Although shellfish are better able to profit from the spring bloom than zooplankton, phytoplankton concentrations will often be high enough to prevent food limitation during spring. The 'surplus' production of the spring bloom will mainly settle and be used by higher trophic levels through the microbial food web, and consequently with a low ecological efficiency.
Field data also show that growth of shellfish is related to summer production of the phytoplankton.
Hence, a relatively large part of the spring phytoplankton bloom will die and be recycled through the microbial food web. Only indirectly, and with relatively high energy losses, will the spring bloom be transfered to higher trophic levels.

Several uncertainties are involved in a reduction of the nitrogen loading to the North Sea. As a precautionary measure, reduction of the anthropogenic nittrogen loading is necessary to
1) reduce the risks of anoxia at the Oyster Grounds in the central North Sea
2) reduce the risk of harmful algal blooms
However, reduced nitrogen loading may affect the level of secondary production, as in most of the areas of the North Sea primary production shows N-limitation during summer. Zooplankton grazing rates on the phytoplankton are high during summer, which means that there is a relatively high transfer of production to higher trophic levels.
The mesocosm results presented in this report show, that a 50% reduction in N-loading did not have a significant effect on secondary production of the macrozoobenthos. Although microzooplankton showed a positive relation with nitrogen loading, this was not the case for mesozooplankton. These results indicate that the bottom-up effects of nitrogen loading on secondary production, within the range that was used in the experiment, was fairly small.

Concluding, a reduction of the anthropogenic N-loading to 50% of the rates in the 1980's will probably have very limited effects on the productivity of higher trophic levels. Such a reduction in N-loading is probably enough to significantly reduce the risk of anoxia.
The long-term policy in the Netherlands is to reduce P loadings with 75% and N-loadings with 70%. The results from this study support the need for a further reduction of the phosphorus loading to reduce the spring bloom of *Phaeocystis sp.* This study indicates that a reduction of the nitrogen loading with 70% may involve a risk of lower productivity at higher trophic levels.