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

Stratification in mesocosms, a pilot experiment

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Contents

1 INTRODUCTION 5

2 MATERIAL AND METHODS 6

2.1 General mesocosm description 6

2.2 Description of the experiment 7
2.2.1 Experimental design 7
2.2.2 Experimental timing and sampling scheme 9
2.2.3 Determination of the mean water column daily irradiance 9
2.2.4 Particulate and dissolved nutrient concentrations 10
2.2.5 Phytoplankton abundance, biomass and production 10
2.2.6 Characterization of the light limitation 11
2.2.7 Determination of potential nutrient limitation 11
2.2.8 Zooplankton abundance and biomass 12
2.2.9 Mussel biomass and growth 13

2.3 Nutrient treatments 14

3 RESULTS 15

3.1 Stratification 15

3.2 Mesocosm conditions compared to *in situ* North Sea conditions 16
3.2.1 Water temperature 17
3.2.2 Light 17
3.2.3 Nutrients 18
3.2.4 Phytoplankton biomass and production 19

3.3 Irradiance and potential light limitation for primary production 20

3.4 Dissolved nutrient concentrations and potential limitation for primary production 24

3.5 Phytoplankton biomass and production 25
3.5.1 Chlorophyll-*a* concentrations 25
3.5.2 Primary production and oxygen concentrations 26

3.6 Phytoplankton species composition 29
3.6.1 Phytoplankton functional groups succession 29
3.6.2 Main diatom species succession 33

3.7 Zooplankton abundance and biomass 36
3.7.1 Microzooplankton 36
3.7.2 Mesozooplankton 36

3.8 Mussel growth 41

4 EVALUATION 42

4.1 Methodological evaluation 42

4.2 Ecological evaluation 43

5 REFERENCES 45

APPENDIX: reeds verschenen BEON-rapporten

Stratification in mesocosms, a pilot experiment

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1 INTRODUCTION

Nutrients, light and turbulence represent key factors for phytoplankton development. The intensity of the response of phytoplankton to nutrient loading (biomass, production increase with N or P-load increase) in mesocosm experiments was closely coupled to the light availability (Escaravage *et al.*,1995; Escaravage *et al.*, 1996). The development of dinoflagellates is promoted by the co-occurrence of low turbulence and high nutrient concentrations (Margalef, 1978; Taylor & Pollingher, 1987). Dinoflagellate blooms occurring in saline stratified marine environments regularly include toxic species (Peperzak *et al.*, 1995). This justifies the efforts made to better understand the conditions for their wax and wane.
Lack of turbulence could, under certain circumstances, favour the establishment of a stratification (saline and/or thermal). The higher light availability in the upper layer induces an intensification of primary production and related nutrient uptake (Peeters *et al.*, 1995). The lack of turbulence promotes the sedimentation of non-motile phytoplankton (mainly diatoms) (Peeters & Peperzak, 1990) whereas active swimming species, including toxic dinoflagellates, could maintain their position in the upper and well illuminated layer (Peperzak *et al.*, 1995).
The hypothesis is made that anthropogenic eutrophication could interfere with this turbulence-related process and amplify its effects on the phytoplankton species composition. Anthropogenic eutrophication mainly consists of the increase of N and P loadings to the sea observed during the second half of this century, whereas the Si-loading, stricly dependent on "natural" sources, did not increase in the same period.
Consequently, non-silicon using phytoplankton (including dinoflagellates) could increase their concentration and bloom duration whereas silicon using phytoplankton (mainly diatoms) development remained limited by the Si-availability (Van Bennekom *et al.*, 1975; Officer & Ryther, 1980; Radach & Berg, 1986; Smayda, 1990; Escaravage *et al.*, 1995). As a result, the co-occurrence of stratification and eutrophication could favour toxic blooms. The incomplete implementation of the sanitation program for the North Sea due to sustained high nitrogen loadings (10% reduction instead of the planned 50% reduction of the N-loadings in 1995) has led to an increase of the N/P ratio in the coastal waters and possibly increases the probability of toxic blooms (Peperzak, 1994).

The questions raised above provided the framework for a series of mesocosm experiments in which salinity stratification was induced. Treatments combined different mixing regimes and nitrogen loadings. A dinoflagellate (*Prorocentrum micans*) was added to the mesocosm water and served as a "model species". The present document presents the results obtained during a three-months pilot experiment, run from 01/05/96 to 06/08/96 in order to evaluate the specific aspects involved with stratification in mesocosms.

2 MATERIAL AND METHODS

2.1 General mesocosm description

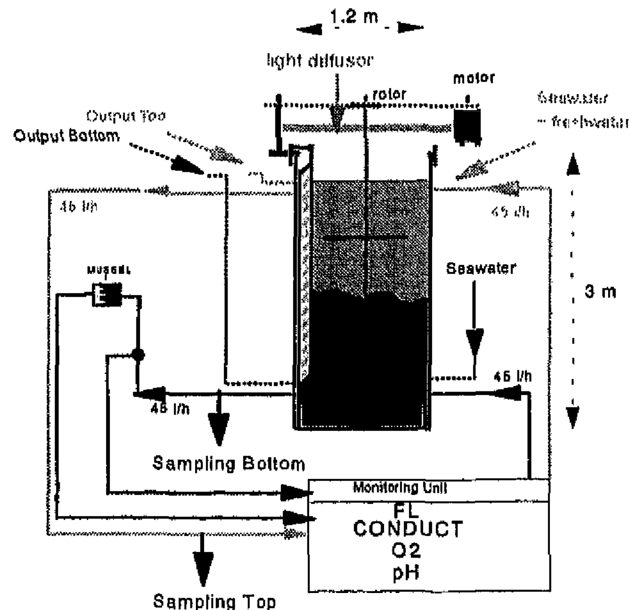
The experiment was carried out with 6 land-based mesocosms, located at the field-station of RIKZ near the mouth of the Oosterschelde estuary (SW Netherlands). The mesocosms consist of black solid polyethylene tanks (height 3 m, diameter 1.2 m, volume 3000 l). Water in the mesocosm was continuously mixed with a rotating mixer. The speed of the mixer was adjusted to allow stratification. 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 a 16 l Perspex benthos chamber. Benthos chambers were designed especially to contain a benthic compartment with macrofauna, and to enable measurements of the exchange of particulate and dissolved matter between the pelagic system and the benthos. The chambers were shielded from light. A 5 cm sand layer was added to each chamber. The benthos chambers were flushed with mesocosm water at a rate of 45 $l.h^{-1}$ with a 701 VB/R Watson & Marlow tubing pump. With an automated system in- and outflow of the benthos chambers were alternately pumped through a bypass containing a Turner fluorometer and a Stork-Servex Datasonde 3 with multi parameter water quality data logger for the registration of fluorescence, oxygen, temperature, conductivity and pH. Data were stored on a personal computer. Heating of the mesocosms by solar radiation was diminished by spraying sea water on the outer wall of the tanks and by shelling the tanks with foil.
A sediment container of 150 l was placed on the bottom of the tanks and filled with artificial sediment (azoic sand with a median grain size of 210 μm). Figure 1 gives a schematic view of a mesocosm unit. An extensive description of the mesocosms is given in Prins *et al.* (1995).

2.2 Description of the experiment

2.2.1 Experimental design

The design of the mesocosm as used during the years 1992 to 1995 was slightly modified to allow the establishment and maintenance of a salinity stratification. New connections were made to allow an independant flushing of the top and the bottom layer of the water column (Figure 1). In order to allow the maintenance of the stratification, pure sea water (+nutrients) was injected in the bottom layer whereas the top layer was flushed with sea water (+nutrients) diluted with fresh water to a final salinity of 2‰. In both cases the flushing rate induced a 30 days turn over of the water compartments. Connections to the peristaltic pump and the monitoring unit were adapted to allow independent measurements for each water compartment. Water from the bottom layer of each mesocosm was circulated at the rate of 45 $l.h^{-1}$ through a benthos chamber containing 40 mussels (17 to 20 mm long).
Stratification was established by spraying freshwater at the surface of the mesocosm under gently mixing of the top-layer until a conductivity probe placed at 1.5 m recorded a salinity decrease of 2‰. Vertical profiles of salinity were recorded each day.
Three mixing and two nutrient regimes were combined into six treatments applied to the six mesocosms (Table 1).

Figure 1
Schematic view of a stratified mesocosm unit with benthos chamber and the continuous measurement unit providing monitoring for fluorescence, conductivity, oxygen and pH. Sampling position (for lab analysis) of the top and bottom layers are indicated.



The used nutrient loadings were representative for actual loadings to the Dutch coastal zone. The high nitrogen regime corresponded to the present year with nitrogen and phosphorus loadings equalling respectively 90% and 50% of the loadings of the eighties. The low nitrogen regime corresponded to a scenario in which nitrogen loadings should be 25% of the loadings in the eighties. These loadings were estimated from outputs of the DYNAMO model (M.

van der Tol, pers. comm.).
Treatments were randomly assigned to 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).

Table 1
The six treatments used during the mesocosm experiment.The table gives the abbreviations used in this document for the different treatment and corresponding mesocosm (number).

Mixing regimes	**High nitrogen loadings** DIN = 3.70 $\mu mol.l^{-1}.d^{-1}$ DIP = 0.07 # Si = 0.85 #	**Low nitrogen laodings** DIN = 1.00 $\mu mol.l^{-1}.d^{-1}$ DIP = 0.07 # Si = 0.85 #
Continuously stratified	CSTRAT-H (1)	CSTRAT-L (3)
Periodically stratified	PSTRAT-H (2)	PSTRAT-L (6)
Not stratified	NOSTRAT-H (4)	NOSTRAT-L (5)

Batch cultures (3x2 l erlenmeyers) of *Prorocentrum micans* were run in medium PEP-Si, at 15°C, 36 $W.m^{-2}$ and 12/12 day/night cycle (L. Peperzak, pers. comm.) for inoculation in the mesocosms. The development of the culture was followed by microscope and/or analysis of particles size distribution with a Coulter-Counter®. When *Prorocentrum micans* concentrations reached a stationary phase, half (3 l) of the culture was distributed over the mesocosms, the rest was diluted with fresh medium and the culture was left to grow again. Table 2 gives the date and the final concentration of the inocula in cells per liter mesocosm. After julian day 191, the culture (in the 3 erlenmeyers) appeared to be contaminated with *Emiliania huxleyi* and the inocula were stopped by this date.

Table 2
Date and final concentration (cells per l mesocosm) of the *Prorocentrum micans* inoculation in the mesocosms.

Julian day	Inocula final concentration
124	737
131	760
141	784
157	408
165	220
177	220
191	250

2.2.2 Experimental timing and sampling scheme

Day 121: the six mesocosms were filled with Oosterschelde water and continuous flushing with nutrients-enriched sea water was started. In order to increase homogeneity among each nutrient treatment, the mesocosms with the same nitrogen loadings were continuously mixed through their connections to the measurement unit. Weekly samples were taken at mid depth for SPM, POC, PN, PP, chlorophyll-*a*, inorganic nutrients, phytoplankton, microzooplankton and mesozooplankton.
Day 143: connections between mesocosms with the same nutrient loadings were disabled, stratification was established in mesocosms CSTRAT and PSTRAT; mesocosms NOSTRAT remained well mixed. From this day on, samples were taken for particulate and dissolved matter (2x week^{-1}), phytoplankton and microzooplankton composition (2x week^{-1}), primary productivity (1x week^{-1}) at two depths (Top and Bottom). Mesozooplankton was sampled once a week, but only in the non-stratified mesocosms. This sampling scheme went on unchanged until the end of the experiment.
Day 170: water columns in the mesocosms PSTRAT were mixed again whereas mesocosms CSTRAT remained undisturbed.
Day 191: stratification was established again in mesocosms PSTRAT, these mesocosms remained undisturbed until the end of the experiment.
Day 215: last sampling date.
Fluorescence, oxygen, pH, temperature and conductivity were measured continuously. Chlorophyll-*a* data were used to calibrate fluorescence measurements.

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) 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.

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

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

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

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

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

2.2.5 Phytoplankton abundance, biomass and production

Chlorophyll-*a* and phaeophytin-*a* were extracted on GF/C filters according to Gieskes & Kraay (1984) and analyzed with a HPLC method using a 85-100% acetone/water-water gradient and a reversed phase RP18 Novopack column (Waters) in a Spectra Physics Chromatography station. Chlorophyll-*a* was detected with a Perkin Elmer LS-2B fluorometer (excitation: 410-430 nm; emission: >530 nm). A standard chlorophyll-*a* solution was used for calibration. The observed chlorophyll-*a* values were used to convert the continuously measured fluorescence data to chlorophyll-*a*. Phytoplankton samples were fixed with acid Lugol's iodine solution. Phytoplankton cell numbers and species composition were determined by the Utermöhl technique (Utermöhl, 1958). Hydrobios

10 ml sedimentation counting chambers were filled with 5 to 10 ml mesocosm water or 1 to 5 ml of concentrated (factor x10) samples. Samples were examined at 200x and 500x magnification with an inverted microscop. Carbon biomass was estimated from appropiate biovolume geometric approximations and standard allometric equations as in Smayda (1976).
P/I curve parameters were determined by ^{14}C-incubations twice a week. Water samples were incubated for 2 hours with 185 kBq ^{14}C-bicarbonate (Amersham) at irradiances of 0, 2.3, 5.3, 11.7, 29.6, 61.1, 144.3 and 332.3 W m^{-2} in a thermostated incubator. Samples were processed according to Peeters *et al.* (1991a). Irradiance (I, in W m^{-2}) and production (P), in mgC mg^{-1}Chl h^{-1}) were fitted to the model (Eilers & Peeters, 1988):

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

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

2.2.6 Characterization of the light limitation

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

2.2.7 Determination of potential nutrient limitation

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

Zevenboom (1986):

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

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

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

2.2.8 Zooplankton abundance and biomass

The phytoplankton samples were also used to analyse microzooplankton (20-200 μm, mainly heterotrophic dinoflagellates and ciliates) as well. Samples (1 l) were taken twice a week (Monday and Thursday) from the top and bottom layers (see Figure 1), always between 7 and 8 a.m. Only samples, taken simultaneously with the mesozooplankton samples, were analyzed; this resulted in a frequency of once a week.
Samples were fixed with acid Lugol's iodine solution (Throndsen, 1978) to a final concentration of ca. 0.3 %.
After concentration (ca. 10x) by sedimentation 5 ml was taken from the residu and was decolorized with sodium thiosulphate and stained with Bengal rose. Microzooplankton species composition and dimensions were determined using an inverted microscope. The used magnifications were 400x and 500x. Depending on organism abundance between 75 and 200 fields of view were counted. Microzooplankton dimensions were measured with a video image analyzing system. 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.
Carbon biomass was estimated from appropiate biovolume geometric approximations, using a conversion factor of 0.19 pgC μm^{-3} for the ciliates (Putt & Stoecker, 1989) and 0.14 pgC μm^{-3} for the heterotrophic dinoflagellates (Lessard, 1991).
To correct for cell shrinkage in acid Lugol's solution, a volume (biomass) correction factor of 1.33 for ciliates and 1.18 for heterotrophic dinoflagellates was estimated from literature data.
In general, sample analysis was done within 6 months after sampling.

Depth-integrated mesozooplankton (200-2000 μm, mainly copepods) samples 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. This sampling strategy might have disturbed the pycnocline and was too time-consuming for routine sampling during stratification. Therefore, the PVC-tube was used only once in the continuously stratified mesocosm at the end of the experiment. In general, samples were taken once a week, always between 10 and 12 a.m. Each sample was fixed with borax-buffered formalin (Steedman, 1976) to a final concentration of ca. 4 % in 50 ml glass tubes with snap-caps.
After staining the samples with Bengal rose, zooplankton species composition and dimensions (small amounts) were determined with a stereomicroscope (magnification 40-50x). Nauplii were counted in a subsample of 5 ml in a 10 ml cuvet; copepodites and other (large) species were counted in the remaining 45 ml of the sample. From calanoid copepods all stages from each species were counted. For the adult copepod stages distinction was made between males and females. From naupliar stages body-length and from calanoid copepodite and adult stages cephalo-thorax length was measured. Length of harpacticoid and cyclopoid copepodites and adults was measured as body-length. From other groups like larval stages of *Mollusca* and *Annelida* body-length was measured; from nematodes length (if possible) and width were measured.
To calculate copepod (all stages) carbon biomass the length-weight equations given by Klein Breteler *et al.* (1982) were used.
In general, sample analysis was done within 6 months after sampling.

For a more comprehensive overview (with respect to micro- and mesozooplankton), including all procedures to estimate biovolumes and biomasses, conversion factors and used literature for species identification, the reader is referred to Wetsteyn *et al.* (1996) and Wetsteyn & Vink-Lievaart (1997).

2.2.9 Mussel biomass and growth

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

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

with

μ_w = growth rate in day^{-1}
W_0, W_t = AFDW at begin and end of the experiment.

2.3 Nutrient treatments

The actual nutrient loadings used in 1996 are given in Table 3 and compared with the planned values.
The actual nutrient loadings were in the same order of magnitude as the values planned. Beside the inorganic dissolved nutrients, organic dissolved and particulate nutrients were also introduced in the mesocosms with the sea water. The total nutrient loadings, sum of all fractions (organic/inorganic, particulate/dissolved) brought into the mesocosms are shown in Figures 2 and 3.

Table 3
Average actual nutrient loadings in the 1996 mesocosm experiment compared with planned values (in brackets).

Treatment	DIN (μmol l^{-1} d^{-1})	phosphate (μmol l^{-1} d^{-1})	silicate (μmol l^{-1} d^{-1})
H	3.91 [3.70]	0.08 [0.07]	0.94 [0.85]
L	1.12 [1.00]	0.08 [0.07]	0.94 [0.85]

Figure 2
Planned and average of the actual Si-loadings and P-loadings to the six mesocosms.

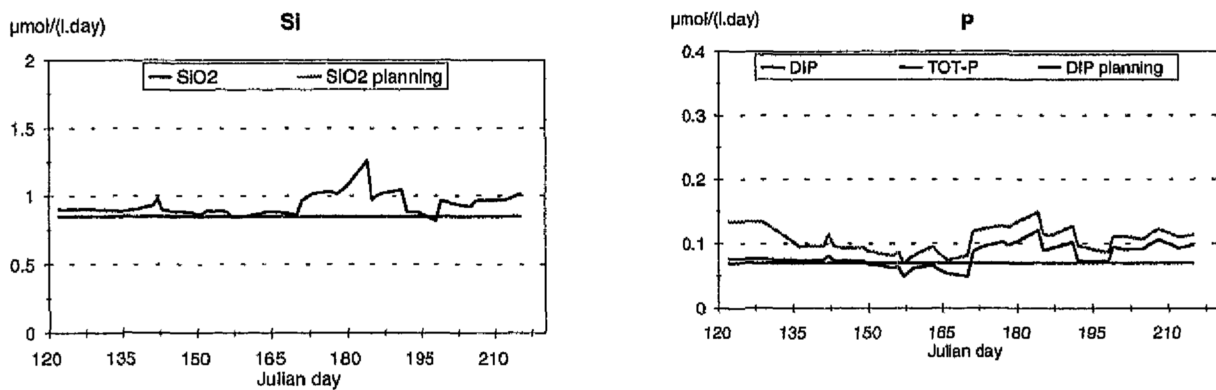
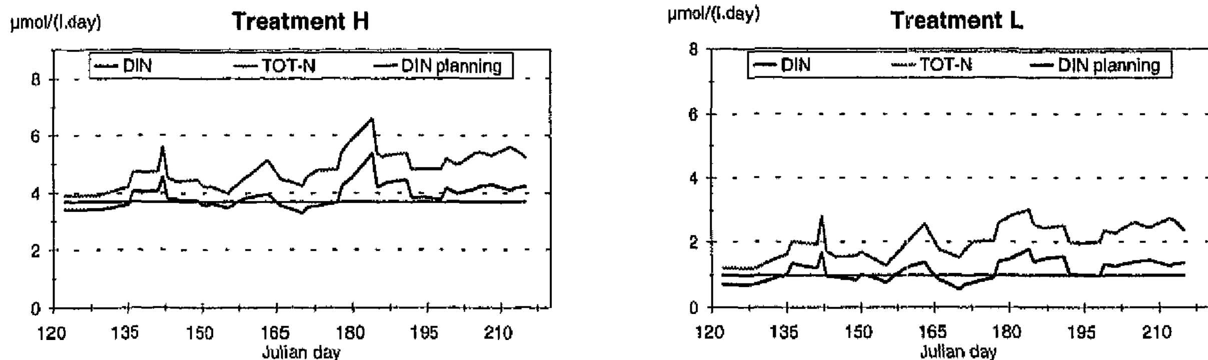


Figure 3
Planned and actual averaged N-loadings in treatments H and L.



3 RESULTS

3.1 Stratification

The salinity stratification measured in the mesocosms through the experiment is illustrated in Figures 4 and 5. Among the continuously stratified mesocosms (CSTRAT) only CSTRAT-L presented a quasi-constant stratification situated at 1.5 m depth from julian day 143 till the end of experiment. In mesocosm CSTRAT-H, the stratification appeared less stable. In order to compensate for this relative lability of the salinity gradient, extra additions of fresh water were performed around julian days 165 and 195. However, these additions did not succeed in establishing a stable stratification.
No long-lasting stratification could be established in mesocosm PSTRAT-H nor in PSTRAT-L (Figure 5). When present, the stratification remained very superficial (20cm) (PSTRAT-L, julian days 140-170) or not clearly pronounced (PSTRAT-L after julian day 190).

Figure 4
Distribution of salinity along depth through the experiment in mesocosms CSTRAT-H and CSTRAT-L.

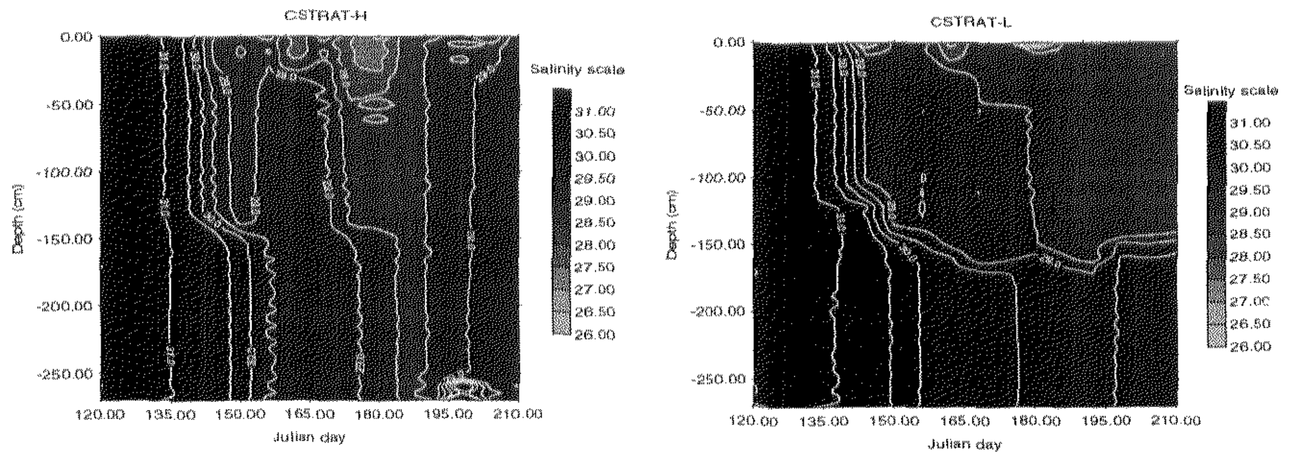
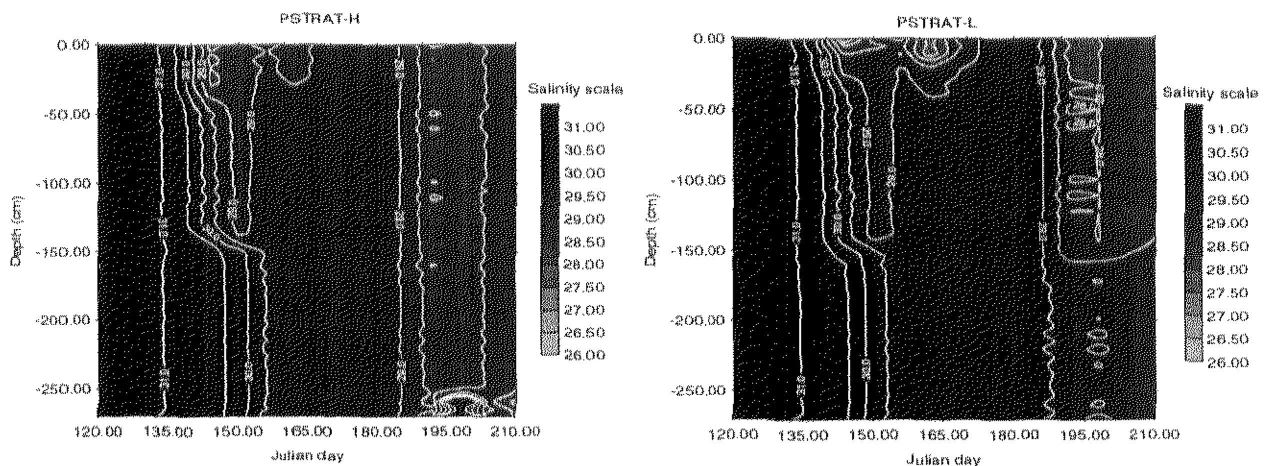
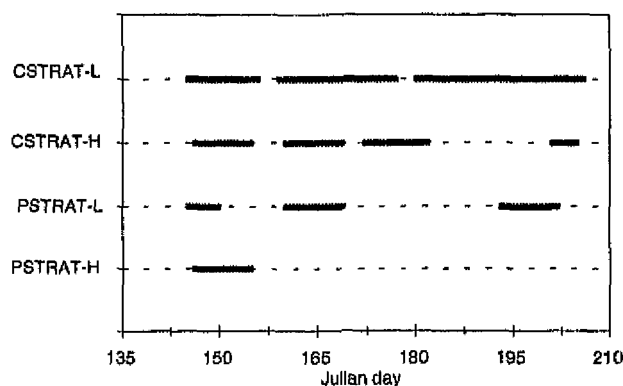


Figure 5
Distribution of salinity along depth through the experiment in mesocosms PSTRAT-H and PSTRAT-L.



In mesocosms NOSTRAT-H and NOSTRAT-L, the salinity remained homogeneously distributed over the water column throughout the experiment. Observations at a finer scale revealed that CSTRAT-L differed from all the other mesocosms at julian day 143 (the first day of stratification establishment) by a steeper salinity gradient: 2‰ over 25 cm in CSTRAT-L, whereas this difference was spread over 50 cm to 1 m in the other mesocosms. The hypothesis is put forward that the salt gradients established at day 143 in all mesocosm except CSTRAT-L were to weak to prevent the mixing of the top and the bottom layers (René Jansen, pers. comm.).

Figure 6
Maximum salinity difference (dS) measured between 20 cm depth intervals over the mesocosm water column. The black areas show periods when dS is larger than 0.03‰ cm^{-1}.



The actual experimental design could be formulated as: One mesocosm was continuously stratified (CSTRAT-L), three mesocosms were periodically stratified (CSTRAT-H, PSTRAT-H and PSTRAT-L) and two mesocosms were not stratified (NOSTRAT-H and NOSTRAT-L).
Stratification was objectively characterized by the maintenance of distinct salinities in the Top and Bottom compartments. An attempt to scale the strength of stratification was made by calculating the salinity gradients. Mean salinities were calculated for each 20 cm depth interval over the whole water column and the maximum value of the differences in mean salinity between adjacent intervals (dS) was computed. During periods when stratification was observed (steady Top/Bottom salinity difference), dS was larger than 0.03‰ cm^{-1}. These periods are shown in Figure 6.

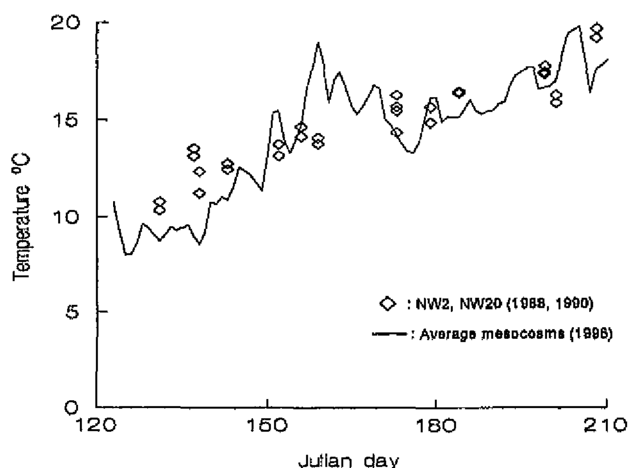
3.2 Mesocosm conditions compared to *in situ* North Sea conditions

Monitoring stations Noordwijk 2 and Noordwijk 20, situated in the Dutch coastal zone at a distance of 2 and 20 km respectively off the coast, served as reference for our mesocosm studies. Because many data were available for the years 1988-1990, these years were used for a comparison of mesocosm values with *in situ* values.

3.2.1 Water temperature

Water temperatures measured in the mesocosms were very close to temperatures measured *in situ* (Figure 7). The average daily temperature range was ± 2°C.

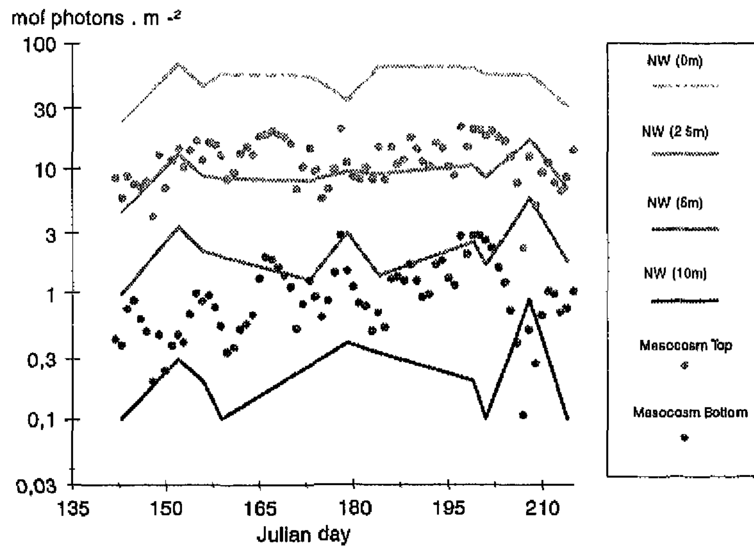
Figure 7
Daily averaged water temperature in the mesocosms (full line). The symbols show temperatures measured at Noordwijk 2 and Noordwijk 20 during surveys made in 1988 to 1990.



3.2.2 Light

Irradiance in the mesocosms was measured under the light diffusor of mesocosm CSTRAT-H. Measured surface irradiance was combined with averaged light attenuation values to estimate mean daily irradiance in compartments Top (0 to 1.4 m) and Bottom (1.4 to 2.8 m) of each mesocosm according to formula 2. These values were compared with mean daily irradiances estimated for the stations NW2 and NW20 at different depths (0, 2.5, 5 and 10 m) (Figure 8). The latter were obtained according to formula 1 by combining values of light attenuation measured during surveys in 1988 to 1990 and the series of surface irradiance measured during the present experiment.
The mean daily irradiances measured in the mesocosm compartments Top and Bottom could be encountered *in situ* around 2.5 m and 7 m respectively. This observation converges with previous analyses that concluded that the light climate of a 10 m water column (and consequently the *in situ* photic zone at Noordwijk stations, *ca.* 0 to 9 m) could be reproduced in the mesocosms.

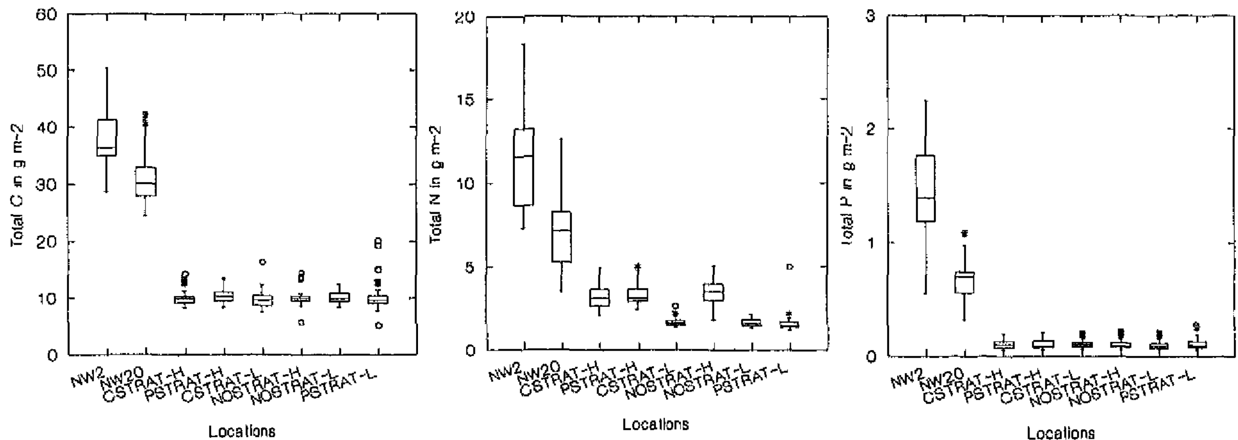
Figure 8
Mean water column irradiance in mesocosm compartments Top and Bottom (symbols) compared with estimates of *in situ* mean daily irradiance at different depths at North Sea stations NW2 and NW20 (solid lines).



3.2.3 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 9.

Figure 9
Box-plots of the total amounts of carbon, nitrogen and phosphorus in the water column of the mesocosms, compared to values observed at NW2 and NW20 in the period March-September 1988-1990.



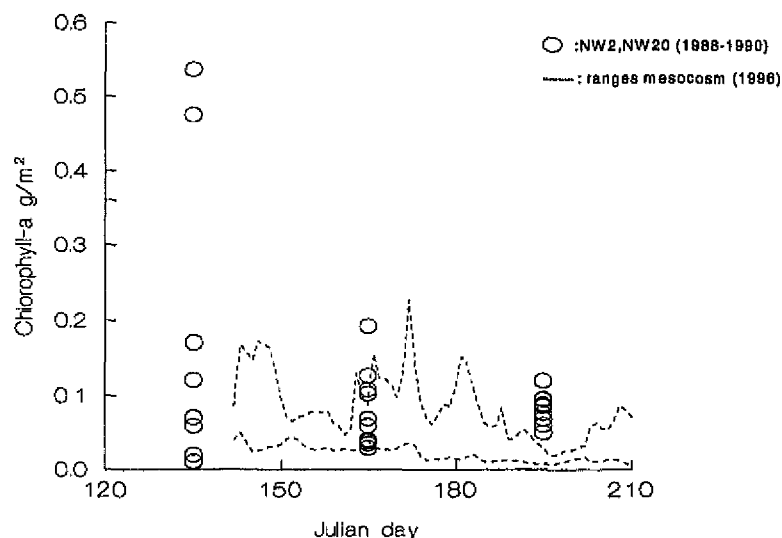
The box contains the middle 50% of the data, and the horizontal line through the box represents the median. Extending from either side of the box are the 'whiskers', representing data points within 1.5 times the interquartile range (length of the box) beyond the edges of the box on either side. The ends 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 (3 m depth) contained less detritus expressed per m^2 than a 10 m deep North Sea water column.

3.2.4 Phytoplankton biomass and production

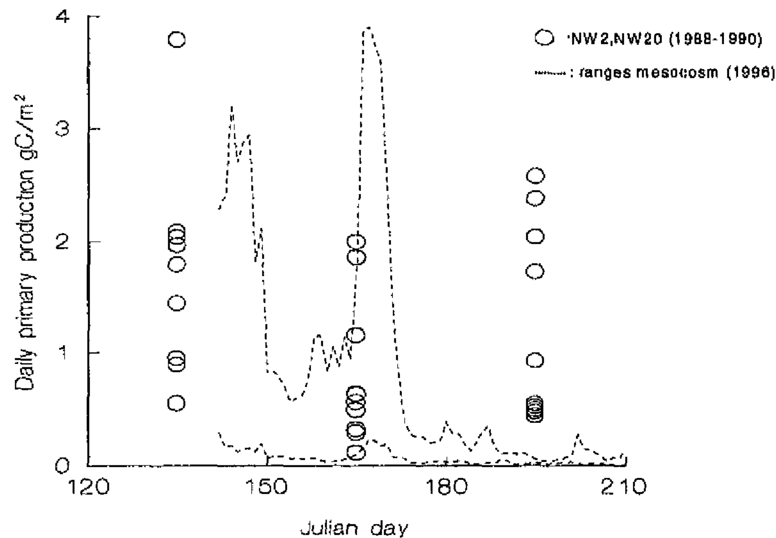
Phytoplankton biomass and production measured during the present experiment were compared with corresponding data collected at North Sea stations NW2 and NW20 during cruises in the years 1988-1990 (Figures 10 and 11).

Figure 10
Ranges of chlorophyll-*a* concentrations (g/m^2) measured during the present experiment (dashed lines) compared with the concentrations measured at NW2 and NW20 during the years 1988-1990 (open circles).



With the exception of the two exceptionally high concentrations measured at NW2 at the beginning of May (associated with a *Phaeocystis* bloom), the concentrations measured in the mesocosms in spring fell within the range of observations from the North Sea stations. Whereas chlorophyll-*a* concentrations in the North Sea stations increased or stabilized between June and July, they tend to decrease in the mesocosms during the same period. Fouling flora was not taken into account in this biomass estimate but may have significantly contribute to the algal biomass in the present mesocosm experiment (See section **3.6.2**).

Figure 11
Daily primary production (gC/m²) in treatment H compared with the average of the values measured at NW2 and NW20 during the years 1988-1990.



Whereas *in situ* primary production in May and June was in the same range as measured in the mesocosms, field values in summer were higher than in the mesocosm. Production by fouling flora on the mesocosm wall was not taken into account but may have be important in summer (See section **3.5.2**)

3.3 Irradiance and potential light limitation for primary production

Four main periods could be distinguished in the evolution of the surface irradiance. 1.-an increase (twofold) from day 150 until day 170, 2.-a sharp decrease between day 170 and day 180, 3.-a smooth increase from day 180 until day 200 and 4.-a decrease after day 200 (Figure 12).

Figure 12
Surface daily 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.

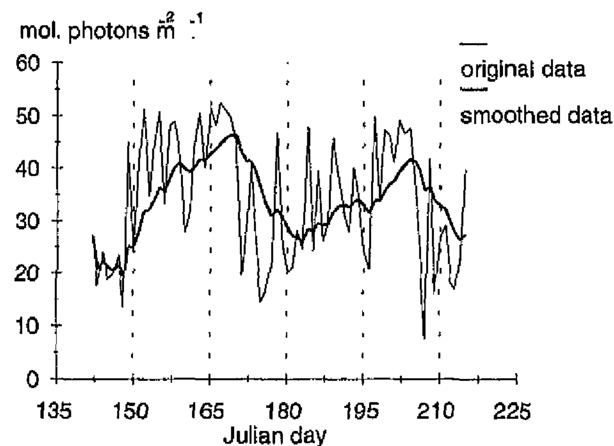
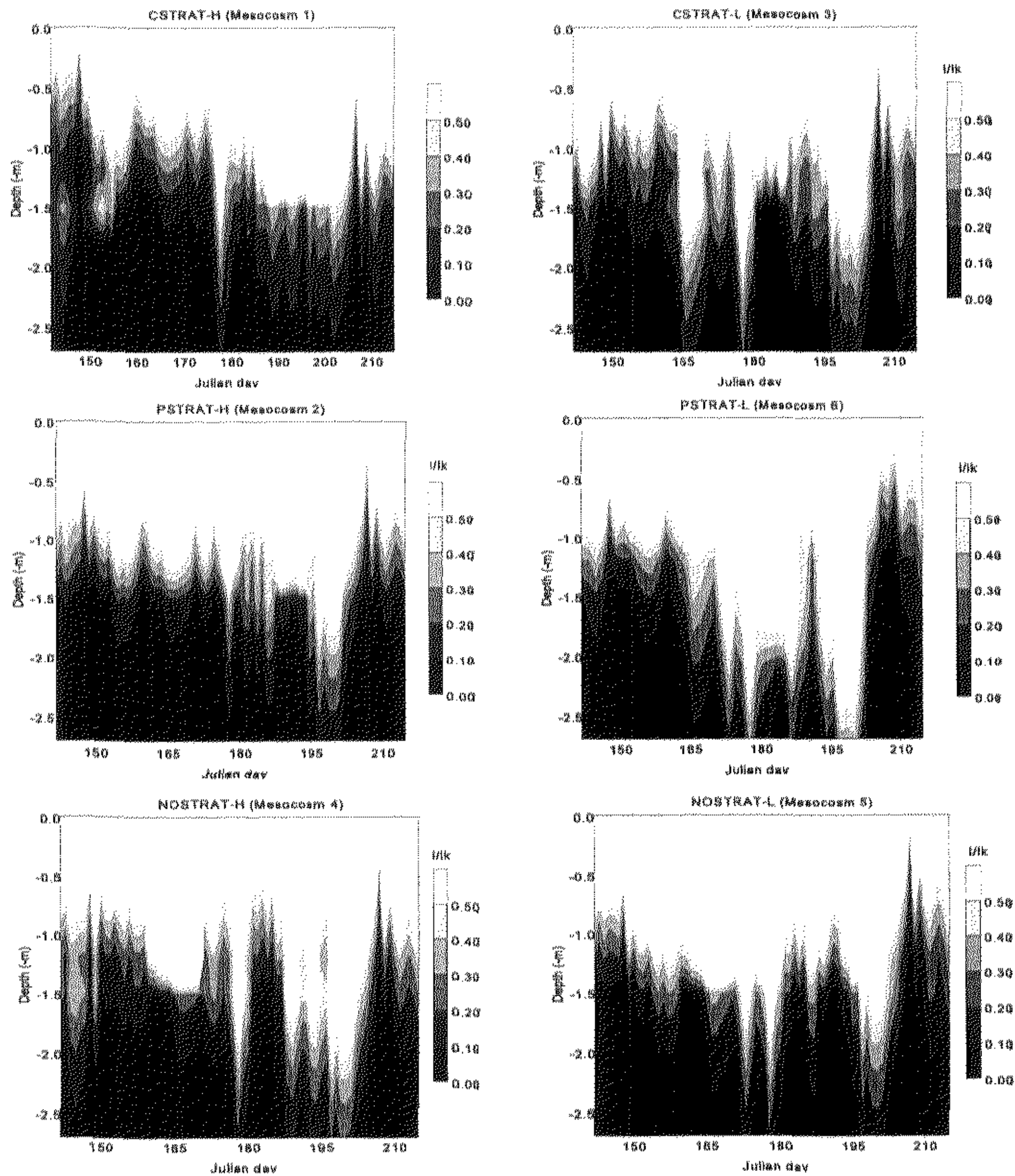


Figure 13
Vertical distribution of the light limitation index (I/I_k) over the mesocosm water column during the present experiment. I: mean daily irradiance, I_k: photosynthetic characteristic irradiance ($I_k = P_{max}/\alpha$, P_{max} highest point and α slope of the P/I curves obtained by the ^{14}C incubations). When I/I_k is between 0.1 and 0.5, light limitation is considered to be moderate; when $I/I_k < 0.1$, light limitation is considered to be severe (See Chapter 2.2.6 for further explanation).



Variations in surface irradiance were not clearly found back in the evolution of the index of light limitation (Figure 13). Each mesocosm showed a specific light climate mainly dependent on its K_d and on the photosynthetic characteristics of its phytoplankton. All mesocosms had in common that severe light limitation($I/I_k < 0.1$) was restricted to the Bottom compartment whereas Top compartment remained most of the time well illuminated.

Figure 14
DIN concentrations and periods of potential nitrogen limitation in the six mesocosms in the upper layer (Top) and in the lower layer (Bottom). When concentrations decrease below 2 μM (Ks value from literature sources, see Chapter 2), DIN *becomes potentially limiting for the primary production as indicated by the shaded bars under the main graphs.*

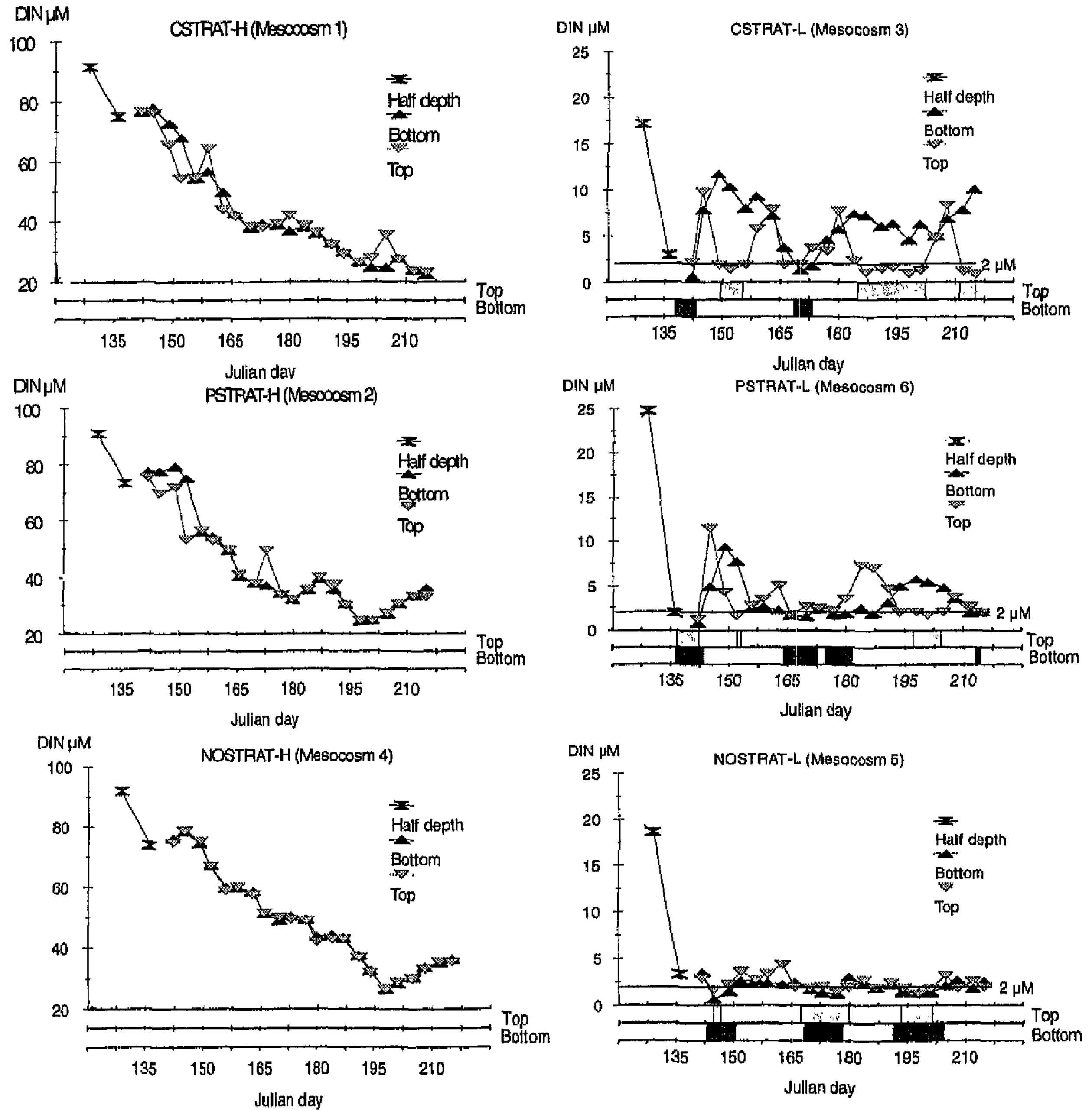


Figure 15
DIP concentrations and periods of potential phosphorus limitation in the six mesocosms in the upper layer (Top) and in the lower layer (Bottom). When concentrations decrease below 0.1 μM (Ks value from literature sources, see Chapter 2), DIP becomes potentially limiting for the primary production as indicated by the shaded bars under the main graphs.

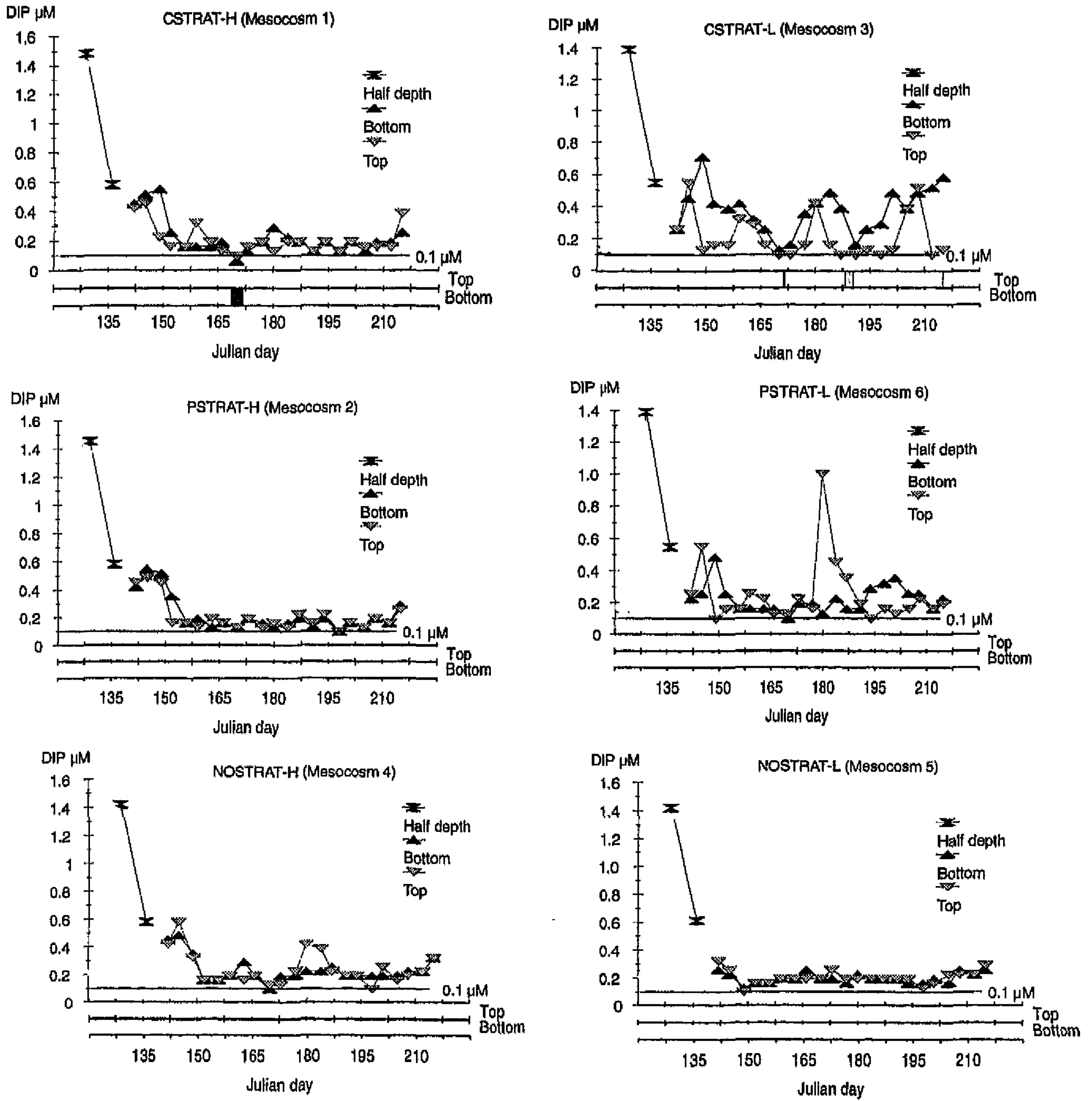
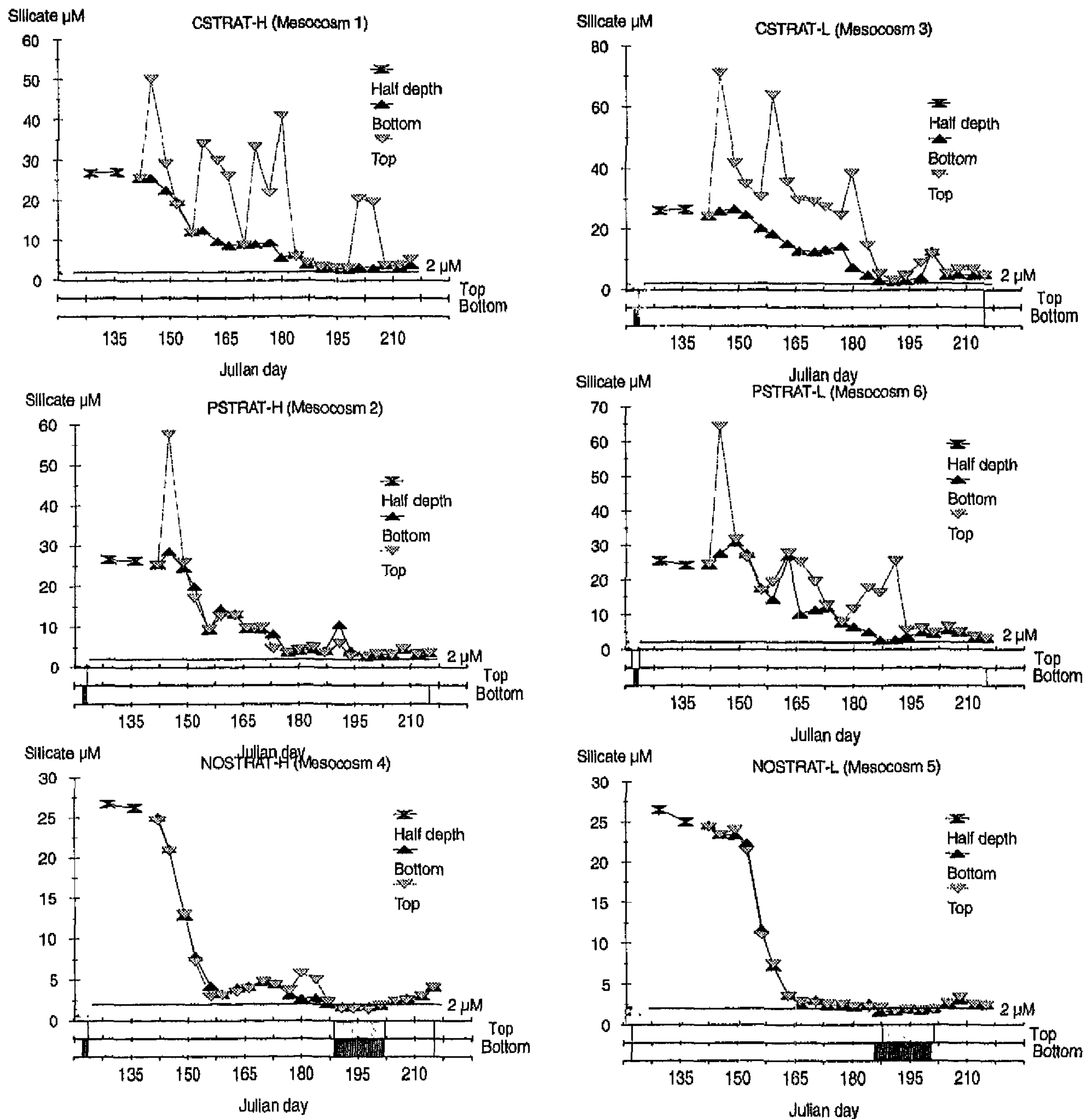


Figure 16
Silicate concentrations and periods of potential silicon limitation in the six mesocosms in the upper layer (Top) and in the lower layer (Bottom). When concentrations decrease below 2 μM (Ks value from literature sources, see Chapter 2), silicate becomes potentially limiting for the primary production as indicated by the shaded bars under the main graphs.



3.4 Dissolved nutrient concentrations and potential limitation for primary production

After the first week of the experiment, DIN concentrations in the three mesocosms with a high N-load (CSTRAT-H, PSTRAT-H and NOSTRAT-H) regularly decreased but never reached limiting values and were similar in both compartments Top and Bottom (Figure 14). In mesocosms with a low N-load, DIN concentrations occasionally

reached limiting values from day 140 onwards. Whereas DIN concentrations did not vary much in mesocosm NOSTRAT-L, they sharply increased in mesocosms PSTRAT-L and CSTRAT-L around days 140, 160, 180 and 200 (only in CSTRAT-L). Periods during which mesocosms CSTRAT-L and PSTRAT-L were stratified (See Figure 6), corresponded with a lowering of the DIN concentrations in Top, at some occasions lower than concentrations in Bottom. This resulted in N-limitation in Top not reproduced in Bottom around days 150 and 195 in PSTRAT-L and around day 150, from days 185 to 205 and after day 210 in CSTRAT-L. In mesocosm NOSTRAT-L periods of N-limitation occurred synchronously in Top and Bottom.

In mesocosms with a high N-load and in NOSTRAT-L, DIP concentrations decreased rapidly and remained between 0.1 and 0.2 μM after day 150 (Figure 15). DIP concentrations showed more variations in mesocosms CSTRAT-L and PSTRAT-L. During periods when DIN concentrations were lower in Top than in Bottom DIP reached also lower concentrations in the upper compartment than in the lower compartment. The very sharp increase in DIP concentrations in PSTRAT-L around day 180 remains unexplained.

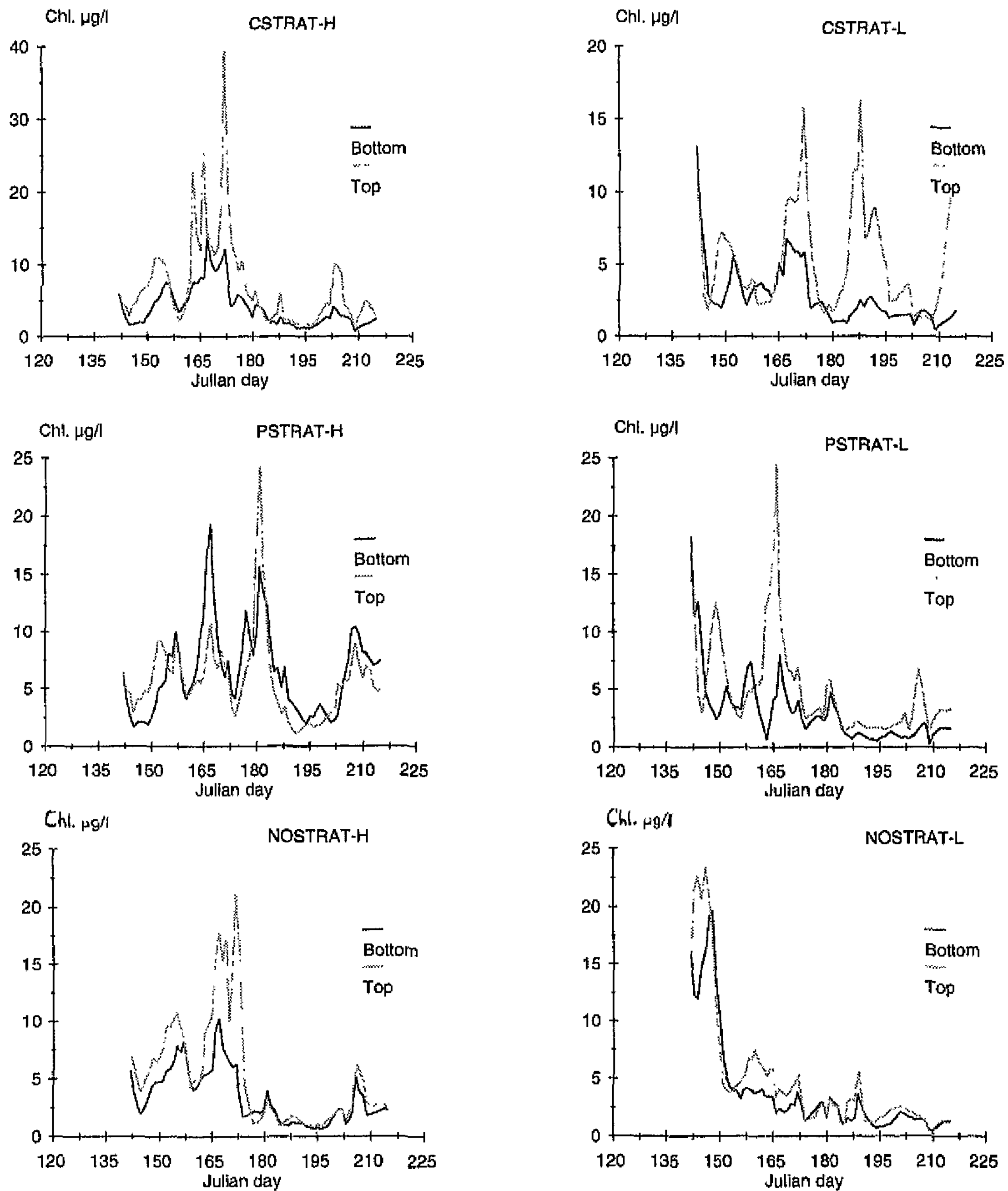
In mesocosms NOSTRAT-H and NOSTRAT-L, silicate concentrations decreased rapidly and reached concentrations near 2 μM (theoretical limitation threshold) around day 160 (Figure 16). Silicate became limiting in both mesocosms around day 195 in Top as well as in Bottom. In the mesocosms aimed to develop salinity stratification, silicate concentrations showed a succession of peaks in Top whereas concentrations in Bottom resembled those in the NOSTRAT mesocosms. The peaks in silicate concentration occurred on the same dates as the DIN concentration peaks shown in Figure 14 . On these particular dates demineralized water was introduced into the mesocosms in order to install or restore the salinity stratification. Probably, these nutrient peaks resulted from contaminations due to a low purification grade of the demineralized water used for this experiment. This additional nutrient input to the CSTRAT and PSTRAT mesocosms prevented the establishment of silicate limitation and could have reduced the intensity of the nitrogen limitation.

3.5 Phytoplankton biomass and production

3.5.1 Chlorophyll-*a* concentrations

Chlorophyll-*a* concentrations showed a succession of peaks varying in number and height among the mesocosms (Figure 17). Within each group of stratification mode, the mesocosms with a high N-load in general showed higher chlorophyll-*a* concentrations than the mesocosms with the low N-load. In most cases chlorophyll-*a* peak concentrations were higher in Top than in Bottom during periods when stratification was present in CSTRAT-H, CSTRAT-L and PSTRAT-L. A similar gradient in chlorophyll-*a* concentrations was also observed in the mesocosm NOSTRAT-H between days 165 and 180, although no stratification was present in this mesocosm during this period.

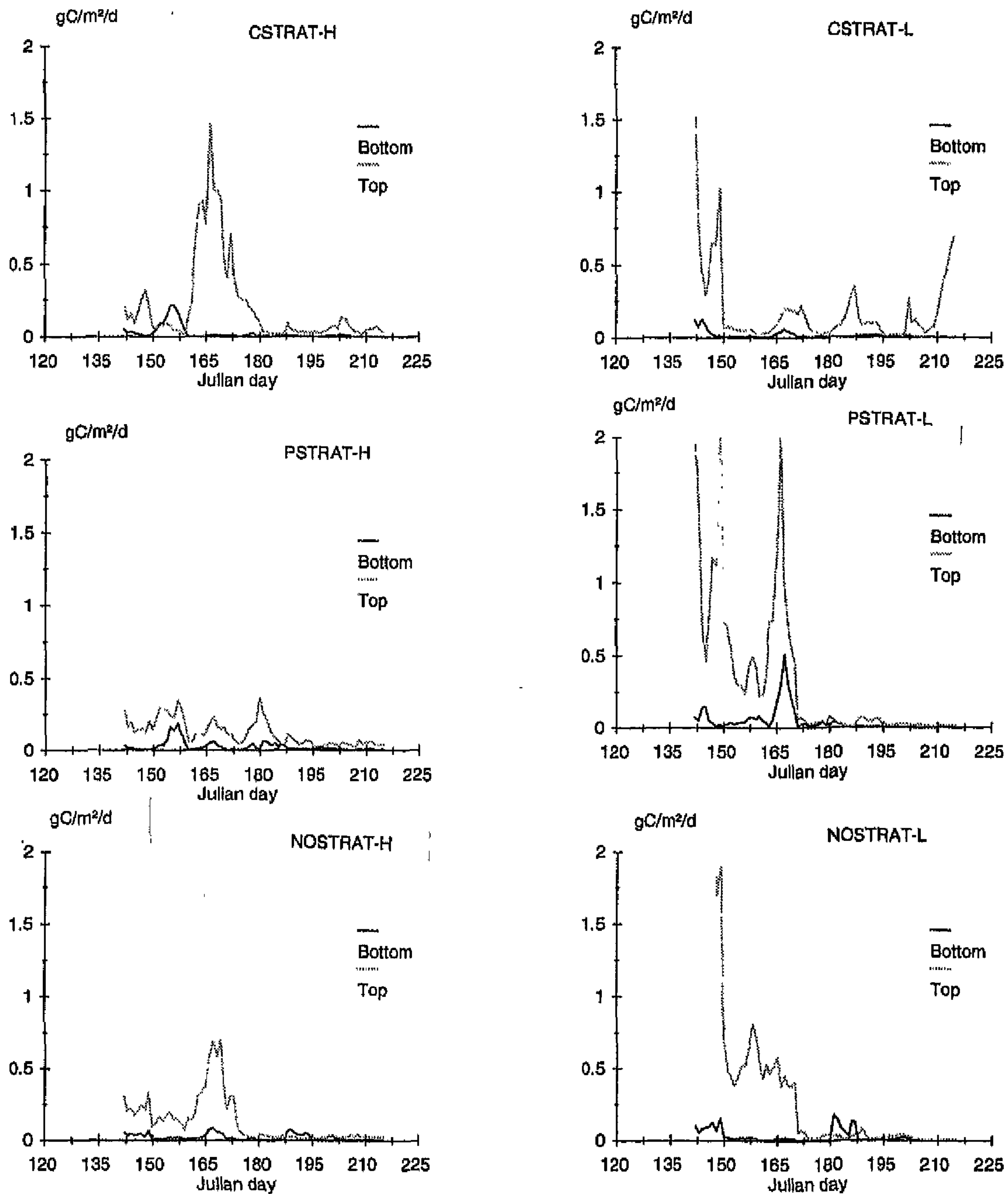
Figure 17
Chlorophyll-a concentrations in the six mesocosms.



3.5.2 Primary production and oxygen concentrations

Due to better light conditions, primary production rates calculated for Top were generallly much higher than those calculated for Bottom in all mesocosms (Figure 18). These graphs illustrate the prominent role played by the Top compartment for the mesocosm primary production when compared to the minor contribution from the Bottom compartment. Despite some small peaks, primary production rates were very low.

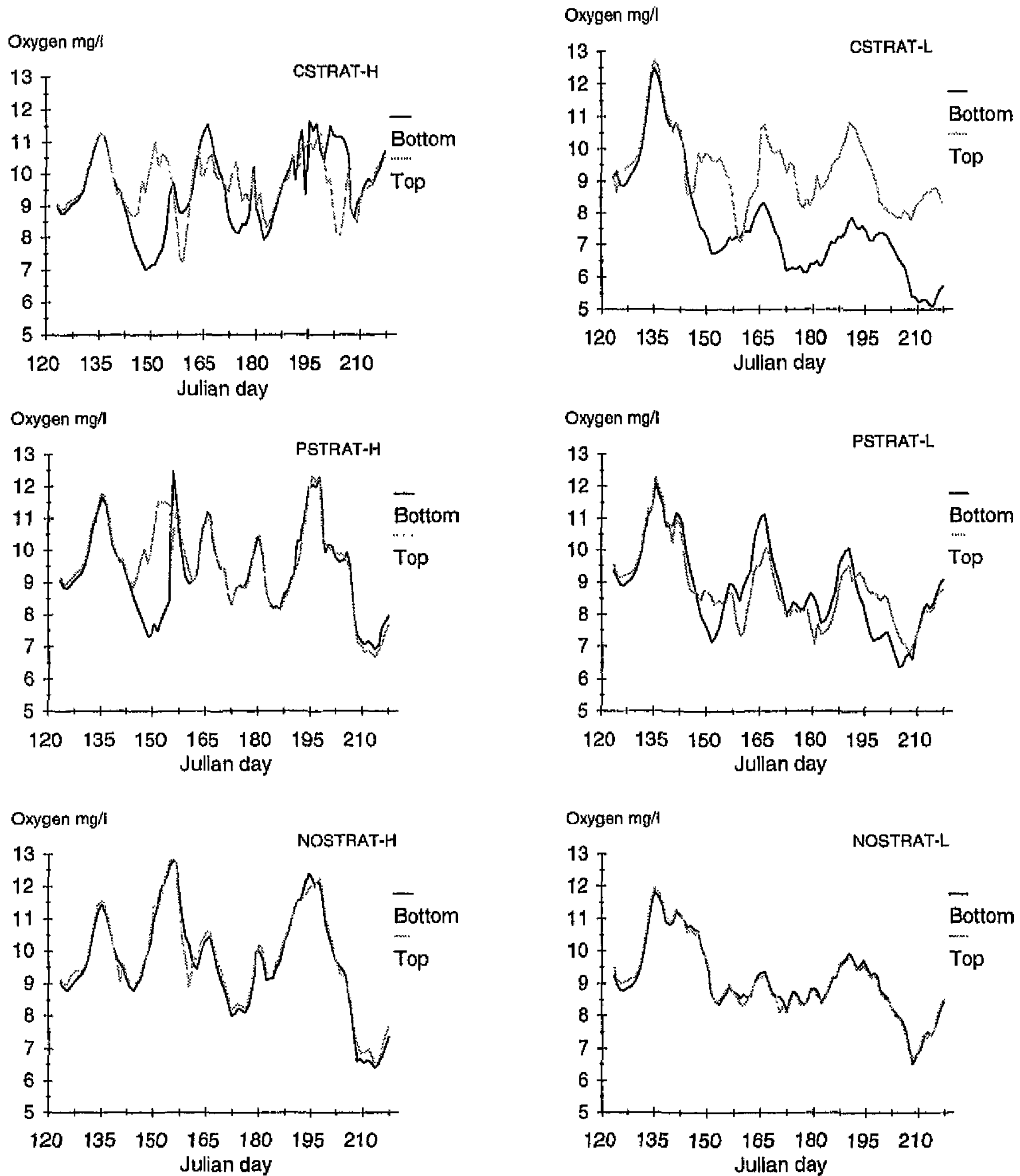
Figure 18
Daily primary production in the six mesocosms (H1, H2, M1,M2,L1,L2)



In CSTRAT-H, PSTRAT-L, NOSTRAT-L, primary production rates followed a similar evolution as the chlorophyll-*a* concentrations (compare Figures 17 and 18). Conversely, in mesocosms PSTRAT-H, CTRAT-L and to a less extent NOSTRAT-H some chlorophyll-*a* concentration peaks (between days 165 and 190) did not coincide with an increase in primary production rates. This observation suggested that part of the chlorophyll-*a* biomass increase measured in these mesocosms did not originate from the primary production

occurring in the pelagic compartment. A possible origin for this biomass could be fouling algae growing on the wall of the mesocosm and possibly brought in suspension during the cleaning of the mesocosm.

Figure19
Continuously measured oxygen concentrations in the six mesocosms.



Without stratification, the oxygen concentrations remained homogeneous throughout the water column (mesocosms NOSTRAT in Figure 19). When stratification was present, oxygen concentrations remained stable or increased in the Top

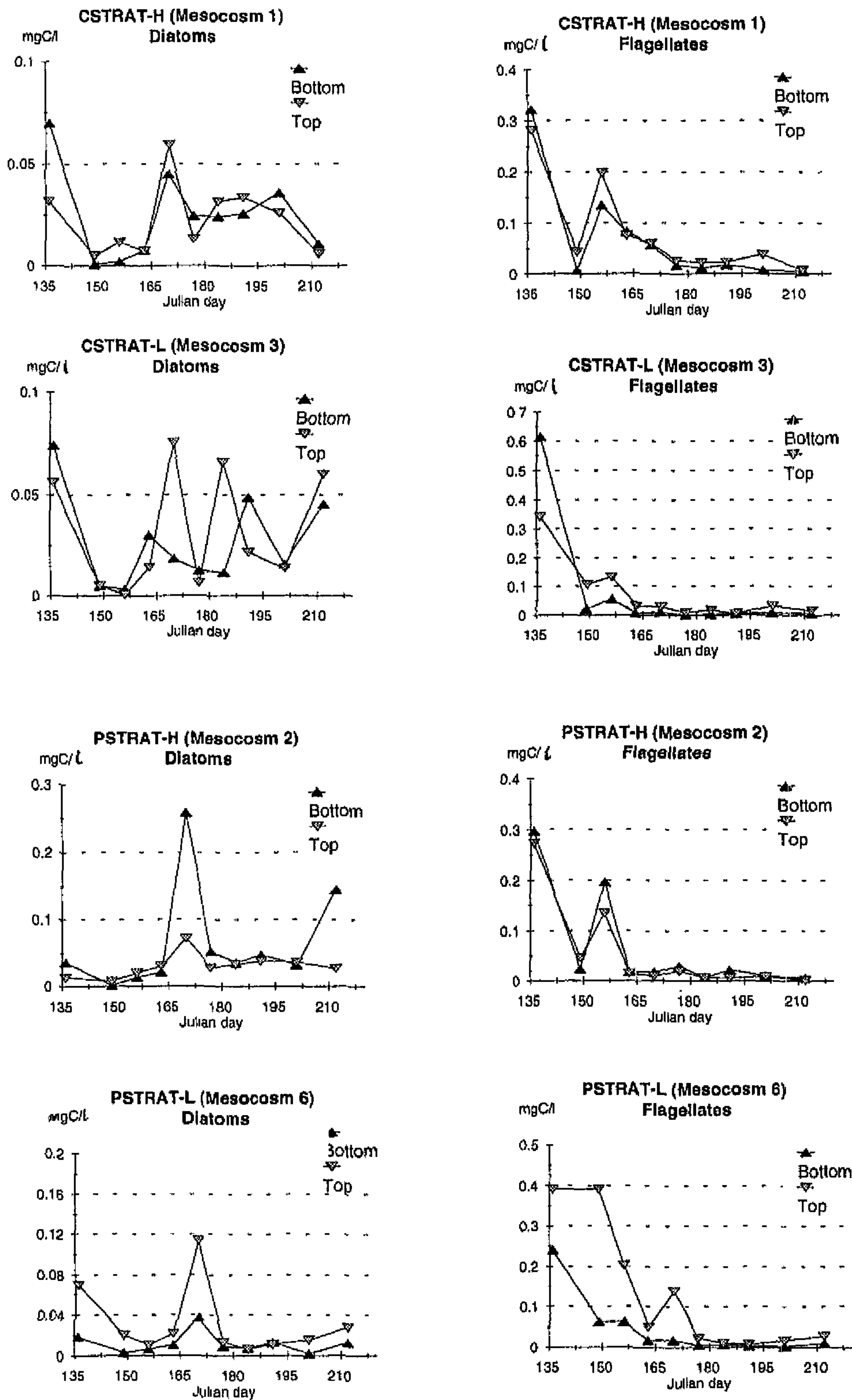
compartments and decreased in the Bottom compartments. The amplitude of this decrease was mainly dependent on the duration of the stratification period (maximum in CSTRAT-L). Changes (increase) in mean daily oxygen concentrations are mainly determined by the activity of all primary producers present in the system. Until day 150, oxygen concentrations evolved in a similar manner in the six mesocosms (Figure 19). From this date on and within each mixing regime, more and generally higher oxygen concentration peaks were observed in mesocosms with a high nitrogen load. Comparison between the changes in mean daily oxygen concentrations and the chlorophyll-*a*/primary production evolution should give an idea about the importance of the 'wall system' relatively to the pelagic system. The chlorophyll-*a* concentration peaks in PSTRAT-H (day 180), CTRAT-L (day 190), and to a lesser extent NOSTRAT-H (day 170), for which no corresponding primary production increase was observed, coincided well with peaks in oxygen concentrations. That could confirm the hypothesis that part of the primary production taking place on the wall of the mesocosm was exported to the water column. The increasing importance of the fouling flora during the course of the experiment is furthermore indicated by the development of oxygen concentration peaks occurring during the last three weeks of the experiment, together with a large uptake of nutrients, while chlorophyll-*a* concentrations in the water column were extremely low.

3.6 Phytoplankton species composition

3.6.1 Phytoplankton functional groups succession

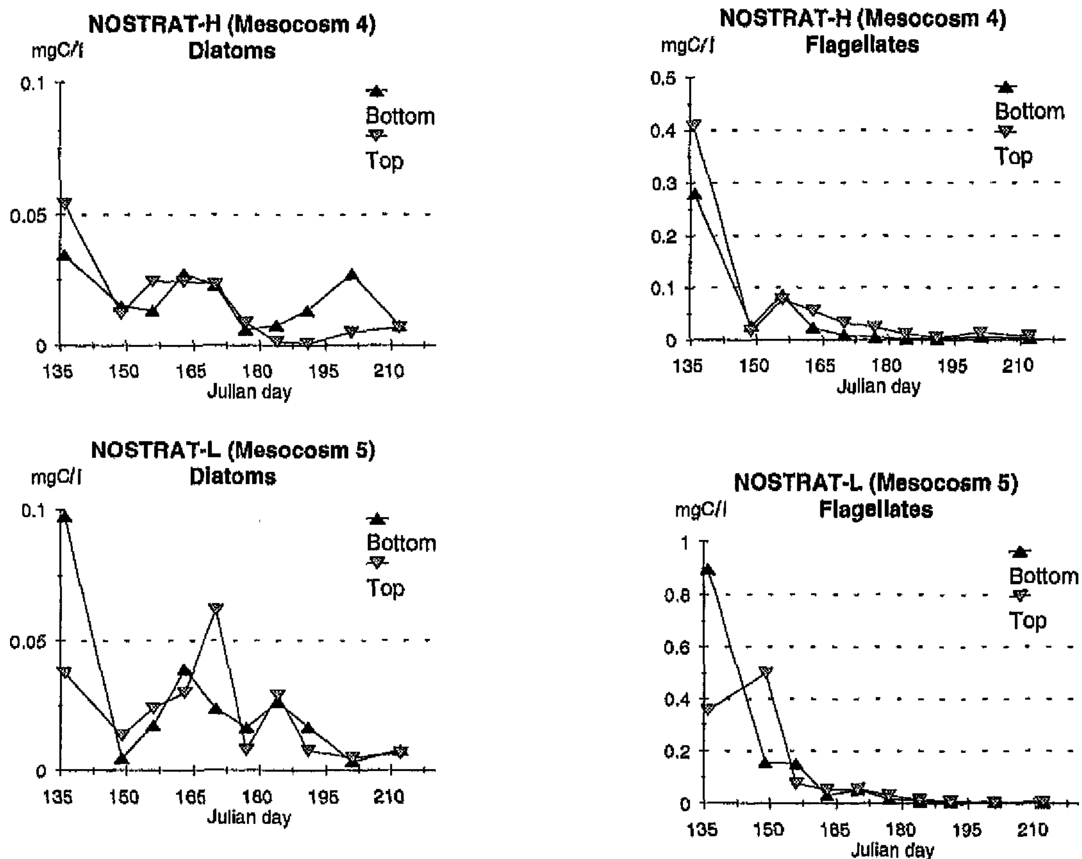
Two different functional groups were distinguished among the phytoplankters: flagellates and diatoms (respectively non-silicon and silicon using phytoplankton). Flagellates consisted mainly of three sub-groups: autotrophic nanoflagellates (2 to 20 μm), Cryptophyceae and *Phaeocystis sp.* represented more than 95% of the total flagellate biomass throughout the present experiment and in all mesocosms.
Flagellates had a relatively high biomass before day 150 in both mesocosms CSTRAT-H and CSTRAT-L (Figure 20). After that date flagellate biomass showed a second minor peak in CSTRAT-H and a very small one in CSTRAT-L. After day 165 in CSTRAT-L and day 180 in CSTRAT-H, the flagellate biomass remained very low. Flagellate biomass in Top and Bottom were similar.
After a drop up to day 150, diatom biomass increased again and formed two and three main peaks in CSTRAT-H and CSTRAT-L, respectively (Figure 20). The biomass values, measured in Top and Bottom, were close to each other in CSTRAT-H, whereas higher flagellate concentrations were observed in Top than in Bottom in mesocosm CSTRAT-L. Periods of strong stratification in CSTRAT-L (days 170 and 185) corresponded to higher diatom biomass in Top than in Bottom.

Figure 20
Total diatoms and total flagellates biomass (mgC/l) in mesocosms CSTRAT and PSTRAT.



Flagellate biomass in PSTRAT-H and PSTRAT-L (Figure 20) showed a similar development as in the CSTRAT mesocosms with relatively high levels up to days 165 and 180 in PSTRAT-H and PSTRAT-L, respectively. Before day 180, flagellate biomass was two times higher in Top than in Bottom in PSTRAT-L, whereas both compartments showed similar biomass in PSTRAT-H. Diatom biomass began to increase in both mesocosms after day 150 and reached peak values in both mesocosms on day 170 (Figure 21). Diatom peak concentration was higher in Top than in Bottom in PSTRAT-L, whereas the opposite pattern was observed in PSTRAT-H. On this date the water column was strongly stratified in PSTRAT-L, whereas it was well mixed in PSTRAT-H. Diatoms seemed to react positively on conditions in stratified mesocosms as suggested by the results in the CSTRAT mesocosms.

Figure 21
Total diatoms and total flagellates biomass (mgC/l) in mesocosms NOSTRAT-H and NOSTRAT-L.

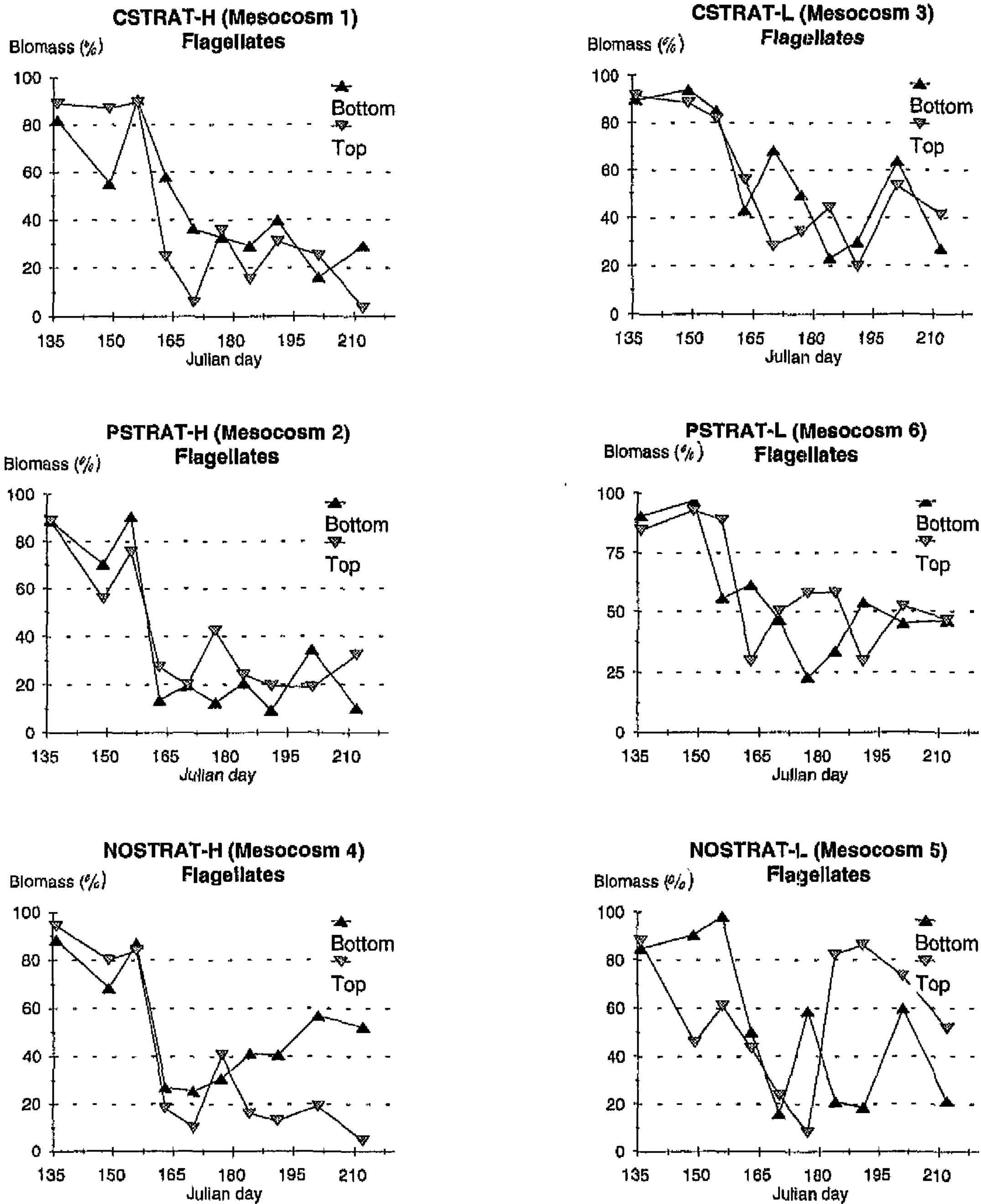


As in the other mesocosms, flagellate biomass in NOSTRAT mesocosms was highest before day 150 and did not significantly increase again after day 165 (Figure 21). After an initial decrease, diatom biomass increased in both NOSTRAT mesocosms and reached peak concentrations around day 170. In NOSTRAT-L, the peak of diatom biomass was higher in Top than in Bottom.
In most mesocosms, the relative proportion of flagellates in the

phytoplankton biomass decreased until day 165 (Figure 22). Periods when mesocosms CSTRAT-L and PSTRAT-L were stratified were characterized by a decrease of the flagellate fraction in the phytoplankton biomass.

Figure 22

Percentage of phytoplankton biomass due to flagellates in the six mesocosms.



3.6.2 Main diatom species succession

The relative importance of the first eleven diatom species (ranked per biomass) is illustrated by the Kite diagrams in Figures 23 a,b,c.

Figure 23a
Kite diagrams of the diatom biomass composition in mesocosms CSTRAT (Bottom and Top). The width of the dashed area is proportional to the contribution of the corresponding species or group of species to the total diatom biomass (See scale).

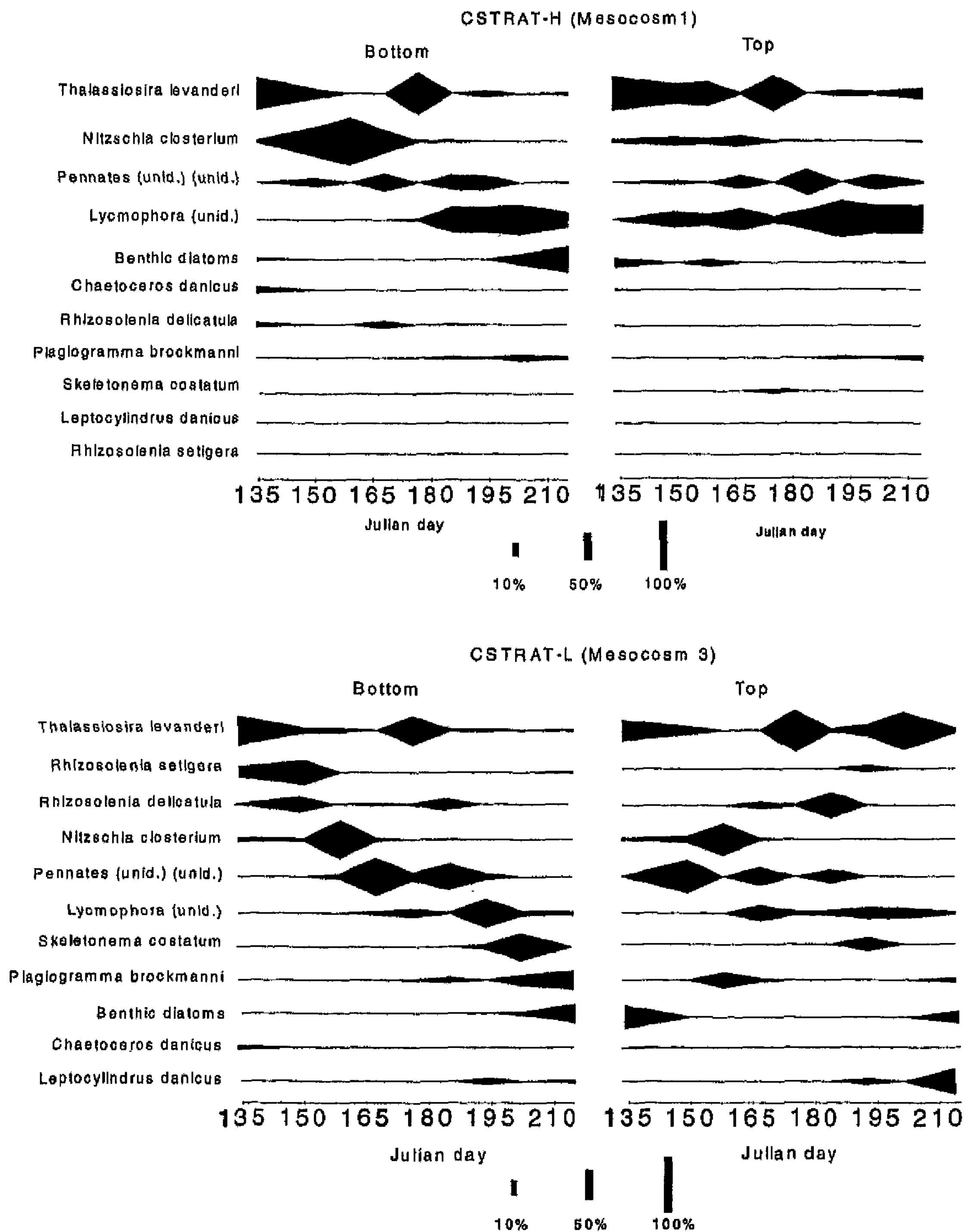


Figure 23b
Kite diagrams of the diatom biomass composition in mesocosms PSTRAT (Bottom and Top). The width of the dashed area is proportional to the contribution of the corresponding species or group of species to the total diatom biomass (See scale).

PSTRAT-H (Mesocosm 2)

Bottom

Top

Thalassiosira levanderi
Chaetoceros danicus
Nitzschia closterium
Pennates (unid.) (unid.)
Plagiogramma brockmanni
Lycmophora (unid.)
Benthic diatoms
Rhizosolenia delicatula
Leptocylindrus danicus
Skeletonema costatum
Rhizosolenia setigera

135 150 165 180 195 210 135 150 165 180 195 210
Julian day Julian day

10% 50% 100%

PSTRAT-L (Mesocosm 6)

Bottom

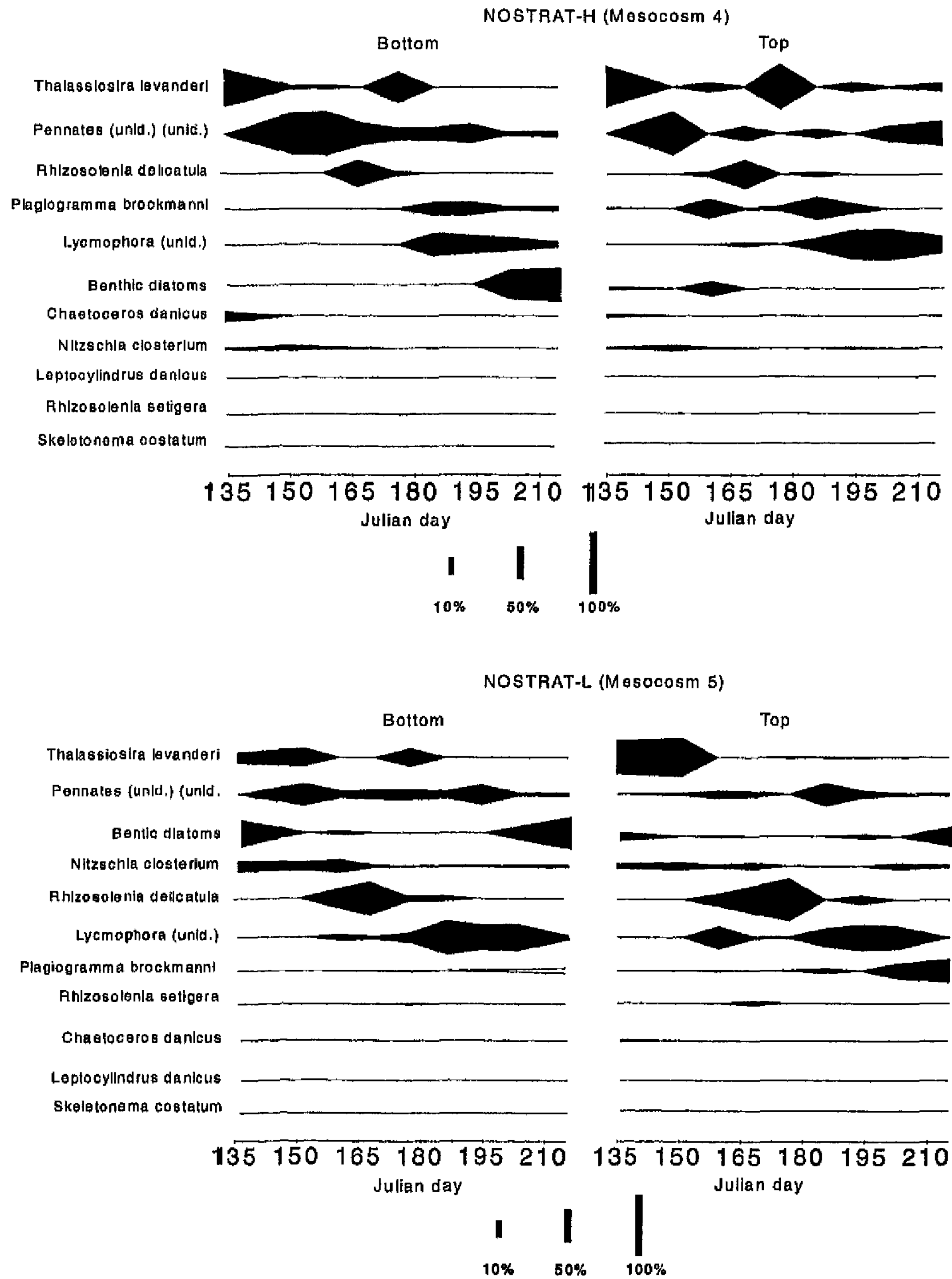
Top

Thalassiosira levanderi
Pennates (unid.) (unid.)
Nitzschia closterium
Chaetoceros danicus
Rhizosolenia delicatula
Plagiogramma brockmanni
Lycmophora (unid.)
Benthic diatoms
Rhizosolenia setigera
Skeletonema costatum
Leptocylindrus danicus

135 150 165 180 195 210 135 150 165 180 195 210
Julian day Julian day

10% 50% 100%

Figure 23c
Kite diagrams of the diatom biomass composition in mesocosms NOSTRAT-H and NOSTRAT-L (Bottom and Top). The width of the dashed area is proportional to the contribution of the corresponding species or group of species to the total diatom biomass (See scale).



In all mesocosms, whatever compartment considered, a similar diatom species succession was observed. *Thalassiosira levanderi* dominated the diatoms until day 150 and was gradually replaced by diatoms with benthic affinities (*Lycmophora sp., Plagiogramma brockmannii,* and benthic pennates). The higher diatom biomass observed in CSTRAT-L and PSTRAT-L in Top during the period when these mesocosms were stratified was due to *T. levanderi.*

3.7 Zooplankton abundance and biomass

3.7.1 Microzooplankton

Data for total microzooplankton biomass (6 mesocosms, Top and Bottom, without eggs and cysts) are presented in Figure 24. Biomass values for Top and Bottom samples were very comparable. Between days 130 and 150 biomass peaked in all mesocosms with maximum values between ca. 150 and 250 μgC/l. After this first peak the lowest biomass values in all mesocosms were found at day 165. After day 170 rather high biomass values were found in the three H-mesocosms with maxima between ca. 250 and 300 μgC/l, while in the three L-mesocosms maximum biomass values remained lower, especially in mesocosm CSTRAT-L.
Figure 25 presents the biomass data for the distinguished microzooplankton groups, analyzed in the Bottom samples. The results from the Bottom samples form the most complete data-set. Besides that, the results in terms of microzooplankton composition and biomass the same pattern existed for the analyzed Top samples. During the first peak important contributors to microzooplankton biomass were heterotrophic dinoflagellates (especially *Gymnodinium sp.* and *Gyrodinium sp.*) and aloricate ciliates (mainly *Strombilidium sp.* 2. After day 170 microzooplankton biomass almost exclusively consisted of the heterotrophic dinoflagellate *Oxyrrhis marina*, especially in the three H-mesocosms and in mesocosm NOSTRAT-L. *Oxyrrhis marina* hardly occurred in mesocosm CSTRAT-L. The remaining groups (other heterotrophic dinoflagellates, loricate and peritrichous ciliates) hardly contributed to microzooplankton biomass.

3.7.2 Mesozooplankton

As explained in section 2.2.8 our long PVC-tube allowed no routine sampling during stratification. Only the NOSTRAT-mesocosms were sampled during the entire experimental period. Mesozooplankton biomass in mesocosm NOSTRAT-H reached a maximum value of ca. 75 μgC l^{-1} during the first half of the experiment and remained low after day 175 (Figure 26). In mesocosm NOSTRAT-L mesozooplankton biomass declined steadily from the initial maximum of ca. 60 μgC l^{-1} and reached a low value at day 170, after which biomass values remained very low (Figure 26).
In both NOSTRAT-mesocosms large (ca. 400-600 μm) cirriped larvae represented a high biomass at the start of the 1996 experiment, but biomass of cirripeds decreased within two to three weeks. Because we use one biomass value for the cirriped larvae $<$ 400 μm and one for larvae $>$ 400 μm (2.5, respectively 11 μgC animal^{-1}, compare with Rodhouse & Roden, 1987), there is some uncertainty whether or not cirriped biomass values are overestimated.

Figure 24
Microzooplankton biomass (without eggs and cysts) in µgC/l during the 1996 experiment in the six mesocosms in the upper (Top) and lower layers (Bottom). Day 130 = 9 May and day 220 = 7 August.

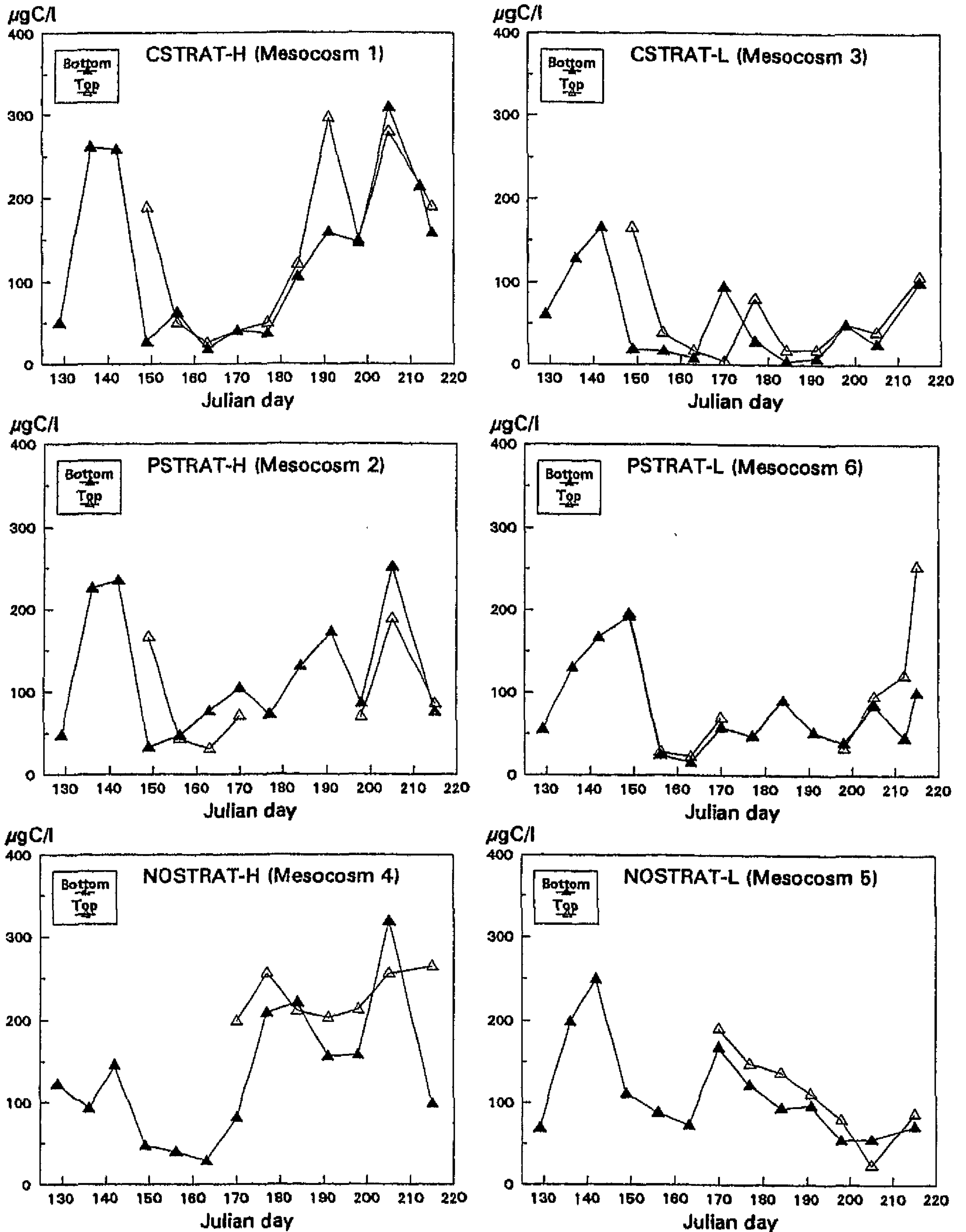
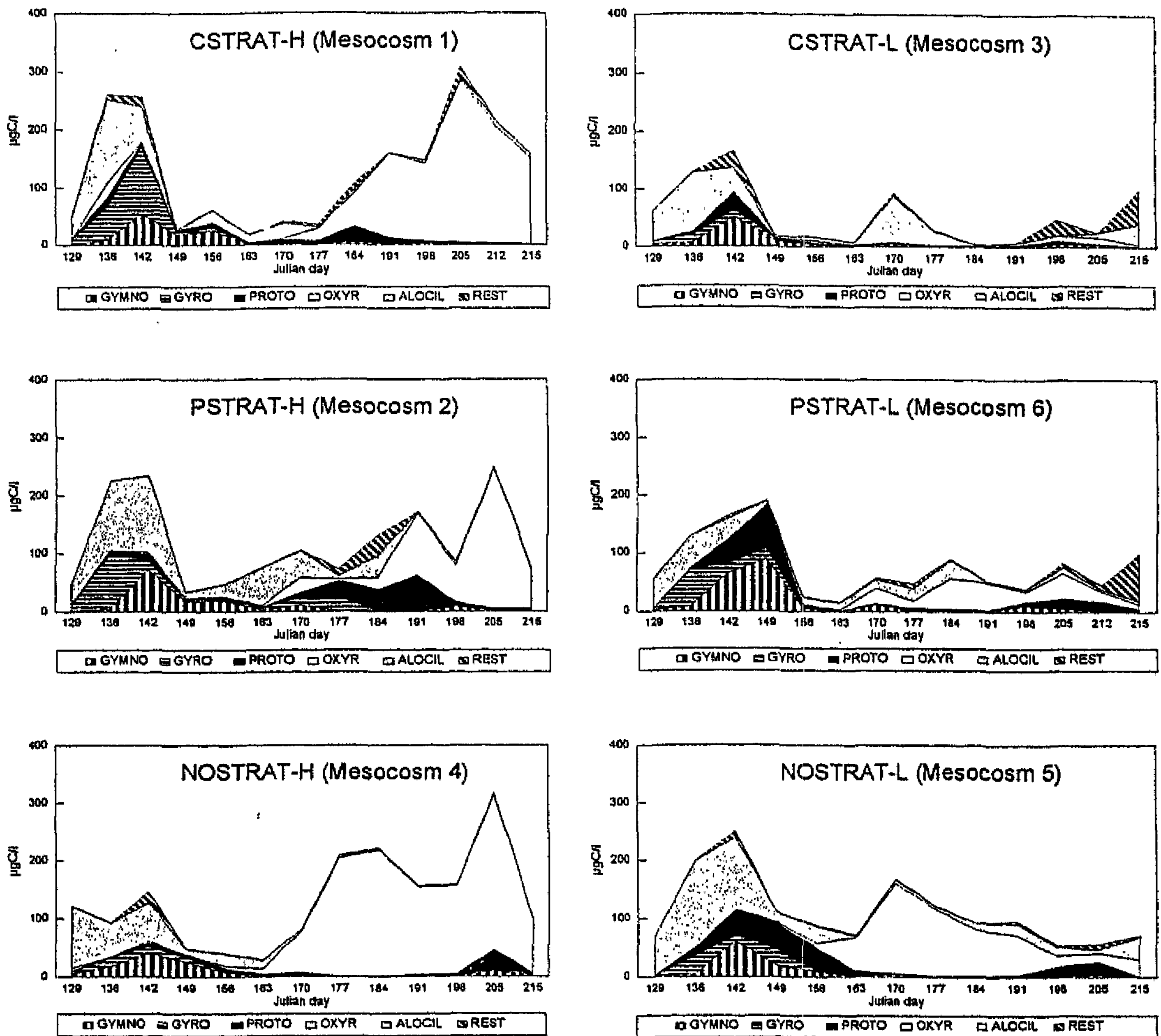
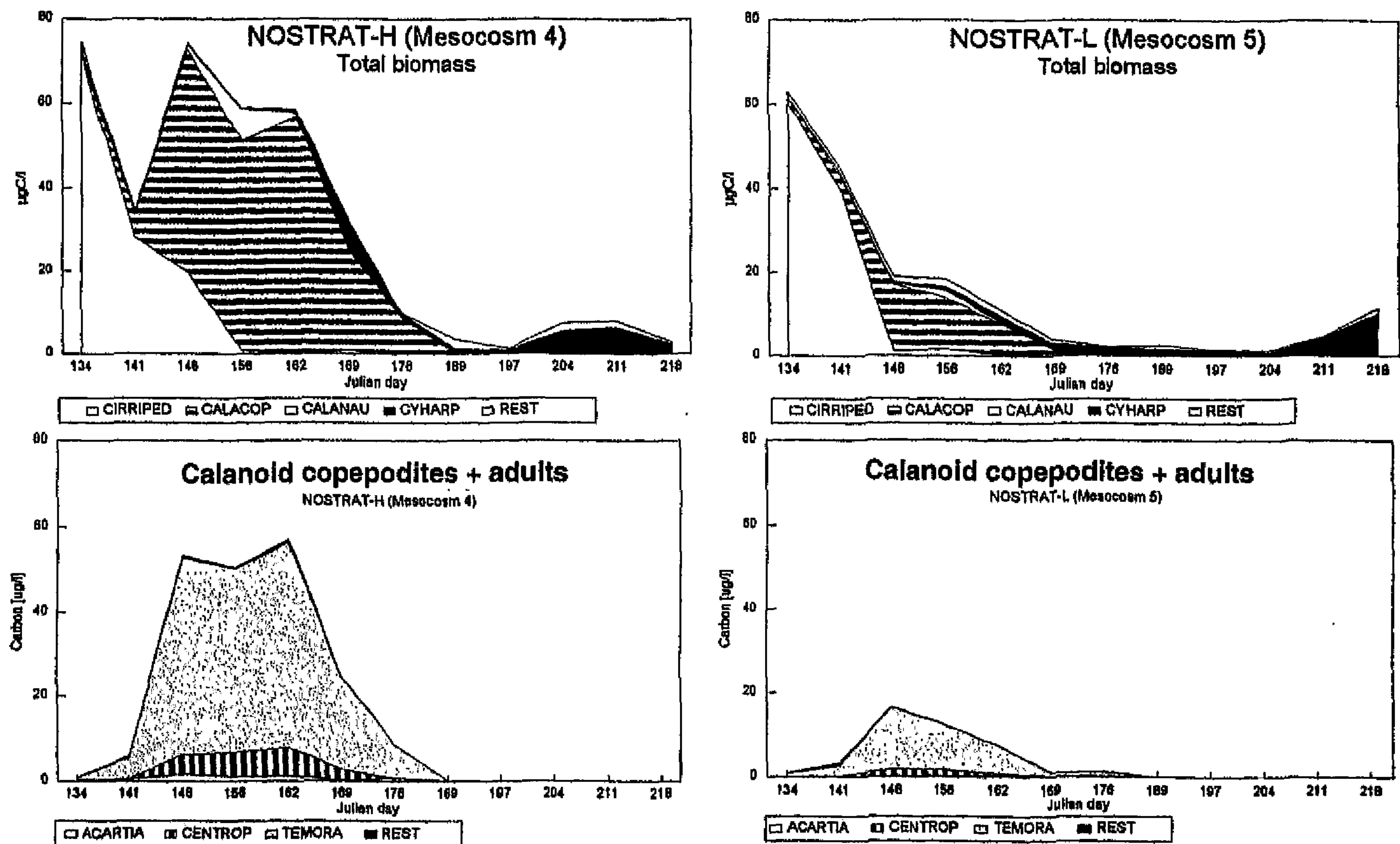


Figure 25
Microzooplankton biomass (without eggs and cysts) in µgC/l during the 1996 experiment in the six mesocosms in the lower (Bottom) layer. Day 129 = 8 May and day 215 = 2 August. GYMNO = *Gymnodinium sp.*, GYRO = *Gyrodinium sp.*, PROTO = *Protoperidinium sp.*, OXYR = *Oxyrrhis marina*, ALOCIL = aloricate ciliates and REST = other heterotrophic dinoflagellates + loricate and peritrichous ciliates.



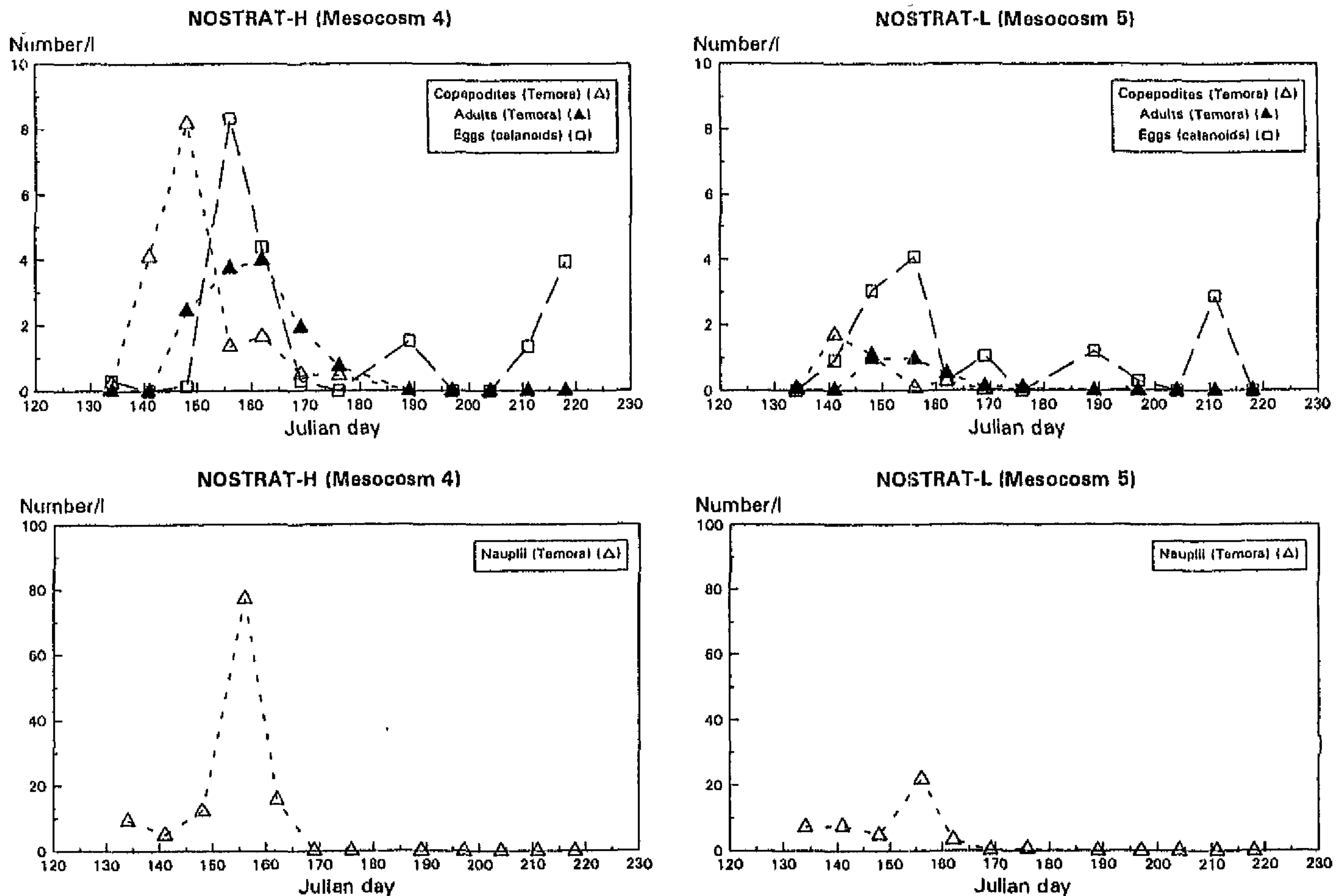
Also at the start of the experiments in 1997 a high biomass (and numbers) of (small) cirriped larvae occurred, but not at the start of the 1994 and 1995 experiments.
Parallelling the decrease of cirripeds, calanoid copepodites + adults gain in importance and between days 140 and 175 (mesocosm NOSTRAT-H), respectively day 170 (mesocosm NOSTRAT-L) almost all mesozooplankton biomass is formed by calanoid copepodites + adults. Biomass of cyclopoid and harpacticoid stages increased somewhat at the end of the experiment to values of ca. 10 µgC l^{-1}.

Figure 26
Total mesozooplankton biomass (without eggs and cysts, upper panels) and calanoid copepodites + adults biomass (lower panels) in μgC/l during the 1996 experiment in the two NOSTRAT-mesocosms. Day 129 = 8 May and day 215 = 2 August. Upper panels: CIRRIPED = cirriped nauplii and cypris larvae, CALACOP = calanoid copepodites + adults, CALANAU = calanoid nauplii, CYHARP = cyclopoid + harpacticoid nauplii + copepodites + adults and REST = rest. Lower panels: ACARTIA = *Acartia sp.*, CENTROP = *Centropagus hamatus*, TEMORA = *Temora longicornis* and REST = other calanoids.



Biomass data on calanoid copepodites + adults in both NOSTRAT-mesocosms, subdivided to the species level, are also presented in Figure 26 (lower panels). *Temora longicornis* is the most important contributor to mesozooplankton biomass, *Centropagus hamatus* en *Acartia sp.* are of less importance.
Figure 27 shows data on calanoid copepod eggs and *Temora longicornis* stages in both NOSTRAT-mesocosms. The observed numbers in mesocosm NOSTRAT-H are two to three times larger than in mesocosm NOSTRAT-L. In mesocosms NOSTRAT-H and NOSTRAT-L, the number of eggs l^{-1} are much lower when compared with maximum values of ca. 50-80 eggs l^{-1}, reported by van Rijswijk *et al.* (1989) for the Oosterschelde (western part); the observed amounts of *Temora* nauplii, copepodites and adults are very similar to values that occurred (during a comparable period) in the western part of the Oosterschelde (Bakker & van Rijswijk, 1987).

Figure 27
Observed number of calanoid copepod eggs (eggs/l) and *Temora longicornis* stages (number/l) during the 1996 experiment in the two NOSTRAT-mesocosms. Day 129 = 8 May and day 215 = 2 August.



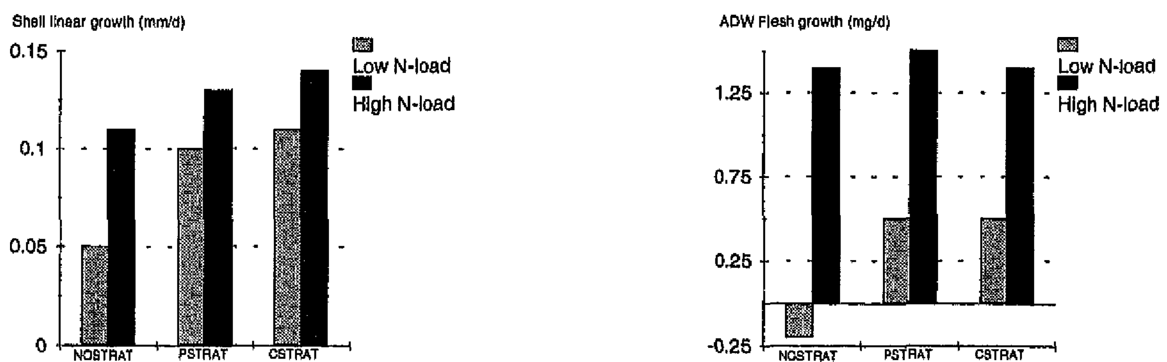
In the samples with highest amounts of calanoid eggs (between days 140 and 170), most of the adult females were *Temora* females, leading to the assumption that most of the calanoid eggs were *Temora* eggs. Thus the development of *Temora* from eggs to adults can most easily be explained starting at the copepodite stages. Adults, developed from these copepodites, are producing eggs from day 140 onwards, leading to hatched nauplii from day 145 onwards. The number of eggs in the water column is much lower than the number of nauplii found in the samples; very probably, this is due to sedimentation of eggs to the bottom of the mesocosms.
The origin of the copepodites, cannot be explained easily. Some of

the copepodites (I-III) may have developed between days 134 and 140 from the larger nauplii, which comprised one third of the total number of nauplii during this period. Unfortunately, samples of the water used to flush the mesocosms (100 l day^{-1}) are lacking for this period. However, it can be estimated that for the observed increase of *Temora* copepodites (in mesocosm NOSTRAT-H) a number of ca. 15 copepodites l^{-1} flushing water is needed. Bakker & van Rijswijk (1987) reported maxima between ca. 8 and 12 *Temora* copepodites l^{-1} for this time of the year in the Oosterschelde mouth. Thus, it is possible that the increase of *Temora* copepodites between days 134 and 148 can also partly be explained by import from the sea water used to flush the mesocosms.
Continuous stratification was most successful in mesocosm CSTRAT-L (see Figure 4). On the last experimental day mesozooplankton in this mesocosm was sampled with the PVC-tube, taking ca. 27 l in each layer (instead of ca. 50 l). Unfortunately, mesozooplankton biomass appeared to be very low: 8.9 μgC l^{-1} (a.o. 1.6 μgC l^{-1} harpacticoids and 6.6 μgC l^{-1} polychaete larvae) in the Top sample and 3.8 μgC l^{-1} (a.o. 1.4 μgC l^{-1} harpacticoids and 1.5 μgC l^{-1} polychaete larvae) in the Bottom sample. These values are too low to draw conclusions whether or not mesozooplankton biomass differed in the Top and Bottom layers of this continuously stratified mesocosm.

3.8 Mussel growth

An estimate for the mussel growth was provided by length/weight measurements performed before and after the experiment on the mussels placed in the grazing chamber of each mesocosm (See Figure 1).

Figure 28
Mean mussel shell and flesh growths estimated from measurements made before and after the experiment expressed as shell linear growth (mm/d) and ADW flesh growth (mg/d).



Shell growth reacted positively to both nutrient loading and intensity of stratification (Figure 28). Shell length growth in NOSTRAT-L was significantly lower than growth in all other mesocosms; growth in CSTRAT-H was significantly higher than growth in all other mesocosms.
The growth in ash-free dry weight showed a similar pattern of

effects of both nutrient loading and stratification. In NOSTRAT-L growth was negative and rates differed significantly from all other mesocosms. The growth rates in PSTRAT-L and NOSTRAT-L were significantly lower than the growth rates in the mesocosms with high nutrient loading. With high nutrient loading, there were no significant effects of stratification.
Data on concentrations of particulate matter (POC, PN and chlorophyll-*a*) at the inflow of the benthos chambers showed no differences between mesocosms at all. Rates of primary production, measured by the ^{14}C-method, were different between treatments, but these differences did not show any correspondence with the observed pattern in growth rates.

4 EVALUATION

This pilot experiment provided much information about critical aspects related to the study of stratification in experimental mesocosms. The main items highlighted in this report are listed below together with the measures to be taken to optimize the experimental set-up to be used in 1997.

4.1 *Methodological evaluation*

Stratification
Although the maintenance of stratification was not always successful, phytoplankton biomass and activity positively reacted to the treatment. The fact that differential vertical distribution of phytoplankton biomass was also observed in the NOSTRAT mesoscosms is due to the relatively low mixing rate (3 rpm) used in all mesocosms during this experiment. At such a low mixing rate (requested to maintain stratification), the specific buoyancy of phytoplankton could allow its differential distribution over the water column in the NOSTRAT mesocosms.
A series of tests has been run during autumn 1996 to define a standard procedure for the establishment of stratification. This goal has been successfully reached and we have now developed a standard procedure for the establishment of a salinity gradient (2‰ over 20 cm) steeper than the minimum $dS = 0.03‰\ cm^{-1}$ computed from the present experiment.
During the next experiment (1997), the rotation speed of the mixer in the NOSTRAT mesocosms will be set to 10 rpm (similar to previous well-mixed mesocosm experiments) whereas it will remain 3 rpm in the STRAT-mesocosms.

Fresh water quality
Great attention will be given in 1997 to the quality of the fresh water used for the mesocosms experiment to avoid any new pollution with exogenous nutrients.

Anti-fouling system
A new anti-fouling system has been developed and successfully tested this autumn. It should prevent any important development of fouling organisms (mainly benthic diatoms) on the inner wall of the mesocosm. All parts of the mixer will be coated with a new teflon based product which should hamper the fixation of fouling algae on

its surface.

Zooplankton
The PVC-tube used last years for depth integrated sampling of mesozooplankton could not be used in stratified mesocosms for routine sampling.
During the 1997 experiments a submersible pump (flow ca. 200 l minute^{-1} has been used to sample mesozooplankton at a fixed depth; mesozooplankton, collected in this way, remained undamaged.

Miscellaneous
Mass culture plastic bags will be used in 1997 to culture *Prorocentrum micans* and insure a sufficient size of the inocula and to limit the risks of contamination.
The experiment planned for 1997 will include duplicated treatments to allow further statistical analysis.

4.2 Ecological evaluation

Phytoplankton
Phytoplankton significantly reacted on stratification as indicated by fluorescence and oxygen concentrations. Some phytoplankton samples were checked to estimate the development of *Prorocentrum micans* in the mesocosm. Very few individuals could be found, pointing at a poor development of this dinoflagellate in the mesocosm. One reason for this poor development could have been the relatively low temperature this summer. In May, the temperature of the Oosterschelde was 3°C lower than the average for this time of the year.
Since nutrients (mainly N and Si) were accidentally added in the stratified mesocosms, it is difficult to estimate to what extent the observed effects were due to the mixing regime or to the extra nutrient additions. Interpretations were even complicated by the presence of a fouling flora that successfully competed for nutrients with phytoplankton under the low mixing regime used in this experiment.
Nevertheless, some response of phytoplankton biomass and primary production could be ascribed with a large degree of confidence to one of the factors: nutrient addition or mixing regime. Chlorophyll-*a* peak concentrations were generally higher in mesocosms with a high N-load (treatments NOSTRAT and PSTRAT). In the same vein, the amplitude of the mean daily oxygen concentration peaks indicated an increase of the primary production rates with the nitrogen loading (treatments NOSTRAT and PSTRAT).
The low mixing regime used in this experiment allowed a concentration of the phytoplankton biomass in the Top compartments of the mesocosms, stratified as well as not stratified. The main specific effects due to the stratification were the larger chlorophyll-*a* and oxygen concentrations measured in the Top when compared to the Bottom compartment in mesocosm CSTRAT-L. This last observation illustrated the efficiency of the physical obstacle created by the saline stratification against the exchanges between the two compartments.
Nutrient additions and mixing regime did also have evident effects on phytoplankton species composition. The additional silicate loading

brought with the fresh water was certainly one of the main factors explaining the low flagellate/diatom biomass ratio in mesocosms with stratification. The relative low flagellate concentrations also observed in the non stratified mesocosms could result from the low N and P concentrations since flagellates have been shown to be bad competitors against diatoms under nutrient limitation in comparable mesocosm experiments (Escaravage *et al.*,1995, 1996).
The low mixing regime used in this experiment could have been partly responsible for the shift observed within the diatom community towards species with benthic affinities. The low mixing regime used in this experiment acted as a selective process in favour of fouling organisms, able to literally stick to the well illuminated Top compartment, whereas pelagic diatoms experienced losses through sedimentation.

Zooplankton
In most cases microzooplankton biomass values for Top and Bottom samples were very comparable. This was, for example, not the case in samples from the (thermally) stratified Oyster Grounds (depth ca. 45 m), where in many cases the Top samples contained much less microzooplankton. Besides that, in the Top layer more smaller species were found than in the Bottom layer (see for example in Tripos, 1997). Data on *Oxyrrhis marina* (see Figure 25) suggest that this species does not thrive well under stratified conditions.
At this moment a point of discussion is whether or not the high initial numbers of cirriped larvae originate from fouling cirripeds in the tubing system of the field-station.

Mussels
The mussels showed significant treatment effects on the growth rates. First of all, both shell length growth and growth in AFDW reacted positively to nutrient loading. Secondly, growth rates in the systems with stratification were generally higher than in the well-mixed systems. The positive effect of nutrient loading was probably caused by higher primary production in these systems. This is not apparent from the measured primary production rates, as a consequence of the high production by benthic algae on the walls of the tanks, which is not included in the ^{14}C-measurements.
The positive effect of stratification may be attributed to several factors. More stratification may have caused a higher flux of settling material towards the benthos, resulting in higher food availability for the mussels. However, data on particulate matter concentrations at the inflow of the benthos chambers did not clearly indicate differences in concentrations between mesocosms. The stratified systems showed a change in phytoplankton composition with a higher dominance of diatoms. This was mainly due to the additional input of nutrients with the addition of demineralized water, which still contained considerable amounts of Si. This change in phytoplankton composition may have had a positive effect on mussel growth, but the enhanced growth may also have been caused by increased primary production related to the higher nutrient input.

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