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III

ENERGY FLOW THROUGH THE MEIOBENTHOS

C. HEIP, P.M.J. HERMAN, N. SMOL, D. VAN BRUSSEL and G.VRANKEN

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Abstract.

The estimation of energy flow through meiobenthic populations is particularly difficult as only a few measurements on production and respiration, mainly of harpacticoid copepods and nematodes, exist. The use of a single production-biomass P/B-ratio for meiobenthos is still a common practice. However, studies presented in this paper for a variety of harpacticoid copepods, ostracods and nematodes demonstrate that there exists an extreme diversity in life-cycle histories and respiration rates in these groups and that meiobenthic production is in many cases much higher than the value P = 9B proposed by Gerlach (1971).

Introduction.

Energy flow through the benthos is the most important force structuring this system. In the North Sea, without benthic primary production, the input of organic material is by sedimentation from the water column. In the classical model of Steele (1974) this sedimentation was estimated at one third of primary production, which in the northern North Sea amounts to 90 g $C.m^{-2}.y^{-1}$. In the Southern Bight a similar model was based on primary production estimates of 110-187 g $C.m^{-2}.y^{-1}$ and a sedimentation rate of 37-62 g $C.m^{-2}.y^{-1}$. Energy flow through the benthos was based on very indirect measurement of biomass and literature data on production and respiration (Van Damme & Heip, 1977) ; it was calculated that total benthic production amounted

to 7.7 g $C.m^{-2}$, total benthic respiration (excluding bacteria) to
11.1 g $C.m^{-2}$. Energy consumption by benthic animals should then
be about 31 g $C.m^{-2}.y^{-1}$, amounting to 50-90% of sedimentation.
However, when bacteria are added as an additional trophic level
between detritus and benthos, with an ecological efficiency of
20%, the required input in the system should be 150 g $C.m^{-2}.y^{-1}$.
Clearly then, when the basic model is accepted some of the 1977
estimates should be revised.

As will be demonstrated in other reports in this volume, pri-
mary production and sedimentation in the Southern Bight of the
North Sea are indeed higher than previously estimated. The re-
search efforts in our laboratory were concentrated on the pro-
duction and respiration of meiofauna, as the estimates existing
in 1977 were extremely inaccurate, whereas figures of macro- and
epifauna were based on much more solid grounds and have been re-
tained in recent calculations.

Despite their numerical abundance in all benthic ecosystems,
the functional role of meiobenthos in these systems is still
poorly known. Several potentially important roles have been
ascribed to the meiofauna : food for higher trophic levels, nu-
trient regenerators and intermediates in detritus transfer to
macrofauna (Coul & Bell, 1979).

None of these roles, however, has been quantified. Even the
most basic information on the energy flow through meiobenthic
populations is almost entirely lacking : namely how much energy
enters the population, and in what form is it subsequently lea-
ving it?
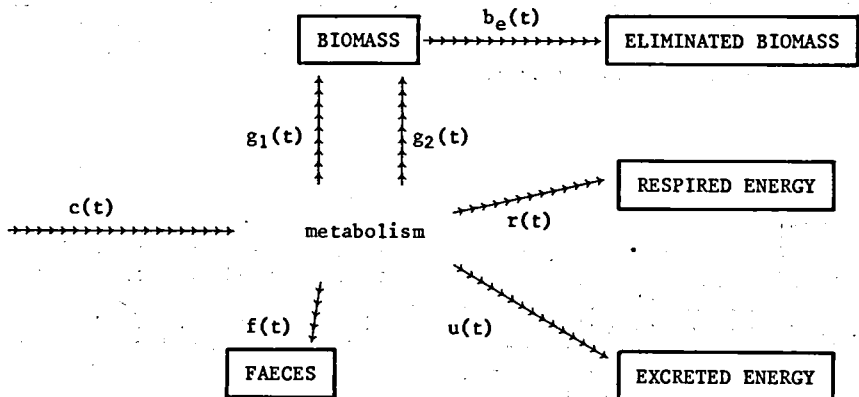
fig. 1.

Energy flow through biological populations

In figure 1 the partitioning by metabolic processes of the
energy entering a population as consumption [consumption flux
$c(t)$] is shown. Part of this energy is used for somatic growth
[flux $g_1(t)$] and reproductive output [flux $g_2(t)$]. Both fluxes
increase the biomass (standing stock) of the population which is
decreased by the elimination of individuals or parts of indivi-
duals from the population. This eliminated biomass goes to higher
trophic levels or to the decomposer food chain. It consists of
high energy content organic material. The rest of the incoming
energy is rejected as faeces $f(t)$, lost in low energy content
excretion products $u(t)$ or dissipated as heat in respiratory
processes $r(t)$. In terms of energy content, conservation
principles require that

(1) $c(t) = g_1(t) + g_2(t) + f(t) + u(t) + r(t)$

Secondary production of a population is the integration of the
fluxes $g_1(t)$ and $g_2(t)$ over the interval of time $t_2 - t_1 = \Delta t$
considered :

(2) $P = \dfrac{1}{\Delta t} \displaystyle\int_{t_1}^{t_2} [g_1(t) + g_2(t)] \, dt$

Production thus depicts the flow of the ecologically "usefull"
energy through the population. Losses of energy in respiratory
activity and excretion are the "price" at which this flow-through
is realized.

The direct measurement of production involves the use of
theoretical models fitted to density and biomass data of the
population. These models take a simple form in the case of co-
hort populations, where reproduction is synchronuous and occurs
over relatively short periods. However, when reproduction is
continuous, as is the case in most meiobenthic populations, the
estimation of production is highly complicated. For different
instars or size classes one has to estimate either recruitment
and mortality, or the duration of the stage. Alternatively,
growth rates determined in laboratory experiments can be used.
In either case, the field population must be sampled frequently.

Probably because of these technical difficulties only two
production estimates of meiobenthic animals have appeared to
date : Feller (1977) (cited in Banse & Mosher, 1980) reports on
the production of the harpacticoid copepod *Huntemannia jadensis*,
and Fleeger & Palmer (1982) studied the production of *Microar-
thridion littorale*, another harpacticoid. Although nematodes
are the numerically most important meiobenthic taxon, direct pro-
duction estimations of nematode populations have not yet been
reported. In this report we present direct production estimates
of some populations from a brackish water habitat : two harpacti-
coid copepod species, *Tachidius discipes* and *Paronychocamptus na-
nus*, and one ostracod species, *Cyprideis torosa*.

It is practically impossible to sample marine populations as
frequently as needed for direct production estimates. Therefore

indirect methods, approximating the production from easily mea-
surable parameters should be of great practical interest. Two
approaches may be useful in this respect : the estimation of the
yearly production/biomass ratio from the body weight at sexual
maturity (Banse & Mosher, 1980) and the estimation of production
from population respiration (McNeill & Lawton, 1970 ; Humphreys,
1979). These (log-log) relationships are purely empirical, so
it is necessary that enough data on production, body mass and
respiration are available to estimate their parameters accurate-
ly. Therefore we measured the respiration of the species men-
tioned. In addition, these respiration data complete the picture
of the energy flow through the populations.

When even less information is available production estimates
may be based on an annual P/B-ratio based on the life-cycle turn-
over (which, according to Waters (1969), lies between two and
five with a modal value of 3.5) multiplied by the annual number
of generations the populations produces in the field. The annual
number of generations itself may be estimated directly from the
life history of the species in the field but when this is also
impossible laboratory experiments in which the influence of tem-
perature on generation time is measured can be used. In this
last case it is clear that the outcome should be treated with
caution, but in the case of many marine nematodes cultivation in
laboratory conditions is unavoidable when one wants to obtain
accurate knowledge about dynamic aspects of their life cycles
(e.g. fecundity, embryonic and postembryonic development, morta-
lity, growth).

Material and methods.

The Southern Bight area has been described in our first pro-
gress report (Heip *et al.*, 1979) where also the field methods
were explained in detail. Production estimates of North Sea
subtidal meiobenthos are all based on density and biomass data
from a number of stations sampled in principle four times a year.
The detailed results of these cruises will be published later and
only a synthesis of some of the more important results will be
given in Herman *et al.* (this volume).

The populations used in direct production estimates, were
studied in the "Dievengat", a very shallow (about 10 cm) brack-
ish water pond, situated in a polder in north-western Belgium,
also described in Heip *et al.* (1979).

Samples were taken with a glass core (surface area 6.06 cm^2)
to a depth of 5 cm. After being transferred to the laboratory,
they were fixed in a neutralized isotonic 4% formaldehyde solu-
tion, heated to 70°C. The animals were extracted according to

the method described by Heip *et al.* (1974), except that centri-
fugation was done with LUDOX, a silica-gel, instead of sucrose
(De Jonge & Bouwman, 1977). For *Cyprideis torosa*, samples were
taken fortnightly from 1970 through 1974 ; for *Tachidius discipes*,
every three days during the spring of 1979 ; for *Paronychocamptus
nanus*, every five days from March to November 1980.

In the same habitat two nematode species were studied taking
monthly samples during four years. Both are members of the order
Enoplida. *Oncholaimus oxyuris* Ditlevsen, 1911 is the dominant
predator in the habitat and of primary importance in terms of
biomass (it makes up for the largest part of the total biomass
over the year) ; *Viscosia viscosa* Bastian, 1865 is an important
euryhaline species and member of a cosmopolitan genus. Both are
regarded as omnivorous species.

For cultivation of marine nematodes we used bacto-agar. The
great advantage of this material is that the animals can be ob-
served individually during their entire life. Sixteen species
belonging to thirteen genera were cultured over the last four
years. This paper contains an extensive study of the influence
of temperature on the postembryonic development of four brackish
water species : *Paracanthonchus caecus*, *Monhystera microphthalma*,
Monhystrella parelegantula and *Chromadora nudicapitata*.

Monhystrella parelegantula was collected from the Sluice
Dock of Ostend, a shallow brackish water pond with yearly sali-
nity fluctuations between 24 ‰ and 37 ‰ (see Thielemans *et
al.*, this volume), the other species from the Dievengat.

Meiobenthic organisms and detritus were extracted from the
sand by the method of Barnett (1968) and collected on a sieve
(mesh width = 38 µm) after which the animals were removed and
placed into petri-dishes containing 0.8% bacto-agar. After a
few weeks, nematodes, harpacticoids and other organisms penetrate
into the transparent agar. In this way, it is possible to main-
tain the stocks for several months (Vranken *et al.*, 1981).

Monospecific cultures were set up by transferring a number
of gravid females on enriched agar plates. The enrichment was
realized by adding 1% medium of Vlasblom and 15 g/l silica (= me-
dium used for *Monhystera microphthalma* and *Monhystrella parelegan-
tula*). The constitution of this medium is as follows :

$$
\begin{array}{ll}
0.278 \text{ g} & FeSO_4.7H_2O \\
3 \text{ g} & NaH_2PO_4.2H_2O \\
30 \text{ g} & NaNO_3 \\
0.47 \text{ g} & MnCl_2.4H_2O \\
50 \text{ g} & \text{glycine per liter distilled water.}
\end{array}
$$

For the
other two species (*Paracanthonchus caecus* and *Chromadora nudica-
pitata*), we used a mixture of phosphorus modified Walne medium
and Provasoli medium (ratio 5/1).

The salinity of the cultures was kept constant and was con-
trolled with a Goldberg T/C refractometer.

The experiments were carried out in incubators, without
light, at temperatures ranging from 5°C to 30°C. Per tempera-
ture a minimum of two replica's was examined and per replica a

known number (± 30) of gravid females deposited eggs during max-
imal 24 hours. Time zero was taken 12 hours before removing
the animals. After this period the adults were transferred,
whereupon the number of eggs produced was counted. A daily
check enabled us to observe at which time the eggs hatched and
the juveniles became sexually mature.

Dry weights were determined on a Mettler ME22 microbalance
to a precision of ± 1 µg, using batches of about 100 individuals.
Before weighing the animals were washed in bidistilled water,
and dried for 2 h at 110°C. Only the soft parts (excluding
shells) were weighed for *C. torosa* (Herman & Heip, in press).

Respiration was measured with a stoppered diver Cartesian
Diver respirometer (Klekowski, 1971). The divers had a gas vo-
lume of 1 µℓ or 2 µℓ ; each diver contained one animal.

Results.

Direct production estimates.

C. torosa.

The ostracod *C. torosa* produces only one generation in the
Dievengat (Heip, 1976), but there is considerable overlap between
successive generations, due to overwintering of older larvae.
Reproduction occurs in late spring and throughout the summer :
some of the early larvae become adult before winter, whereas
others overwinter in different larval stages. During winter
there is no development and maturation is postponed until the
next spring.

In the course of their development, the animals pass through
eight moults. At each moulting, they shed their calcareous
shells and build new ones. As the sediment is slightly alkaline,
the shells are well preserved in it. The distribution of the
preserved shells over the instars contains information on the
mortality pattern in the population : an animal dying e.g. in
stage V will leave shells of the stages I-V in the sediment. Thus
one expects to find more shells of younger stages than of older
ones in a sediment sample, and this is exactly what is observed.
Combining this information with density estimates of the stages
from the five-year sampling period allowed the calculation of
recruitment, the duration of the stages, and the stage-dependent
mortality rate. These result are summarized in Table 1. An
increment-summation production estimation could be made giving a
value of 9.69 g dwt.m^{-2}.y^{-1}. Mean biomass was 3.55 g dwt.m^{-2},
hence the annual P/B equalled 2.73.

Table 1

Summary of the data for the production estimate of *Cyprideis torosa*

The survival values were determined from the shell countings : they express the fraction of the animals entering the stage that survive till the next stage. Recruitment data are expressed as numbers per 10 cm² per 5 years.

Stage	Survival	Duration (days)	Recruit-ment	dwt (µg)
IV	0.7478	9.38	4119	0.925
V	0.8521	24.86	3080	1.5
VI	0.8681	56.85	2625	2.9
VII	0.8400	85.94	2278	5.3
VIII	0.9048	76.10	1914	10.3
AD	0.8641	111.88	1732	19.9

The size-frequency method for production estimation (Menzie, 1980 and references therein) was also used to calculate the production of this population. It basically consists of the fitting of a simple (but robust) population dynamical model to the data, and the subsequent calculation of production from the estimated recruitment into the stages. Some information is needed on the relative duration of the stages, and although these were roughly calculated assuming a constant mortality, the resulting production estimate, $P = 9.24$ g dwt.m^{-2}.y^{-1}, is in good agreement with the results obtained with the more elaborated model.

The dependence of respiration on body mass and temperature can be expressed (Herman & Heip, in press) as :

$$\log R = 9.7055 - \frac{2785.122}{T_{abs}} + 0.746 \log w$$

where $R =$ respiration (nl O_2 hr^{-1} ind^{-1})
$W =$ body mass (µg dwt ind^{-1})
$T_{abs} =$ absolute temperature (K).

The oxygen consumption of the population is 20.38 l $O_2 m^{-2} y^{-1}$; giving a production efficiency, $P/(P+R)$, of 0.382 and 0.372 for both production estimates resp.

Tachidius discipes.

The harpacticoid copepod *T. discipes* is a dominant species in many European and North-American brackish waters. Its size, form and epibenthic life style make it a representative species for an important part of the North Sea harpacticoid fauna. The data from the frequent sampling program (three days intervals) in

the spring of 1979 show that there are most probably three gene-
rations. These generations are clearly distinguishable in the
younger stages, but show increasing overlap in older copepodite
stages and adults (Fig. 2). In order to resolve the generation
peaks from these compound curves, we assumed that the peaks take
the form of Gaussian distributions. Then a statistical method
for the resolution of frequency distributions into Gaussian com-
ponents (Bhattacharya, 1968) could be used. Fig. 3 shows the
fit of the summed Gaussians on the density data of copepodite 3.
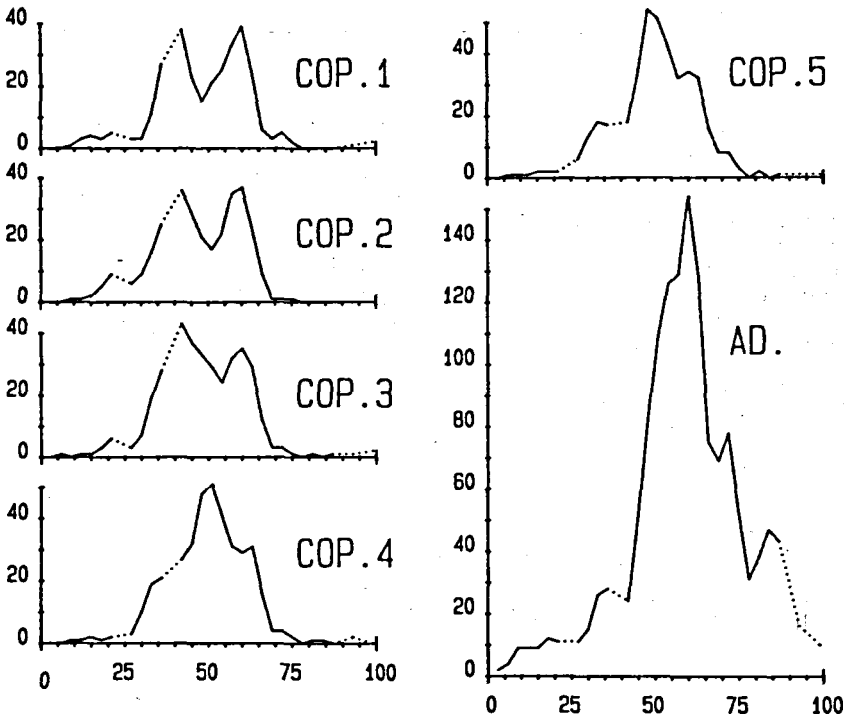
T. discipes : N / sample

fig. 2.

Tachidius discipes : densities (N/sample of 6.06 cm²)
of the different stages during spring 1979
(Day 0 : 27-03-1979)

Tachidius discipes

Copepodite 3 (N/10cm²)
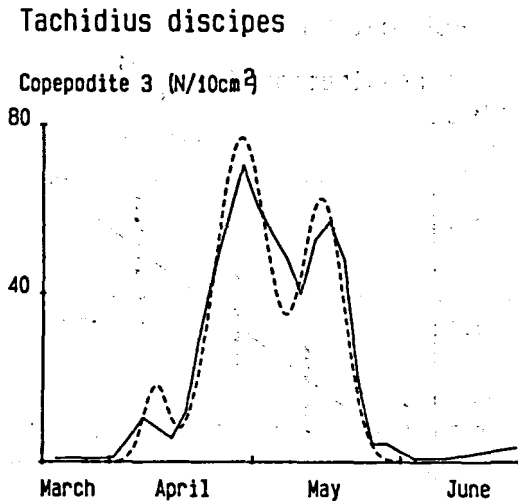
fig. 3.

Tachidius discipes : density (N/10 cm²) of copepodite 3 during spring 1979
with the summed Gaussians (dotted line) superimposed

The means of these Gaussians correspond to the mean pulse time of the generation peaks used in the model of Rigler & Cooley (1974). Their absolute heights correspond to the surface under the abundance curves (number of animal.days). Using Rigler & Cooley's model enabled the estimation of the duration of the stages and of their recruitment : it provided the necessary parameters for a production estimation. The production of copepodites and adults amounted to 1.1 g dwt.m^{-2} in the spring period when the species was present.

Production of nauplii and eggs was based on the number of egg sacs produced and the duration of naupliar development as determined in the laboratory (Smol & Heip, 1974 ; Heip & Smol, 1976). It is 1.3 g dwt.m^{-2}, and thus a very important part of total production. The P/B ratio for the total population was P/B = 9.34 over the sampling period. This amounts to 3.11 per generation.

The dependence of respiration rate on body weight is shown in Fig. 4. As body weight the mean weight of the stage that that the experimental animal belonged to was taken, since length measurements proved to be inaccurate due to the telescopy of the body segments. The relationship can be expressed as :

$$\log R = 1.12 + 0.82 \log W$$

where R = respiration in nl O_2ind^{-1}hr^{-1}
   W = μg dwt ind^{-1}

Tachidius discipes

Log respiration (nl O_2h^{-1})

Log dry weight (ug)
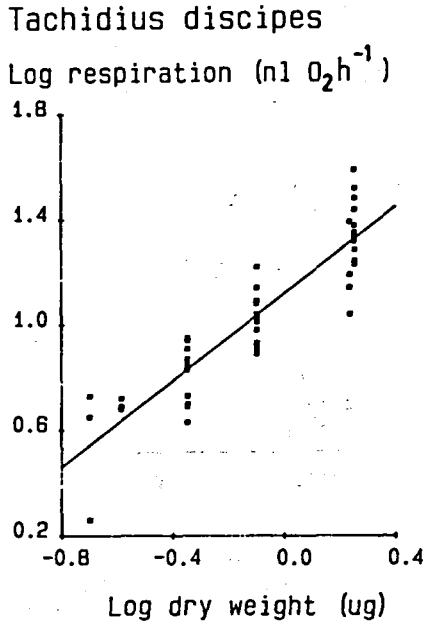
fig. 4.
Tachidius discipes
log — log relationship between dry weight and respiration

Assuming that temperature dependence of the respiration rate can be expressed by Krogh's normal curve, the respiration of co-pepodites and adults amounts to 3.3 ℓ O_2m^{-2} during the sampling period. Production efficiency is then 0.302.

Paronychocamptus nanus.

The production of copepodites and adults of this harpacti-coid species was estimated with the size-frequency method. This requires the estimation of the generation time and of the pro-portion of the life cycle spent in each stage.

Smol & Heip (1974) estimated the dependence of generation time on temperature from laboratory cultures of this species. Their equation $D = 528 \ T^{-1.05}$ was used to establish a physiologi-cal time scale. One unit on this time scale corresponds to one

Fysiological time (generations)
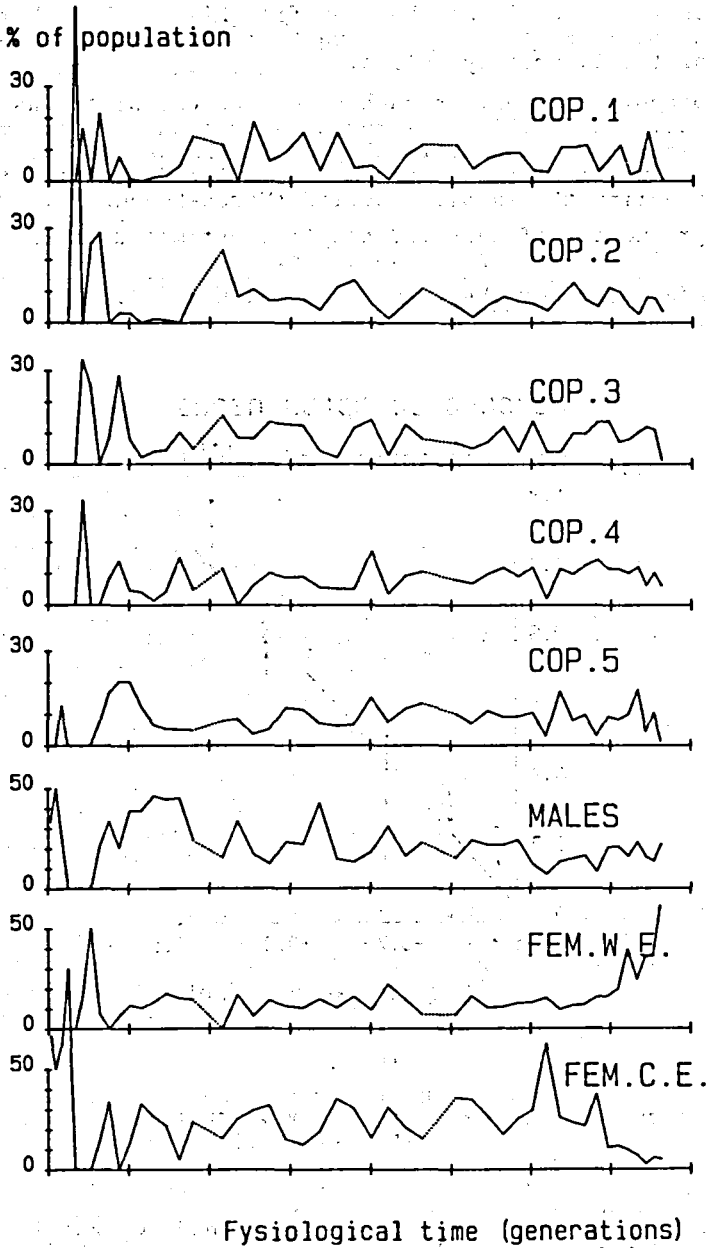
fig. 5.

Paronychocamptus nanus : procentual composition of the population during 1980
on a physiological time scale

generation time, and it can be seen from Fig. 5 that there occurs one peak per time unit in most stages, giving support to the use of the laboratory values.

The proportion spent in each stage was calculated assuming a uniform mortality. This is a rough estimation, but the method does not seem to depend critically on it (Benke, 1979). Production of copepodites and adults thus calculated amounted to 1.92 g dwt.m^{-2} during the sampling period (March-November).

The production of eggs and nauplii, estimated in the same way as for *T. discipes*, is 2.31 g dwt.m^{-2}.

The P/B ratio for the total population is 24.45, or 3.20 per generation.

Paronychocamptus nanus

Log respiration (nl O_2h^{-1})

Log dry weight (ug)
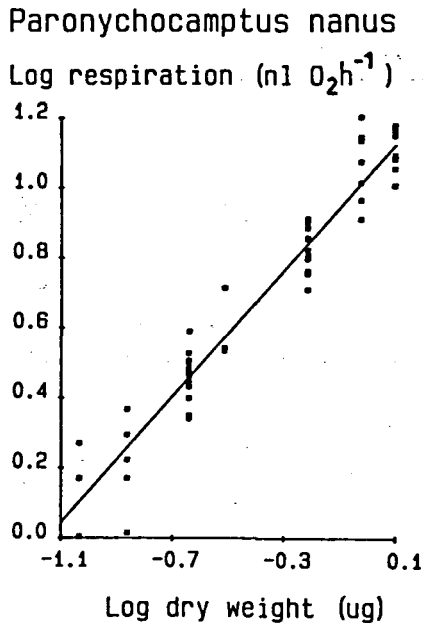
fig. 6.

Paronychocamptus nanus
log — log relationship between dry weight and respiration

The regression of respiration rate on body weight is shown in Fig. 6. It is expressed by

$$\log R = 1.03 + 0.90 \log W$$

The respiration of the copepodites and adults amounts to 7.2 ℓ O_2 m^{-2} during the sampling period. The production efficiency of copepodites and adults is 0.27.

Indirect production estimates.

As the number of annual generation is the critical parameter in indirect production estimates, the life cycle of the species in the field has been studied for two nematode species.

Oncholaimus oxyuris.

Density fluctuations of this species are characterized by bimodal annual curves for all age-classes, with an important peak in spring or summer and winter. Minima occurred during February or March. Because on the average 70% of the population is represented by juveniles, we have a nearly similar curve for the juveniles. The lowest densities of juveniles occur each year during spring, from March till June, a period in which their part in the population is reduced to below 50%. It is clear that most of them moult into adults (Fig. 7).

In winter we note a second but less distinct period of low abundance, but the contribution of juveniles to the population remains high, only few of them have moulted into adults.

An analysis of density of the two sexes (Fig. 8 demonstrates that males are present throughout the year ; they mature earlier and live longer than the females. They constitute a large part of the adult population, with a mean value of 75% and a sex-ratio of 40:60.

The presence of females is restricted to distinct periods. A main peak of high abundance occurs during spring with numbers nearly equal to the males. They appear again during winter, but in much lower numbers. During the rest of the year females are absent, providing a mean sex-ratio of 1:4 over the whole year. When females are present, more than 50% and often 100% of them are gravid.

Summarizing, we find a reproductive period in spring extending from March until June ; the first juveniles then produced are able to complete their life-cycle before winter, whereas the juveniles born later, have to overwinter to attain adulthood next spring. The juveniles that become adults in late summer are able to reproduce so that the overwintering juveniles belong to two generations (Fig. 9), but their offspring are produced during the same main reproductive period in the following spring. Whether these generations remain distinct or not is not clear.
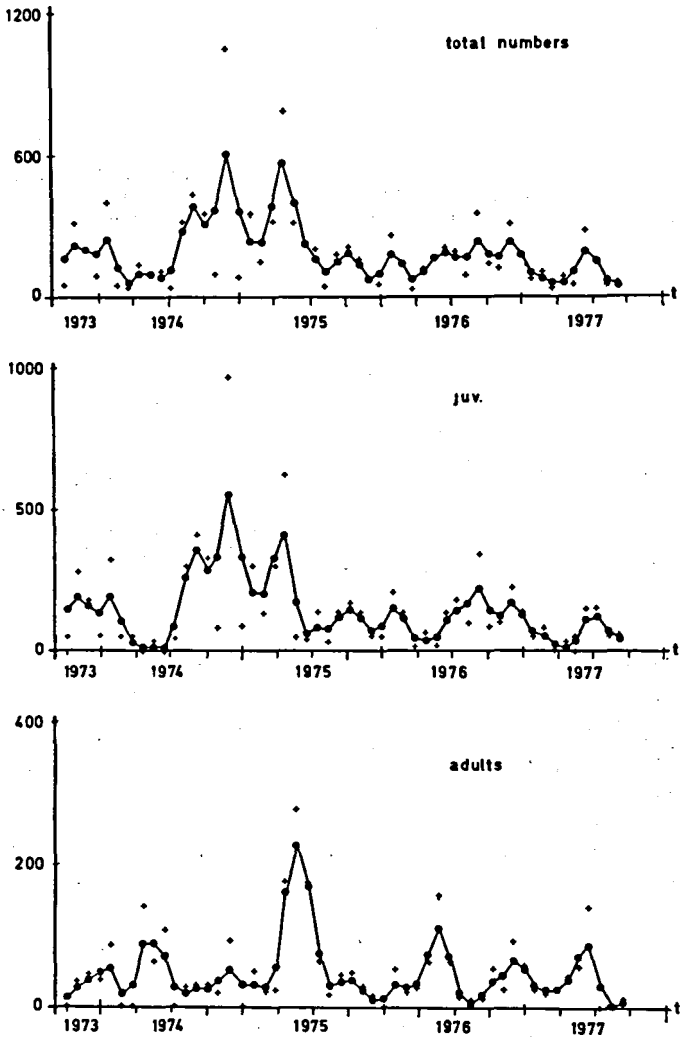
O. OXYURIS

fig. 7.

Density fluctuations of the *Oncholaimus oxyuris* population over four years

O. OXYURIS
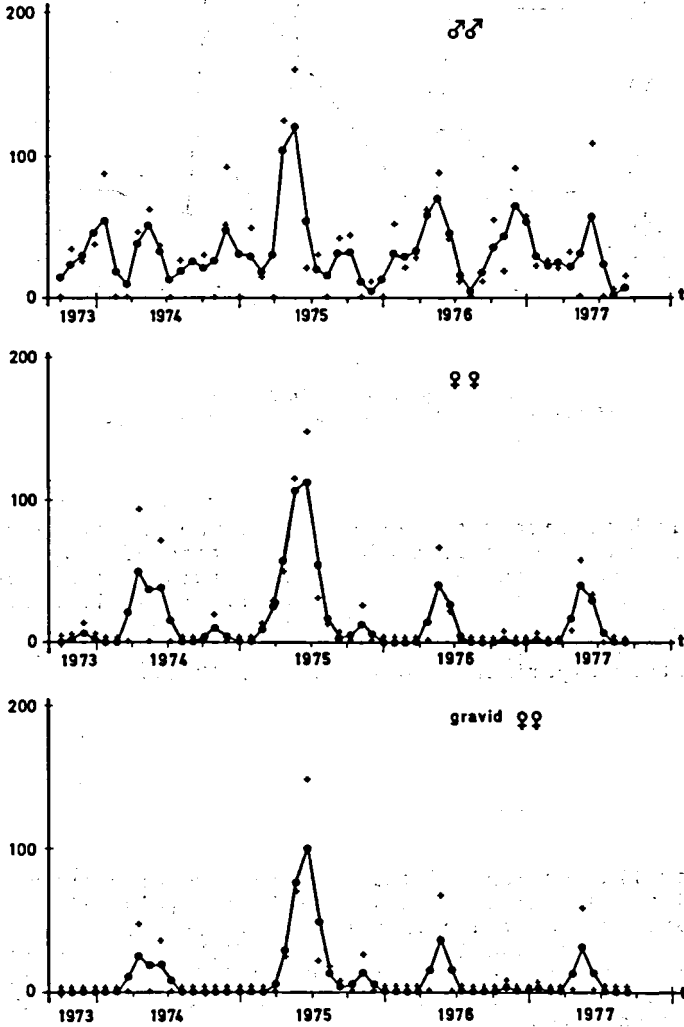
fig. 8.

Density fluctuations of the males, females and gravid females
of *Oncholaimus oxyuris* over four years
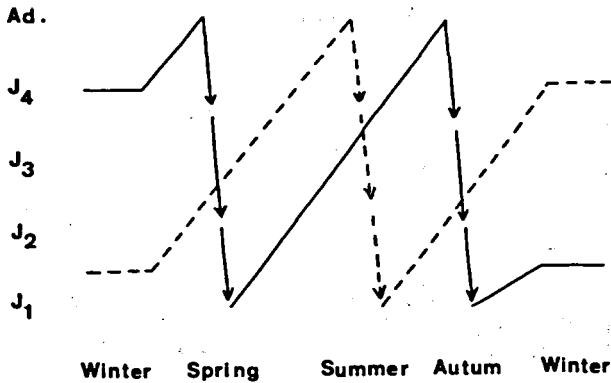
fig. 9.

Scheme of post-embryonic development
of two cohortes of *Oncholaimus oxyuris* over one year

We may thus have either two generations, one in spring and one
in autumn, or more complicated an alternation of two generations
in one year and one in the next year, in this case we have three
generations in two years of 1.5 per year. This slightly compli-
cated scheme (Fig. 9) may nevertheless represent the most exact
picture of the life cycle of *O. oxyuris* as is substantiated by
the laboratory experiments (Heip *et al.*, 1978).

Viscosia viscosa.

Density fluctuations of this species are also characterized
by bimodal annual curves in all age-classes (Fig. 10). Maxima
occur in spring-summer and in winter. Adults predominate in the
population with an annual mean of 60%. Males and females are
present all over the year. The number of females is nearly half
the number of the males, and *Viscosia viscosa* clearly has two
generations each year.

VISCOSIA VISCOSA

fig. 10.

Density fluctuations of the *Viscosia viscosa* population over four years

Laboratory experiments.

Development times until adulthood, at different temperatures, are listed in Table 2 and shown in Figs 11-14. Separate data are given for both sexes, except for *M. parelegantula.*

Table 2

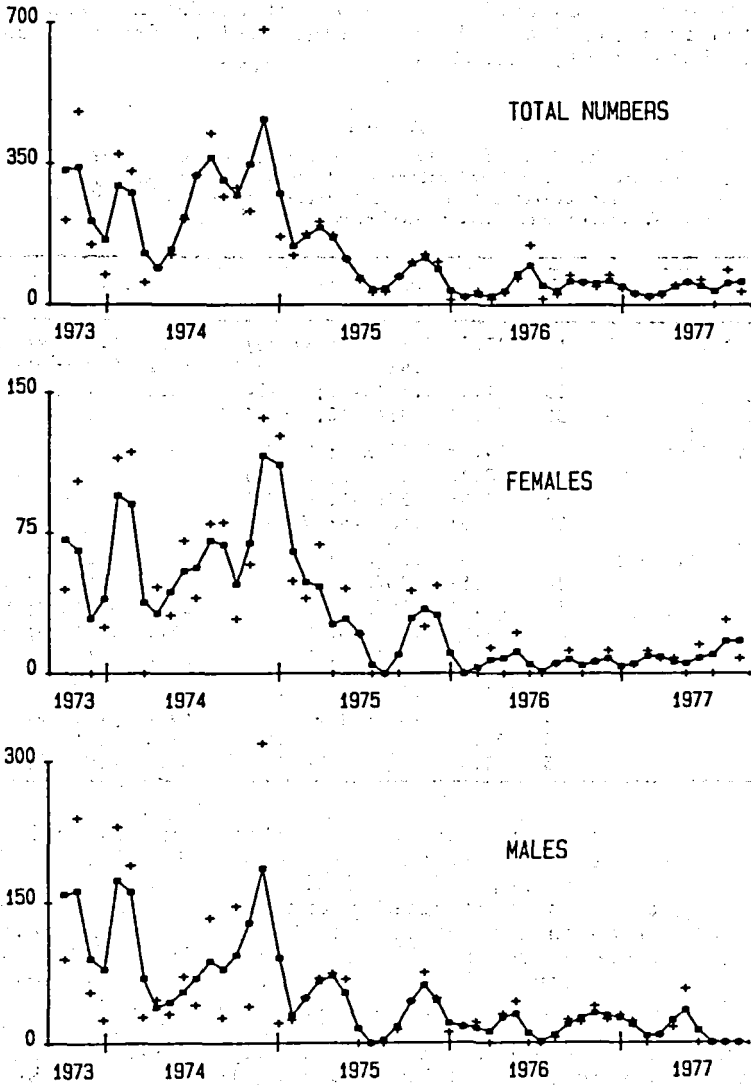Development times of four brackish water nematodes at different temperatures (T) and fixed salinity (S). Mean duration in days (D) with standard deviation; number of experiments (n) and number of females, respectively males (N) studied during the experiments.

Species	S ‰	T	D (females)	D (males)
M. *microphthalma*	20	15	27.8± 2.20(n=7; N=123)	28.1± 1.67(n=7; N=96)
		20	10.2± 0.92(n=11; N=113)	11.2± 0.90(n=11; N=107)
		25	7.8± 1.18(n=15; N=174)	8.0± 1.10(n=15; N=160)
		30	6.6± 1.31(n=10; N=137)	6.4± 1.02(n=10; N=86)
M. *parelegantula*	30	15	54.3± 5.74(n=2; N=30)	–
		20	18.1± 1.52(n=5; N=275)	–
		25	7.9± 0.57(n=6; N=539)	–
		30	6.3± 0.48(n=5; N=467)	–
C. *nudicapitata*	20	5	84.5± 8.20(n=2; N=52)	87.0± 9.0(n=2; N=48)
		10	52.5± 3.80(n=3; N=96)	51.1± 3.4(n=3; N=79)
		15	24.9± 2.70(n=3; N=64)	23.3± 3.1(n=3; N=53)
		20	14.0± 1.40(n=3; N=108)	14.1± 1.6(n=3; N=76)
		25	16.9± 1.70(n=3; N=44)	15.2± 2.1(n=3; N=49)
P. *caecus*	20	10	131.9±14.7(n=2; N=93)	124.0±13.0(n=2; N=35)
		15	65.7± 3.6(n=3; N=82)	65.7± 3.8(n=3; N=68)
		20	51.1± 4.8(n=4; N=66)	47.0± 4.9(n=4; N=60)
		25	41.9± 4.2(n=4; N=92)	37.6± 4.9(n=4; N=87)

From these results we can conclude that there is an important influence of temperature on development. Previous studies (for a summary see Kinne, 1977) did already reach the same conclusion. Another aspect of development studies that has received some attention in literature, is the nature of the relationship between temperature and the duration of development time (MacLaren, 1963 ; Heip, 1974 ; Botrell, 1975 ; Heip & Smol, 1976 ; Sarvala, 1979 ; Palmer & Coull, 1980). Several equations have been investigated, but two equations, namely the simple power equation : $D = at^b$ (Heip, 1974 ; Heip & Smol, 1976 ; Sarvala, 1979 ; Warwick, 1982 ; Heip *et al.*, 1982) and the semilogarithmic quadratic equation $lnD = lna + Tlnb + T^2lnc$ (Botrell, 1975 and Sarvala, 1979) have been proposed and used as models to describe this relationship. Both equations give a reasonable fit to the experimental data. The semilogarithmic quadratic equation has the advantage that after logarithmic transformation, the heteroscedacity among variances can disappear (Sarvala, 1979), although this is not always the case. The function can describe

longer development times at higher temperatures, but the power equation is preferred by several authors due to its simplicity.

Table 3

Curvilinear regressions relating development time (D) in days and temperature (T) in °C, for four species of brackish water nematodes. Parameter values (= constants) are given for two equations. The variance ratio (F) tests the significance of the regression. The coefficient of determination (r^2) estimates the amount of variance of the dependent variable (D) explained by the independent variable (T).

	$D = aT^b$				$D = a + bT + cT^2$				
	a	b	F_s	r^2	a	b	c	F_s	r^2
M. microphthalma									
females	4679	−1.96	126[***]	0.75	115	−8.13	0.15	86[***]	0.81
males	7270	−2.10	209[***]	0.84	116	−8.05	0.15	116[***]	0.85
M. parelegantula	208053	−3.11	241[***]	0.94	241	−17.40	0.32	248[***]	0.97
C. nudicapitata									
females	607	−1.16	102[***]	0.90	134	−10.92	0.25	479[***]	0.99
males	684	−1.22	148[***]	0.93	138	−11.40	0.26	563[***]	0.99
P. caecus									
females	2357	−1.28	92[***]	0.89	304	−22.40	0.48	79[***]	0.94
males	2252	−1.29	131[***]	0.92	275	−19.90	0.42	98[***]	0.95

In this paper we used two equations, the power equation and the untransformed quadratic equation, $D = a + bT + cT^2$. Table 3 gives the values of the constants a and b for the power equation (equation I) and the coefficients a, b and c for the quadratic equation (equation II). Furthermore this table contains the statistic F which is ratio between the mean square due to linear regression and the residual or the unexplained mean square. The coefficient of determination (r^2) which can be consired as a measurement of the variation of the dependent variable (D) explained by the independent variable (T) is also listed. From Table 3, we can conclude that for all the regressions the F value is highly significant ($P < 0.001$, $\beta = 0$). The coefficient of determination (r^2) is always larger for equation II than for equation I. Calculated predictions of the developmenttimes for the females, as results of the application of both functions are listed in Table 4. Both functions give a reasonable fit and can be used for descriptive or predictive purpose, this without giving any theoretical meaning to the fitted equations (Figs. 11-14). Both functions can then be used to obtain an estimation of the number of annual juvenile periods (i.e. approximately the number of generations) produced by the species in the field. Juvenile periods are intervals where the development accumulation $D(t) = 1$.

Table 4

Observed development time (D_{obs}) at different temperatures compared with predicted values (D_{pred}) obtained by application of equation (I) : $D = aT^b$ and equation (II) : $D = a + bt + cT^2$ for female individuals.

| | | Temperatures | | (°C) | | |
	5	10	15	20	25	30
Monhystera microphthalma						
$D_{exp. I}$	–	–	23.2	13.2	8.5	6.0
$D_{exp. II}$	–	–	26.8	12.4	5.5	6.1
$D_{obs.}$	–	–	27.8	10.2	7.8	6.6
Monhystrella parelegantula						
$D_{exp. I}$	–	–	45.8	18.7	9.3	5.3
$D_{exp. II}$	–	–	52.0	21.0	6.0	7.0
$D_{obs.}$	–	–	54.3	18.1	7.9	6.3
Chromadora nudicapitata						
$D_{exp. I}$	93.8	42.0	26.2	18.7	14.5	–
$D_{exp. II}$	85.7	49.8	26.4	15.6	17.3	–
$D_{obs.}$	84.5	52.5	24.9	14.0	16.9	–
Paracanthonchus caecus						
$D_{exp. I}$	–	123.7	73.6	50.9	38.3	–
$D_{exp. II}$	–	128.0	76.0	48.0	44.0	–
$D_{obs.}$	–	131.9	65.7	51.1	41.9	–

$$D(t) = \int_0^t R[T(t)] \, dt$$

where R is the development rate and T the temperature, is the total amount of juvenile development that takes place in a given interval of time. To calculate D(t) we have to know the water temperature of the habitat. Water temperature in the Dievengat and the Sluice Dock can be described as simple sinusoidal functions of time t ; for the Dievengat the equation is

$$T(t) = 11.2 + 8.3 \sin (t - 117)$$

(Heip & Smol, 1976); for the Sluice Dock the mean daily temperature can be estimated with the equation provided by Podamo (1975)

$$T(t) = 11.5 + 8.5 \sin (t - 120) \; .$$

Monhystrella parelegantula

Development time (days)
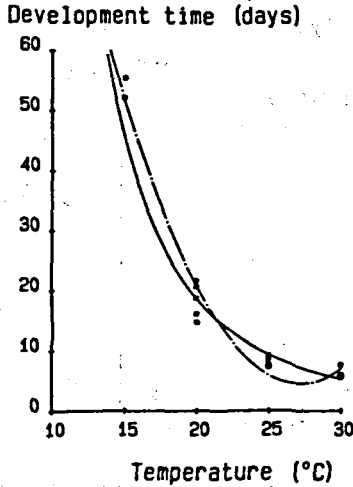
fig. 11.

Monhystrella parelegantula
Relation between development time (days) and temperature (°C)
at 30 % salinity
(Data obtained from cultures of female individuals)

Monhystera microphthalma

Development time (days)   Development time (days)

FEMALES   MALES

Temperature (°C)   Temperature (°C)
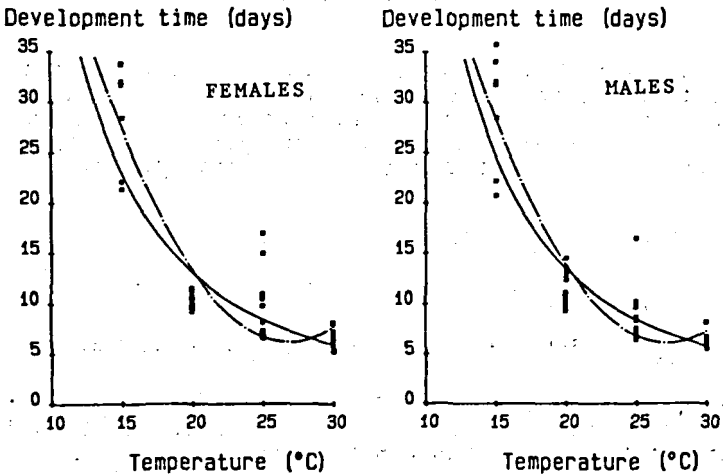
fig. 12.

Monhystera microphtalma
Development time (days) at different temperatures (°C)
and a constant salinity of 20 % under laboratory conditions

Chromadora nudicapitata

Development time (days)

Development time (days)
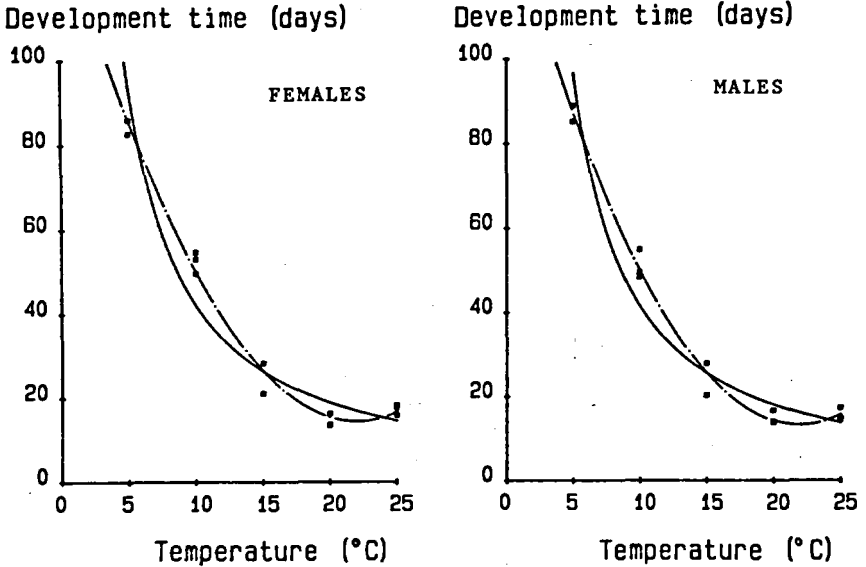
fig. 13.

Chromadora nudicapitata
Relationship between temperature (°C) and development time (days)
at 20 ‰ salinity under laboratory conditions

Knowing the annual temperature regimes in both habitats, we now are able to estimate the development rate R (the reciprocal of the development time D) as a function of temperature. For example for *M. microphtalma* we can give estimations of the development rate R at any time t using the following equations

(3) $R[T(t)] = \frac{1}{4679} [11.2 + 8.3 \sin (t - 117)]^{-1.96}$

for the power equation and (4)

$R[T(t)] = \frac{1}{115} - 8.13[11.2 + 8.3 \sin(t - 117)] + 0.15[11.2 + 8.3 \sin(t - 117)]^2$

for the quadratic equation. The development rate of the three other species can be obtained by using analogous equations. The constants of this equations can be found in table 3.

For *M. microphthalma* the annual development accumulation D(365) can now be estimated by integrating the development rate obtained by application of (3) or (4) over a period of one year.

Paracanthonchus caecus

Development time (days)   Development time (days)

FEMALES   MALES

Temperature (°C)   Temperature (°C)
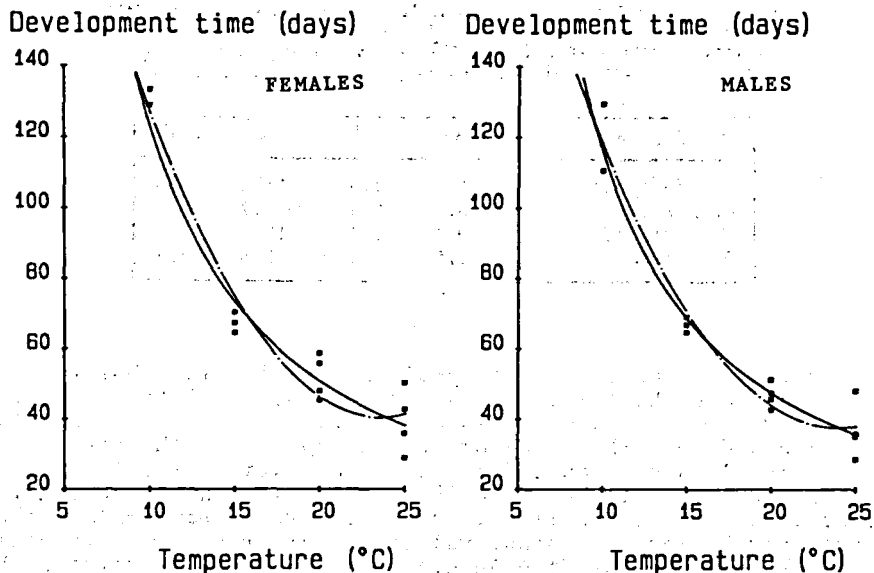
fig. 14.

Paracanthonchus caecus
Duration of development time (days) until adulthood
in relation with temperature (°C) at 20 % salinity
under laboratory conditions

We have calculated this integral

$$\int_0^{365} R[T(t)] \, dt = D(365)$$

using Simpson's method for discrete intervals. The results of
these computations are given in table 5, from which we can see
that the number of annual juvenile periods for any one species is
very similar whether using the power (A) or the quadratic
equation (B).

These results (Table 5) have to be regarded with some
caution, because they are based on development times (= minimum
generation times) and not on mean generation times; the latter
have to contain the mean duration of the egg production period.
Furthermore these estimations are biased because they do not
account for the basal temperature (the temperature below which
development stops).

Table 5

Number of juvenile periods during one year for four brackish water
nematodes (females); using Simpson's approximative method with
development rates predicted by the power equation (A) and the
quadratic equation (B); (A)*, (B)* yearly juvenile periods after
correcting for the basal temperature.

Species	(A)	(A)*	(B)	(B)*
M. microphthalma	11.0	9.9	11.6	9.3
M. parelegantula	6.6	6.4	6.7	5.7
C. nudicapitata	10.1	10.1	10.8	10.8
P. caecus	3.5	3.4	3.8	3.5

Observations on life history of the species in laboratory
conditions gave the following values for the basal temperature :
$10°C$ for *M. microphthalma* ; $±10°C$ for *M. parelegantula* ; $5°C$ for
P. caecus and less then $3°C$ for *C. nudicapitata*. When these bio-
logical zero's are accounted for the computations result in the
numbers indicated with an asteriks (Table 5). The largest dif-
ference, 2.3 juvenile periods, occurs for *M. microphthalma*, the
discrepancy between the other values is rather small. From this,
it is also obvious that the quadratic equation gives more weight
to the lower temperatures. Nevertheless the bias introduced
when basal temperature is not accounted for moderate ; only for
those species where basal temperature is high, precautions are
necessary.

The annual number of generations (Table 5) is somewhat less
than previously published values : 15 for both *Chromadorina ger-
manica* (Tietjen & Lee, 1977) and *Monhystera denticulata* (Tietjen
& Lee, 1972) and 17 generations for *M. disjuncta* (Gerlach, 1971),
but considerably higher than the modal value of three proposed
by Gerlach (1971) for marine meiobenthos, except for *P. caecus*,
the largest species. At the time being we can only confirm the
statement of Heip *et al.* (1982) that it is to early to give a
modal value for nematodes, because develoment data exist only
for opportunistic species belonging to a few genera.

Discussion.

The available results of direct production estimates of meio-
benthic populations (Table 6) already indicate that the use of a
single annual P/B value for meiofauna (Gerlach, 1971) is inappro-
priate. In fact the annual P/B values show a considerable range,
and the high diversity in life histories of meiobenthic popula-
tions is clearly reflected in the diversity of annual P/B values.
In this respect, it is interesting to note that the P/B per ge-
nerations annually is the most important factor in determining
annual P/B-values (Waters, 1969).

Table 6

P/B ratio's per year and per generation for meiobenthic species
for which direct production estimates are available

Species	$(P/B)_a$	$(P/B)_g$	Source
Huntemannia jadensis	3.8	3.8	Feller, 1977
Microarthridion littorale	18.0	?	Fleeger & Palmer, 1982
Tachidius discipes	9.3*	3.1	This paper
Paronychocamptus nanus	24.5	3.2	id.
Cyprideis torosa	2.7	2.7	id.

* The annual P/B for *T. discipes* is calculated over the period of presence in the
habitat.

In nematodes, both field observations and culture experi-
ments show that the generation times range between a few days
and a whole year. Since generation P/B is relatively constant,
the range of annual P/B values will be much wider than the re-
sults of direct production estimation indicate to date. On the
basis of our laboratory experiments on the four nematode species,
and assuming a life cycle P/B of three, we can give a rough ap-
proximation of the annual (indicated yr.$^{-1}$) P/B, 28.8 yr^{-1} for
M. microphthalma ; 18.2 yr^{-1*} for *M. parelegantula* ; 31.4 yr^{-1*}
for *C. nudicapitata* and 10.4 yr^{-1} for *P. caecus*. As *M. parele-*
gantula and *M. microphthalma* do not reproduce and occur only
sporadically during approximately half a year, annual mean bio-
mass will be much lower and the annual P/B-ratio consequently
much higher than these figures, which are based on the period
that the species actually occurs (y^{-1*}). On the other hand, the
large *O. oxyuris* and *V. viscosa* are expected to have a P/B of
between three and six.

The use of a single P/B for the meiobenthos is clearly un-
realistic. However, other shortcut methods may prove more use-
ful. The data presented here indicate that the production effi-
ciencies are rather constant, implying that respiration data may
be used to estimate production. Humphreys (1979) analysed 235
field populations and found significant log-log relationships

between annual production and annual respiration for a number of
species groups. For non-insect invertebrate detritivores he
found $\log P = -0.601 + 1.069 \log R$ (P and R in cal $m^{-2}y^{-1}$. Table
7 compares the productions estimated from this equation to the
productions directly measured for the three populations studied
in this paper. The rather close agreement between these values
encourages further research along this line.

Table 7

Comparison between observed production
and production predicted from respiration
for three meiobenthic crustaceans

	log R	$(\log P)_{pred.}$	$(\log P)_{obs.}$
Cyprideis torosa	4.950	4.691	4.741
Paronychocamptus nanus	4.499	4.208	4.038
Tachidius discipes	4.160	3.846	3.797
(R and P in cal $m^{-2}y^{-1}$)			

Table 8

Respiration per unit body weight (nℓ O_2.dwt^{-1}.h^{-1})
of meiobenthic Crustacea

Species	Respiration	Source
Asellopsis intermedia	3.8	Lasker *et al.*, 1970
Hastigerella leptoderma	3.42	Vernberg *et al.*, 1977
Nannopus palustris	4.99	id.
Thompsonula hyaenae	10	Sellner, 1976
Paraleptastacus spinicauda	4.2	Laserre & Renaud-Mornant, 1973
Enhydrosoma propinquum	2.0	Coull & Vernberg, 1970
Longipedia helgolandica	6.6	id.
Harpacticoids brackish water		
"class I" (9.0 µg dwt)	4.0	Laserre *et al.*, 1975
"class II" (1.5 µg dwt)	10.0	id.
Ostracods	3.0	id.
Hirschmannia viridis	2.0	Hagerman, 1969
Cyprideis torosa	0.8-1.0	this paper
T. discipes	11.8-17.4	id.
Paronychocamptus nanus	10.5-13.7	id
Marine planctonic copepods	30	Ivleva, 1980

The available data show that a considerable range exists in
the respiratory activities of meiobenthic animals. Data for ne-
matodes are reviewed by Warwick & Price (1979). Table 8 summa-
rizes data on crustaceans. In nematodes the variation is one

order of magnitude ; it is somewhat less, but still important in
meiobenthic crustaceans. Several attempts have been made to
correlate the observed variation to ecological variables such as
feeding type of the species or oxygen content of the habitat
(Teal & Wieser, 1966 ; Schiemer & Duncan, 1974 ; Warwick & Price,
1979), but although there appear to be some tendencies, the res-
ults are not very conclusive. Therefore, it remains difficult
to forecast the respiratory rate of a population unless it has
been measured.

   Furthermore, a comparison between the respiration rates of
T. discipes in the Dievengat (this paper) and in an English es-
tuary (Teare & Price, 1979) makes clear that important differ-
ences can exist between two populations of the same species. In
the Lynher estuary, reproduction is continuous throughout the
year. The modal volume of adult animals (value read from a fi-
gure) is about 8.25 nl and the respiration rate per unit body
volume of animals from the field population is almost 2.5 times
lower than for the Dievengat animals. In the Dievengat the po-
pulation is only present in the spring, the modal body volume of
an adult is 12.5 nl, and the respiratory rate is higher. At
least a part of these differences can be attributed to differen-
ces in feeding conditions. In their laboratory reared popula-
tion, Teare & Price (1979) had adults of model body volume =
10.0 nl, with a respiration rate per unit volume twice as high
as in the animals taken from the field.

   Apart from stressing the difference between local populations,
this example illustrates that food availability, and hence pro-
ductivity in the habitat may be an important factor in determi-
ning the respiratory activity of a population. Therefore, look-
ing for a general value for the respiratory activity of meiofauna
may be as spurious as looking for one overall P/B figure.

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IV

MEIOFAUNA OF THE BELGIAN COASTAL WATERS :

SPATIAL AND TEMPORAL VARIABILITY AND PRODUCTIVITY

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Abstract.

Density, biomass and diversity of meiobenthic assemblages have been studied in the Belgian coastal waters of the North Sea since 1972. Near-shore sediments east of Ostend show an extremely poor meiofauna, with nematodes extremely dominant. West of Ostend muddy sand and sands exist with a richer fauna. These zones are clearly delimited on the basis of nematodes, whereas they are more uniform when either macrofauna or harpacticoids are studied (Govaere et al., 1980). This is also true for off-shore sediments. Besides species diversity also trophic diversity, as expressed by the number of feeding types, increases towards off-shore sandy sediments.

The study of density and biomass of nematodes and harpacticoids permits estimations of production of these groups. The total carbon requirements of the meiobenthos can be estimated at 8-9 g C/m^2.year in the coastal zone and a minimum of 5-6 g C/m^2.year on the Kwinte bank, a subtidal sandbank, although sedimentation is very much lower in the sandbank. This indicates that when energy flow is lower a much larger part of it passes through the meiobenthos.

Introduction.

Meiobenthos of the Southern Bight of the North Sea has been investigated on a large spatial scale from 1971 to 1976 (see maps in Govaere *et al.*, 1980) whereas a smaller area covering the Belgian near-shore waters has been sampled regularly from 1978 onwards. A survey of the meiofauna of the greater grid area was given by Van Damme & Heip (1977) and Govaere *et al.* (1980). These earlier papers were based mainly on harpacticoid copepods as far as the meiofauna was concerned and the sampling programme was very heterogenous in time which made detailed studies of temporal variation impossible. In the present report these gaps have been filled as it presents a detailed analysis of the nematodes of the larger area and data on density, biomass and diversity of nematodes and harpacticoids in the coastal area.

Material and methods.

From 1971 to 1975 350 samples from 74 stations were analysed for meiofauna (Govaere *et al.*, 1980). All samples were taken with a 0.1 m² Van Veen grab. The material was collected in a bucket and fixed in 7% neutralised formalin on board of the ship. Meiobenthos samples were taken from the bucket with a core (sandy sediments). In the laboratory they were elutriated by decantation or a sugar-flotation technique was used (muddy sediments).

Eighteen coastal stations were sampled seasonally during ten cruises from June 1977 till September 1979. The coordinates are listed in table 1.

In the first three cruises (June 77, September 77 and March 78), meiofauna was collected by subsampling a Van Veen grab. From April 78 onwards all sampling was done by a modified Reineck box corer (sample surface : 170 cm²). Each box core was subsampled by four plastic cores (surface : 10.2 cm²). Two replicates for meiofauna were fixed with warm formalin (70°C) to a final concentration of 4%. The other cores for chemical and sediment analysis were frozen immediately. All stations were sampled during April, June, September, December 1978 and April, June, September 1979, except st. 10061 and st. 10500 which were not sampled in April 1978 due to bad weather conditions.

Sediment analysis and mathematical methods are as described in the first paper of the series (Heip *et al.*, 1979).

Table 1

Coordinates of the eighteen coastal stations and sediment characteristics
(average per station over the entire study period) : mean % mud and sand,
mean median grain size (in ϕ-units and mm) and mean sorting (ϕ-units) of
the sand fraction.

| Stat. | Latitude | Longitude | % mud | % sand | Grain size | | Sorting |
					Md_ϕ	Md_{mm}	
10061	51°08'21"	02°31'40"	2.95	97.04	2.461	0.191	0.357
10080	51°07'10"	02°31'00"	0.28	98.24	2.168	0.227	0.401
10481	51°12'20"	02°50'14"	20.75	79.10	2.722	0.152	0.385
10500	51°11'06"	02°42'04"	16.28	81.91	2.499	0.177	0.432
10791	51°14'35"	02°55'25"	49.28	49.52	2.627	0.164	0.499
11121	51°16'40"	03°00'30"	9.09	90.90	2.529	0.174	0.370
11150	51°16'32"	02°51'08"	2.03*	97.73*	1.890	0.270	0.459
11312	51°19'10"	03°06'00"	·58.76	36.53	2.600	0.165	0.588
11315	51°19'30"	03°03'00"	56.26	43.60	2.540	0.172	0.526
11331	51°19'01"	02°56'50"	44.81	55.18	2.820	0.142	0.521
11672	51°21'00"	03°14'00"	26.71	71.23	2.383	0.192	0.458
11851	51°23'02"	03°22'56"	44.93	55.27	2.643	0.160	0.575
11860	51°22'38"	03°18'41"	30.93	69.30	2.440	0.184	0.437
11880	51°22'00"	03°09'15"	64.53	34.93	2.600	0.165	0.613
12300	51°26'55"	03°23'24"	25.22	72.90	2.280	0.206	0.449
12501	51°27'17"	03°31'33"	2.29	97.56	2.181	0.221	0.418
12510	51°26'55"	03°21'45"	9.34	91.32	2.381	0.192	0.390
12830	51°29'49"	03°25'45"	18.80	80.91	2.492	0.178	0.461

* March 78 is not considered due to the aberrant value of 76 % mud.

Results and discussion.

The distribution of sediments in the area.

The sediment composition of the sea bottom varies from 100%
silt, over silty fine sand to clean fine sand from the NE to the
SW along the coast and from predominantly muddy in the coastal
water to very pure sandy sediments offshore. Due to the decreas-
ing tidal currents a clear gradient from coarse sands in the
south to fine sands in the north of the area is a very signific-
ant feature of the Southern Bight.

Gullentops *et al.* (1977) gave some data on the origin, trans-
port and on the behaviour of the fine sediments along the Belgian
coast. The material along the Belgian coast is from two differ-
ent origins ; the western part of the coast is influenced by the
IJzer, French coast and by the Channel, the eastern coast is in-
fluenced by the Western Scheldt estuary. Based on sedimentolo-
gical arguments and hydrodynamic indications, transport out of
the Western Scheldt goes to the SW, probably till Ostend. The
silt on the east side of Ostend has its origin in the Western
Scheldt ; the silt between Nieuwpoort and Ostend is derived from
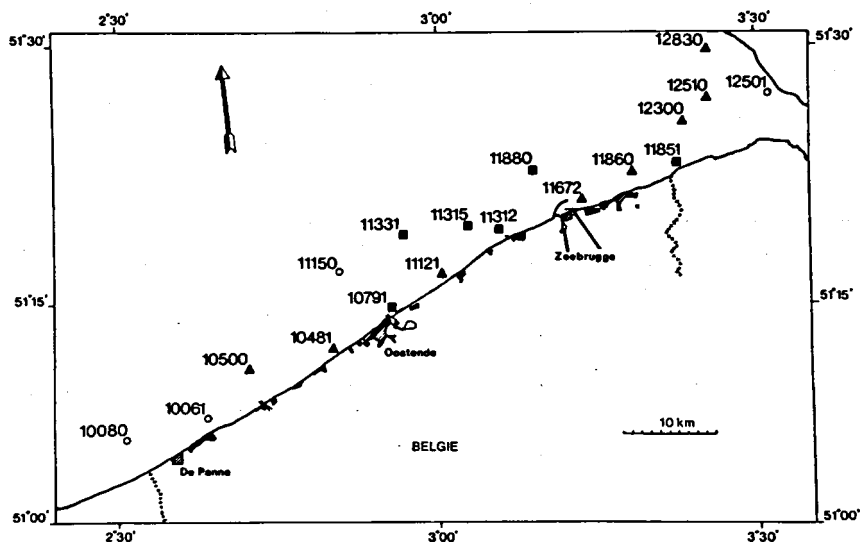the Channel, the French coast and from the IJzer.

fig. 1.

Localisation of the eighteen coastal stations
and their characterisation according to three sediment groups
o sand stations (< 5 % mud)
▲ muddy sand stations (5 % < < 32 %)
■ mud stations (> 32 % mud)

The characteristics of the sediments in the coastal stations
are listed per station in table 1.   The overall median grain size
of the sand fraction here is 0.186 mm.   The eighteen coastal sta-
tions can be classified into three groups (Fig. 1).   Those sta-
tions where the mud content remains below 5% of the sediment over
the ten cruises are called sandy stations : 10080, 10061, 11150
and 12501.   The mean grain size is 0.228 mm for the whole samp-
ling period.   There is no difference between summer and winter
samples.   Also the percentage mud is quite stable both in space
and time.   Except for a peak value of 75.75% mud (st. 11150,
March 78), the mean mud content is approximatively 2% (Table 2).
The muddy sand stations with a mud content between 5% and 32%
are : 10500, 10481, 11121, 11672, 11860, 12300, 12510 and 12830.

Although the mean grain size is equal for summer and winter
samples there is often a great fluctuation in mud content in both
station groups.   These differences are not only seasonal, but
factors such as weather condition, current velocity, fisheries
and public work activities may be important.

Table 2

Mean and summer-winter values of median grain size (Md$_{mm}$),
percentages mud and sand in the three station groups

	Eighteen stations	Sand stations	Muddy sand stations	Mud stations
Median grain size				
mean Md$_{mm}$	0.186	0.228	0.184	0.163
summer	0.187	0.228	0.183	0.164
winter	0.186	0.229	0.184	0.161
% mud (<63 μm)				
mean %	27.52	1.75	19.70	53.85
summer	28.64	2.01	21.05	56.36
winter	25.85	1.25	17.67	50.08
% sand				
mean %	72.06	95.79	79.85	45.87
summer	70.94	97.47	78.53	43.14
winter	73.75	93.28	81.84	49.96

Spatial distribution of nematodes in the Southern Bight.

280 species of nematodes from 121 genera and 28 families have been found, which means a mean number of 20 species per station. Because families group species which are morphologically very similar, an analysis on family basis was attempted. Diagnostic characteristics for families are buccal structures (which reflect feeding preferences) and position and structure of sense organs (which allow reactions to environmental conditions).

Spatial variability was examined from samples obtained during summer 1972, a period in which nearly all stations were sampled. A mean density of 1500 ind./10 cm^2 was found over the whole region. This value should be considered as minimal as the material was subsampled out of a Van Veen grab.

The similarity (Sørensen-index) between the stations was examined. All the families were used for cluster analysis. The matrices of similarity thus obtained were subjected to flexible sorting (Lance & Williams, 1967), with the cluster intensity coefficient β set at -0.25.

The region can be divided into six zones, which are a very good representation of the sediment composition (Fig. 2).
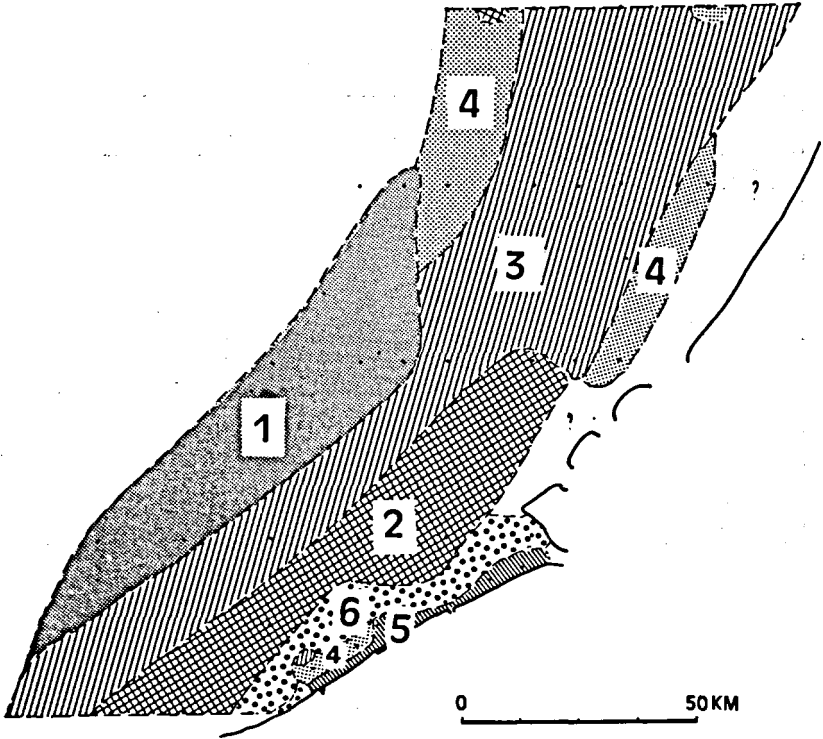
fig. 2.

Spatial distribution of nematode communities in the Southern Bight

Zone 1 :-the open sea region.
          Clean sand with median grain size of the sand fraction
          > 300 µm ; carbon content high (> 12%) ; shell content
          > 8% ; much gravel.
          -The most important nematode families (in decreasing or-
          der of importance) (abundance > 10%) are the Desmodori-
          dae, Epsilonematidae, Xyalidae, Chromadoridae (mostly
          interstitial nematodes).

Zone 2 :-The region of the sandbanks.
          -Sand-gravel bottom ; median size of the sand fraction
          between 200-350 µm ; high carbon content (> 12%) ;
          shell content > 8%.
          -Microlaimidae, Xyalidae, Desmodoridae, Comesomatidae,
          Chromadoridae, Cyatholaimidae.

Zone 3 :-The northern, homogeneous sand zone with megaripples ;
          there is a small extension to the south.

-Clean sand (98-100%) median size of the sand fraction
between 150 and 300 μm ; carbon content is low (0-4%) ;
shell content low (0.2-8.0%).
-Chromadoridae, Desmodoridae, Xyalidae, Microlaimidae,
Cyatholaimidae, Oncholaimidae.

Zone 4 :-In the north-western part of the investigated area and
western Belgian coastal waters.
-Fine to medium sand with a little amount of silt.
-Cyatholaimidae, Xyalidae, Chromadoridae, Desmodoridae.

Zone 5 :-Eastern Belgian coast ; polluted coastal stations.
-Very high amount of silt.
-The Comesomatidae or the Xyalidae are the dominant fa-
milies.

Zone 6 :-More offshore the Belgian coast.
-Silty sand.
-Co-dominance of the Xyalidae and the Comesomatidae.

The same cluster analysis, based on macrobenthic animals and
on harpacticoid copepods from the same sampling sites only
yielded three different zones, which for both groups are more or
less the same : a coastal zone, a transition zone and on open sea
zone (cf. Govaere *et al.*, 1980). Nematodes thus appear to be
more sensitive to slight changes in sediment composition than
other benthic metazoans. A monitoring method may consist in re-
cording the number of higher taxa (families or genera), at least
as a first approach to detect chronical pollution effects

Distribution of the feeding types of nematodes in the Southern Bight.

Free-living nematodes occupy many different roles in aquatic
ecosystems as consumers of bacteria, as grazers of primary produ-
cers and as predators. The trophic diversity in marine nematodes
was first examined by Wieser (1953). He divided nematodes into
four feeding groups according to the structure of the buccal ca-
vity : selective deposit feeders (type 1A), non-selective deposit
feeders (type 1B), epigrowth feeders (type 2A) and predators-
omnivores (type 2B).

Comparing the coastal area with the open sea zone (Fig. 3), a
remarkable difference is noticed in the distribution of the rela-
tive frequency of the four nematode feeding types. The coastal
area is characterized by a large amount of non-selective deposit
feeders (1B). These 1B-nematodes are extremely dominant (>95%)
in the NE region of Ostend (very polluted silty area) ; epigrowth
feeders (2A) and predators-omnivores (2B) gain some importance in
the SW region of Ostend (silty sand).

In the open sea area (fine sand as well as coarse sand bot-
toms), the four feeding types are more equally distributed, al-
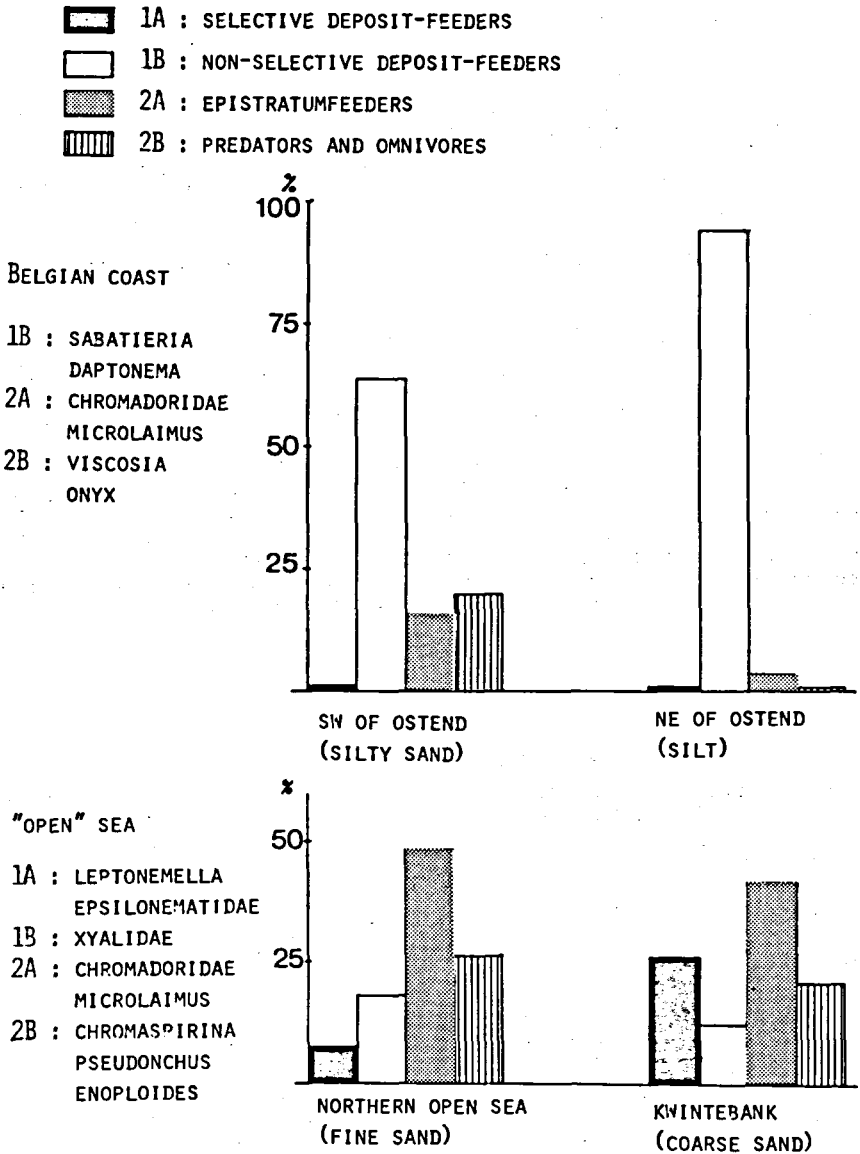though epigrowth feeders are the most numerous group.

fig. 3.

Distribution of nematode feeding types
in different zones of the Belgian coastal water

The diversity in the feeding groups is correlated with sediment characteristics (especially median grain size of the sand fraction and silt-clay content) as far as the sediments of the different regions (e.g. coast - open sea) are compared.

The coastal area : taxonomic group diversity.

The meiobenthic species from the eighteen coastal stations belong to ten taxonomic groups. The four major taxa, occurring in more than 60% of the samples, are Nematoda (frequency 100%), Harpacticoida (74%), Turbellaria (63%) and Polychaeta (60%).

Infrequent taxa are : Halacarida (6.4%), Ostracoda (7.0%), Hydrozoa (3.5%), Tardigrada (5.8%), Gastrotricha (1.2%) and Foraminifera (0.6%). Temporary meiofauna, such as Oligochaeta, Polychaeta and Mollusca occurs in less than 30% of the samples.

The number of taxa over the entire study area tends to increase slightly in time. Starting with a mean of 2.9 taxa/station in June 1977 we found 3.39 taxa per station for September
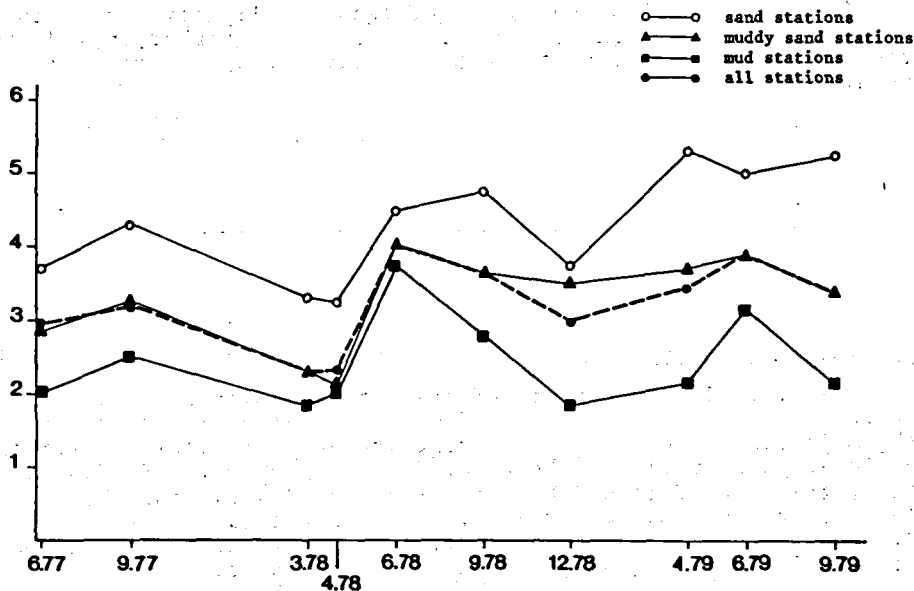

fig. 4.

Mean number of taxa per sample per station group
from June 1977 till September 1979

1979 and an overall mean of 3.23 taxa/station over the whole stu-
dy period. Fig. 4 illustrates this trend for the three sediment
station groups. In the sandy stations an overall mean of 4.26
taxa/sation is found. For the muddy sand stations this value is
3.26 ; for muddy stations it is 2.46 taxa/station (Table 3).

Table 3

Mean number of taxa present in summer and winter per station group

	mean	summer	winter	S/W ratio
sand	4.26	4.50	3.92	1.15
muddy sand	3.26	3.50	2.91	1.20
mud	2.46	2.87	1.96	1.46

Comparing the zone west of Ostend to the Eastern zone of the
study area, we found respectively 4.1 and 2.9 taxa/station.

In sandy stations up to seven different taxa are represented,
while in more than 50% of the other stations only one or two taxa
occur.

In summer samples the number of taxa is slightly higher than
in winter samples. The summer-winter ratio is higher in mud sta-
tions (1.46) than in the two other station groups where it is
about 1.2.

Density of meiofauna in the coastal zone.

The overall mean density of the total meiofauna is 1.8 10^6
ind./m^2. Fig. 5 gives the trend of the relative importance of
the major taxa (Nematoda, Harpacticoida and Turbellaria) over the
whole sampling period. The nematodes contribute for 95.75% to
the total fauna followed by harpacticoids (2.49%), turbellarians
(0.97%) and polychaetes (0.56%). All other taxa represent only
0.23% of total meiofaunal density. The importance of the harpac-
ticoids is incresing slightly in the latter samples.

The meiofaunal composition in summer and winter samples is
rather stable. In summer nematodes dominance is 94.89% against
in winter 97.05%, while for harpacticoids the difference is 3.19%
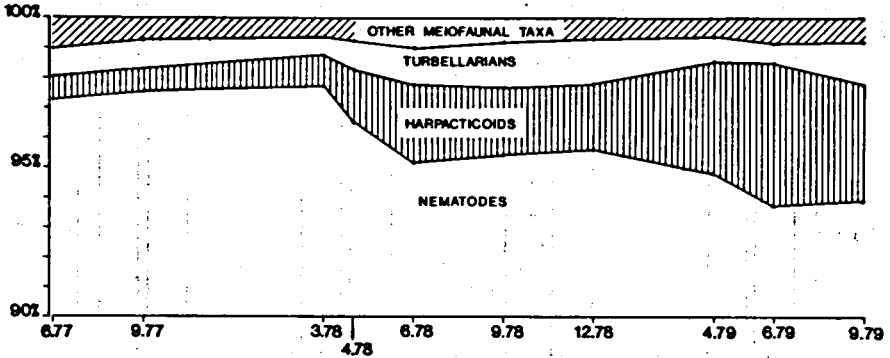to 1.45% (Table 4).

fig. 5.

Composition of the meiofauna for all stations
and per station group in summer and winter

Table 4

Mean composition of the meiofauna
in the eighteen coastal stations in summer and winter

	mean	summer	winter	S/W ratio
% Nematoda	95.75	94.89	97.05	0.98
% Harpacticoida	2.49	3.19	1.45	2.20
% Turbellaria	0.97	1.00	0.94	1.06
% Polychaeta	0.56	0.63	0.45	1.40
% Other taxa	0.23	0.30	0.13	2.31

Through the overwhelming dominance of the nematodes, density
fluctuations of total meiofauna are those of this taxon. Mean
density in summer is 2.3×10^6 ind./m^2, in winter it is 1.1×10^6
ind./m^2, which yields a summer-winter ratio of 2.1.

The mean density of the nematodes over the ten cruises is
1.73×10^6 ind./m . The highest density (Table 5) found is 19×10^6
ind./m^2 at st. 11851 in September '78. In June 1978 in at least
three stations extreme nematode abundances were noted : 12.4×10^6
ind./m^2 at st. 11851, 12.1×10^6 ind./m^2 at st. 11121 and 11.0×10^6
ind./m^2 at st. 12501.

The lowest densities recorded are found in winter samples.
Only 13000 ind./m^2 were found at st. 11672 (March '78) and at st.
11880 (April '78) and 20000 ind./m^2 at st. 12510 in June 1977.
In 10% of the samples densities of less than 100000 ind./m^2 were
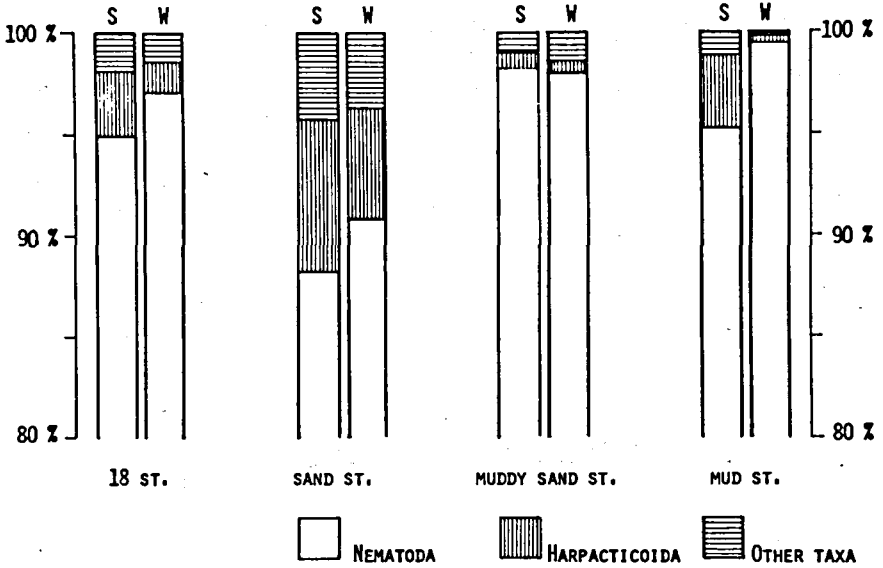noted.

fig. 6.

The relative importance of the major meiofaunal taxa
over the whole sampling period (trand values)

Table 5

Density of nematodes (N/10 cm^2) in the eighteen coastal stations

	6/77	9/77	3/78	4/78	6/78	9/78	12/78	4/79	6/79	9/79	x̄/stat.	+s
10061	713	1326	263	392	857	859	582	–	963	400	706	±336
10080	734	195	286	88	502	1129	3119	324	8534	3150	1806	±2628
10481	3664	5345	1303	596	3010	811	1108	812	575	1552	1878	±1603
10500	3700	2234	1196	397	7467	2368	635	–	3988	2402	2710	±2169
10791	2132	2321	95	3400	4610	7710	1151	229	446	2628	2472	±2347
11121	2946	4259	801	442	12145	2127	1764	955	1043	1098	2758	±3495
11150	56	77	310	239	304	164	309	739	653	442	329	±226
11312	2296	4756	170	433	4273	7050	165	2733	146	828	2285	±2405
11315	604	1188	413	707	1944	103	528	1633	1574	175	887	±652
11331	178	364	100	104	140	164	207	440	141	194	203	±112
11672	255	7750	13	855	1337	145	405	284	1793	534	1337	±2322
11851	1580	5188	547	4063	12401	19020	3107	87	228	93	4631	±6298
11866	45	336	2065	3817	1910	242	855	2250	721	1439	1368	±1170
11880	462	319	674	13	155	67	360	417	2457	84	501	±718
12300	1290	85	105	2150	517	575	130	85	216	308	546	±674
12501	81	2100	1586	1440	11032	181	193	203	2326	182	1932	±3315
12510	20	4120	195	439	968	315	343	2395	7156	734	1669	±2309
12830	1580	4696	6540	688	2430	2271	5100	1944	1306	3913	3047	±1910
x̄/cruise	1241	2592	926	1126	3678	2517	1115	1061	1903	1120		
	±243	±551	±358	±311	±997	±1108	±319	±229	±564	±276	1728	

The mean density in the four sandy stations is 1.19×10^6 ind./ m^2 (Table 6). In summer the mean is 1.54×10^6 ind./m^2 while in winter 0.67×10^6 ind./m^2 are found, which gives a summer-winter ratio of 2.30. In the muddy sand stations, with the highest mean density of 1.92×10^6 ind./m^2, the summer winter ratio was 1.68. For the six mud stations this ratio is 2.69, the mean density is 1.83×10^6 ind./m^2.

Table 6

Mean density of nematodes (in 10^6 ind./m^2)
in summer and winter per station group

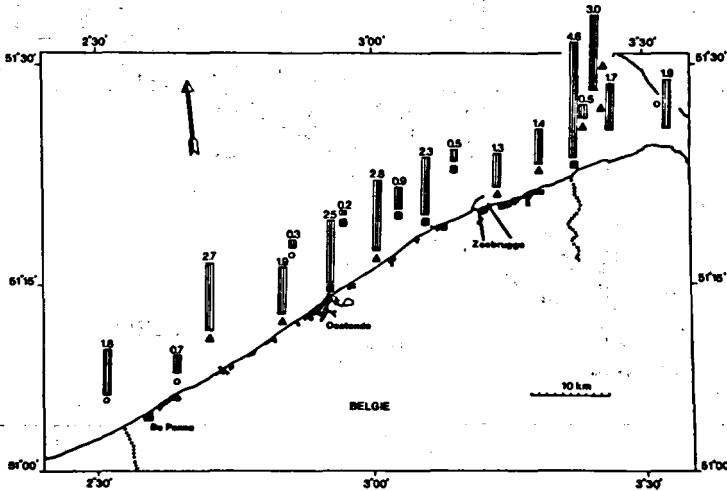|	mean	summer	winter	S/W ratio
18 stations	1.73	2.18	1.06	2.06
sand	1.19	1.54	0.67	2.30
muddy sand	1.92	2.29	1.36	1.68
mud	1.83	2.44	0.91	2.69

fig. 7.

Mean nematode density per station (in 10^6 ind./m^2)
Symbols of the stations are the same as in fig. 1

Fig. 7 gives the spatial distribution of average nematode density per station over the whole study period. Stations with the highest density are mostly localized near the coast. Stations with a low mean density are spread throughout the area.

St. 10061 and st. 11150 are typical sandy stations, st. 12300 is
localized in the middle of the river Westerschelde out-flow and
st. 11880 lies at the border of the traffic channel to Zeebrugge
harbor. The lowest mean density is 0.2×10^6 ind./m^2 at the sandy
mud station 11331.

From the other meiofaunal taxa, only harpacticoids were trea-
ted in detail. In nearly 25% of the samples no harpacticoids, in
13% only one specimen and in 9% two harpacticoid copepods were
present. Thus in nearly half of the investigated samples less
than three harpacticoids were found, which illustrates the extre-
me poverty of the coastal area. The influence of the river Wes-
terschelde deposition zone on the spatial distribution of the
Harpacticoida is clearly demonstrated in Fig. 8. In most of the
stations between the mouth of the estuary and De Haan the mean
density is less than 10 ind.10 cm^2.
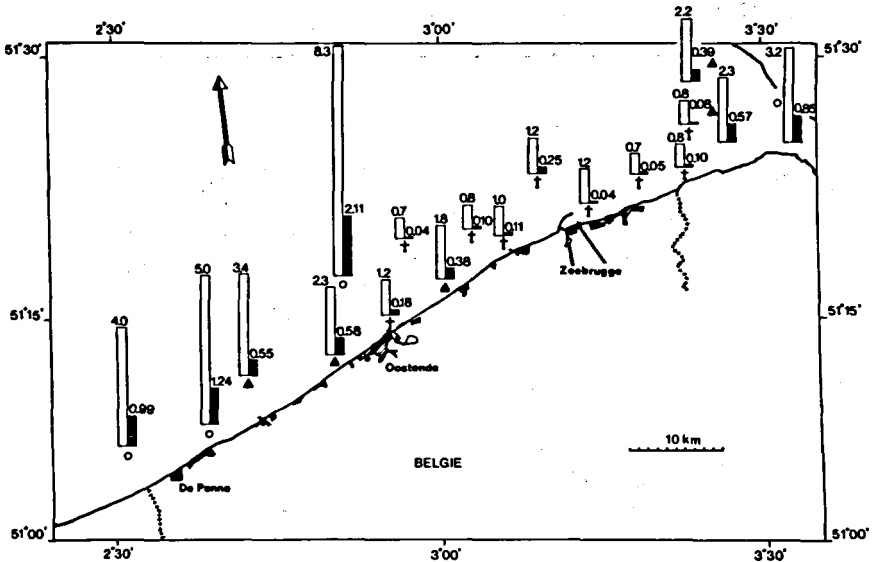
fig. 8.
Mean number of species $\overline{s}$ (open bars), mean diversity H (shaded bars)
and mean density of harpacticoid copepods per station
($\dagger$ < 10 ind.; ▲ 10 < < 20 ind.; o > 20 ind./sample)

In June '79 maximal densities were recorded : 835 ind./10 cm^2
at st. 10791 and 540 ind./10 cm^2 at st. 11150. Only in 5% of the
samples densities of more than 100 ind./10 cm^2 were noted. Ex-
cluding these high values the mean density for the coastal area
is 14300 ind./m^2.

Biomass of meiofauna in the coastal zone.

Because nematodes always comprise more than 95% of the total
meiofauna, only the biomass fluctuations of this taxon will be
considered here.

Individual biomass was determined at the eighteen stations
over four sampling periods. This resulted in a mean individual
biomass of 0.27 μg dwt in June '78, 0.24 μg dwt in September '78,
0.29 μg dwt in December '78 and 0.34 μg dwt in March-April '79.
The overall mean individual biomass is 0.29 μg dry weight per ne-
matode.

Table 7

Mean individual and total biomass (dry weight) of nematoda
in summer and winter per station group

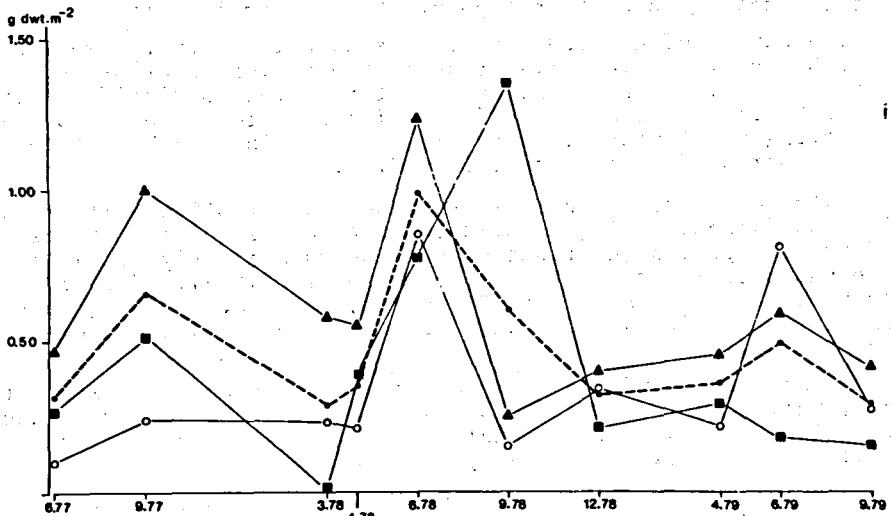|	Ind. Biomass in μg dwt/ind.				Total Biomass in g dwt/m^2			
	Mean	Summer	Winter	S/W ratio	Mean	Summer	Winter	S/W ratio
18 stations	0.290	0.255	0.315	0.81	0.50	0.55	0.33	1.67
sand	0.320	0.260	0.380	0.68	0.38	0.57	0.25	2.28
muddy sand	0.295	0.280	0.310	0.90	0.57	0.64	0.42	1.52
mud	0.245	0.220	0.270	0.81	0.45	0.54	0.24	2.25

fig. 9.

Temporal fluctuations of biomass in the three station groups
(same symbols as in fig. 4)

Table 7 gives the mean individual and total nematode biomass for the whole study period and per station group. In sandy stations the individual weight was highest but total mean biomass is lower as nematodes are less abundant in these sediments. Also the summer-winter differences are greater than in other station groups ; the summer biomass is 2.28 times higher than in winter although the individual weight in summer samples is 68% of the winter values.

Fig. 9 gives the temporal fluctuations of biomass in the three station groups. When sediment particles are smaller the mean individual biomass is less. The higher densities in muddy sand and muddy stations are reflected in the mean total biomass (resp. 0.57 and 0.45 g dwt/m^2). The average mean biomass over the whole study area is 0.50 g dwt/m^2 or 0.2 g C/m^2.

Nematodes of the coastal area.

In Table 8, the dominant species (>5% abundant in at least one station) of the different coastal stations are given.

Species numbers varies from 1 to 16, genera numbers vary from 1 to 14 and families from 1 to 13.

Spearman rank correlations show that there is a significant + correlation (>95%) between the position of the stations from south to north and nematode diversity. We did not consider M05 in this rank because at that point the current is north.

The number of nematode species is much lower in the region NE of Ostend ($\bar{x}$ = 5.4 ± 1.5) than in the region SW of Ostend ($\bar{x}$ = 14.3 ± 1.9). Even the number of families is significantly higher SW from Ostend. Very few families (3 to 7) were found in the strongly polluted silt area in the NE. In the SW area the mean number of families is 11.9 (± 3.1).

Sabatieria occurs in every station (except M1080) with relative abundances varying from 12 to 100%. This genus is represented by six species (distinction between them is only possible on male characteristics) : *S. vulgaris* (de Man, 1907), *S. celtica* Southern, 1914, *S. breviseta* Stekhoven, 1935, *S. strigosa* Lorenzen, 1972 *S. hilarula* de Man, 1922 and *S. granulosa* Vitiello & Boucher, 1971.

S. breviseta and *S. vulgaris* are most abundant on silty bottoms, while *S. strigosa*, *S. hilarula* and *S. granulosa* are found irregularly in most of the stations. *S. celtica* has maximum abundances in clean, fine-sandy sediments.

Species of *Daptonema* may also be very abundant, especially : *D. xyaliforme* (Wieser & Hopper, 1967), *D. fistulatum* (Wieser & Hopper, 1967), *S. svalbardensis* (Gerlach, 1965), *D. normandicum* (de Man, 1890), *D. longicaudatum* (Filipjev, 1922) and *D. tenuispiculum* (Ditlevsen, 1918). These species mostly do not occur together.

Table 8

Relative abundance of nematodes in the Belgian coastal area

|	MO1		M1034	M1050		M1080	M10791	M1096	M1114	M1148	M1186	M1207	M05
	6-72	6-73	8-72	8-72	6-72	6-78	8-72	8-72	8-72	8-72	7-76	6-72	6-72
1B *Sabatieria*+ (6 spec.)	42	75	81	34	–	12	17	87	63	96	100	45	64
1B *Daptonema fistulatum*	30	–	–	–	–	–	–	–	–	–	–	–	–
1B *Daptonema longicaudatus*	–	–	–	–	–	–	–	5	–	5	–	–	–
1B *Daptonema normandicum*	–	–	–	–	8	–	75	–	–	–	–	–	–
1B *Daptonema tenuispiculum*	–	–	–	–	75	75	–	–	–	1	–	15	–
1B *Theristus* sp. A	–	–	1	2	3	–	–	6	6	–	–	–	6
1B *Monhystera disjuncta*	–	–	–	–	–	–	–	–	10	–	–	35	–
1B *Metalinhomoeus* n.sp.	–	7	6	3	–	–	–	4	15	–	–	–	–
2B *Mononcholaimus separabilis*	2	–	–	–	–	–	–	–	–	–	–	–	5
2B *Mesacanthion diplechma*	–	–	–	–	–	3	–	–	–	–	–	–	2
2B *Viscosia viscosa*	3	1	–	4	1	–	1	–	–	–	–	–	–
1B *Richtersia inaequalis*	3	1	2	1	–	–	–	–	1	–	–	–	1
2A *Microlaimus marinus*	–	–	–	1	–	–	–	–	4	–	–	–	–
1B *Ascolaimus elongatus*	–	2	–	–	–	3	–	–	–	–	–	–	–
2B *Synonchiella* sp. A & B	–	–	–	1	5	–	–	–	–	–	–	–	–
2A *Hypodontolaimus* sp. A	–	2	–	–	–	–	6	–	–	–	–	–	–
2A *Spirinia parasitifera*	–	–	–	38	–	–	–	–	–	–	–	–	2
2B *Onyx perfectus*	–	–	–	–	62	–	–	–	–	–	–	–	–
N° families/station	13	9	6	11	10	3	5	3	7	3	1	5	10
N° genera/station	14	11	9	14	14	3	5	4	8	4	1	6	14
N° species/station	15	11	11	15	16	3	5	4	8	4	1	6	14
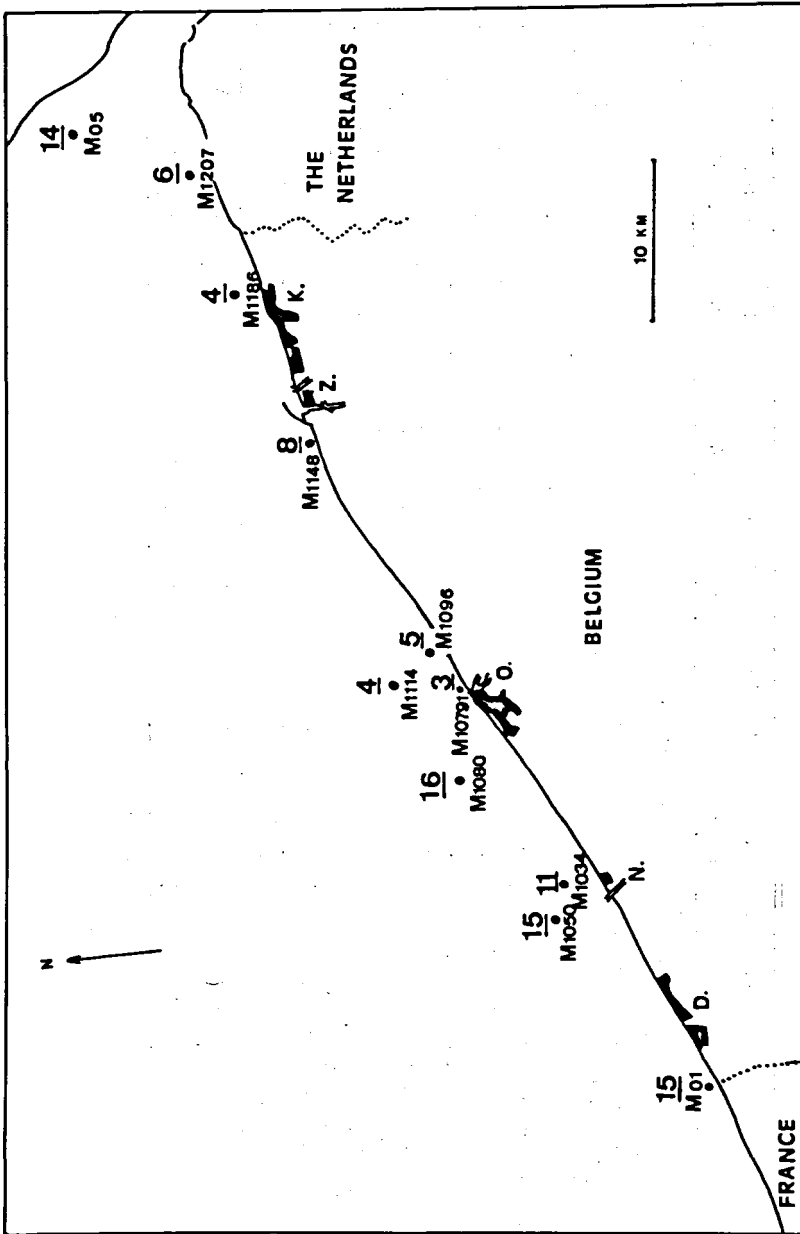
fig. 10.

Mean number of nematode species per station in the Belgian coastal water

Govaere *et al.* (1980) found one faunistic zone along the whole Belgian coast. This zone is characterized by a macroben-thic *Abra alba*-community, and by a meiobenthic *Microarthridion littorale-Halectinosoma herdmani*-community. In their study nema-todes were neglected.

Based on nematode species diversity (nematode family diversi-ty) and on the presence absence of those species, the Belgian coastal waters can be clearly divided into two zones ; the divi-sion between the two zones is at the level of Ostend :
SW of Ostend : higher diversity, silt from the Channel (not so heavily polluted) ; more sandy bottoms ;
NE of Ostend : lower diversity, silt from the Western Scheldt (more polluted) ; more suspended material upon the silty bottom.

Harpacticoida of the coastal area.

Besides nematodes, harpacticoid copepods are good indicators of environmental conditions.

For the description of the harpacticoid communities only box core subsamples are considered. The coastal area, especially the eastern part, is characterized by an extreme poverty in harpacti-coid species. According to Govaere *et al.* (1980) one can distin-guish two communities in the Southern Bight, with a transition zone between them. The coastal assemblage is designated as the *Microarthridion littorale-Halectinosoma herdmani*-community. All our muddy sand and muddy stations are populated by this communi-ty. In the muddy stations only five species, all large epiben-thic or endobenthic forms, were found, while in muddy sand sta-tions up to ten species were noted over the whole period. *Micro-arthridion littorale* is very dominant followed by *Halectinosoma herdmani*, *H. sarsi*, *H. gothiceps* and *Pseudobradya beduina*. The mean diversity is estremely low, as in 60% of the samples only one species (H = 0) or no species at alle were found. This res-ults in a mean diversity of H = 0.13 bits/ind. for the muddy sta-tions and H = 0.33 bits/ind. for the muddy sand stations, with an average number of species per sample of resp. 0.95 and 1.84.

Table 9

Total number of species s, average number of species per sample s̄
and Brillouin's diversity H per station group

	s	s̄	H
sand	26	1.30	1.30
muddy sand	10	1.84	0.33
mud	5	0.95	0.13

Sandy stations are characterized by an impoverished harpacti-
coid community which corresponds well to the transition community
described by Govaere *et al.* (1980). In these stations the most
dominant species are *Paraleptastacus espinulatus*, *Leptastacus*
laticaudatus laticaudatus, *Kliopsyllus holsaticus*, *K. constrictus*
and *Evansula pygmaea*. Large species are *Halectinosoma sarsi*, *H.*
herdmani, *Pseudobradya* spp. and *Canuella perplexa*. The dominant
species are representatives of the *Leptastacus laticaudatus-Para*
mesochra helgolandica-community but *P. helgolandica* is only found
sporadically, and this small interstitial form is replaced here
by species of the genus *Kliopsyllus*.

The average number of species per station is 5.13 and the
mean diversity H = 1.30 bits/ind. The most diverse station
11150, situated near one of the Femish sand bars, yields an ave-
rage of 8.3 species per sample and a diversity H = 2.11 bits/ind.
(Fig. 8).

Productivity of meiobenthos.

It is impossible to measure production of meiofauna in the
field with the means that were at our disposal. Indirect calcu-
lations are based on biomass and density measurements and the
relationships between these parameters and respiration and pro-
duction (Heip *et al.*, this volume).

Two contrasting situations are compared. In the Belgian
coastal waters nematodes are extremely dominant and average den-
sities are high. On the Kwintebank (Willems *et al.*, 1982),meio-
fauna is less abundant and nematodes are less dominant. The pro-
duction calculations for these two habitats are shown in Table
10.

Table 10

Production and respiration of nematodes in Belgian coastal waters
(Summer values above broken line)

	Near-shore sediments	Kwintebank
Temperature (°C)	16	16
Ind. weight (µg)	0.26	0.26
Density (N.10 cm^{-2})	2175	628
Biomass (g dwt.m^{-2})	0.57	0.17
Respiration (nl.h^{-1}.ind^{-1})	0.47	0.77
Respiration (1.half y^{-1}.m^{-2})	4.50	2.12
Respiration (1^{-1} O$_2$.y^{-1}.m^{-2})	5.75	2.71
Production (g dwt.m^{-2}.y^{-1})[1]	4.10	1.22
Production (g dwt.m^{-2}.y^{-1})[2]	2.92	1.31
Consumption (g C.m^{-2}.y^{-1})	6.57	2.62

Based on temperature in the habitat (on average 16°C in summer and 6°C in winter), mean individual weight and total density, individual respiration can be calculated taking the partitioning of feeding types into account (Table 11). This explains why individual respiration is higher on the Kwintebank than in the coastal area, where non-selective deposit-feeders dominate.

Table 11

Metabolic intensity of different meiobenthic species
(respiration of an individual weighting 1 µg dwt)
Values calculated from log R = log a + b log W, taking b = 0.75

	nl $O_2 \cdot \mu g$ dwt$^{-1} \cdot h^{-1}$	Source
Nematoda		
Non-selective deposit-feeders	1.77	Warwick & Price (1979)
Epigrowth-feeders	2.56	Warwick & Price (1979)
Omnivores-predators	3.78	Warwick & Price (1979)
Ostracoda		
Cyprideis torosa	1.78	Herman & Heip (in press)
Copepoda		
Canuella perplexa	3.72	Herman (unp.)
Mesochra lilljeborgi	10.47	Herman (unp.)
Tachidius discipes	12.59	Herman (unp.)

Annual respiration, as the sum of respiration in summer and in winter, can be converted to g C using 1 l O_2 = 0.402 g C and to calories using 1 g C = 12 kcal. Using the relationship between respiration and production established by Humphreys (1979) for non-insect invertebrates log P = 1.069 log R - 0.601 (in cal) and converting back to g dwt, one obtains the figures shown in Table 11 : 2.92 g dwt.m^{-2}.y^{-1} in the coastal area and 1.31 g dwt.m^{-2}.y^{-1} on the Kwintebank.

These figures can be compared with those obtained using a single P/B = 9 as suggested by Gerlach (1971). Estimations obtained in this (crude) way are 4.10 g dwt.m^{-2}.y^{-1} for the coastal zone and 1.22 g dwt.m^{-2}.y^{-1} on the Kwintebank. The lower figure obtained using respiration as the independent variable for the coastal zone can be explained by the low metabolic intensity of the non-selective deposit-feeders. It must be stressed that, in spite of the good agreement in the case of the Kwintebank, both estimations are subject to considerable uncertainty.

The same calculations can be done for harpacticoid copepods, which have a much higher metabolic intensity (Table 11). The results are illustrated in Fig. 11. Whereas harpacticoids are responsable for only a small part of meiobenthic metabolism in the coastal zone, they become dominant on the Kwintebank where their respiration exceeds that of the nematodes.
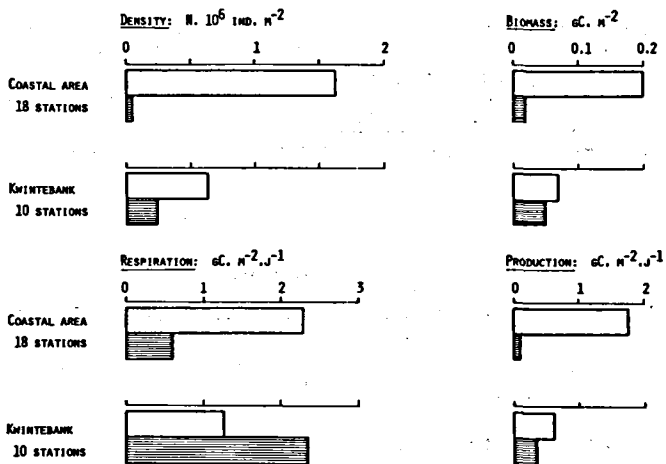
fig. 11.

Comparison between the coastal area and the Kwintebank
based on annual averages of density; biomass, respiration and production
(Nematoda : open bars; Harpacticoida : shaded bars)

Total consumption of the meiofauna can then be estimated when assimilation (P + R) is taken at 60% of consumption. It amounts to 8.6 g $C.m^{-2}.y^{-1}$ in the coastal zone and 5.5 g $C.m^{-2}.y^{-1}$ on the Kwintebank. As the importance of other meiofauna groups on the Kwintebank is considerable, total carbon requirements may approach those of the coastal sediments, indicating that meiofauna plays a much larger role in energy flow through the sandbank.

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BENTHOS OF THE KWINTE BANK

(An exploited sandbank in the Southern Bight)

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Abstract.

This paper summarizes studies on the meio- and macrofauna of a linear subtidal sandbank, the Kwinte Bank, situated in the Belgian Coastal waters, which is exploited for sand and gravel. The distribution of the macrofauna is strongly correlated with the gradient in grain size, but this is much less the case for the meiofauna. Especially nematodes seem to be able to exploit microhabitats more efficiently than the macrofauna.

Introduction.

In the Southern Bight of the North Sea exists a series of pa-
rallel sublittoral sandbanks, the Flemish Banks (Middelkerke Bank,
Kwinte Bank, Buiten Ratel and Oost Dijck) situated in a south-
west north-east direction (Fig. 1). These banks resulted from
the accumulation of sandy deposits of glacial origin, transpor-
ted by the stream draining the waters from the present rivers Rhi-
ne, Meuse, Scheldt and Thames, before the Flandrian marine trans-
gression. During and after the postglacial transgression of the
North Sea these deposits got their present sandbank-shape (Hou-
bolt, 1968).
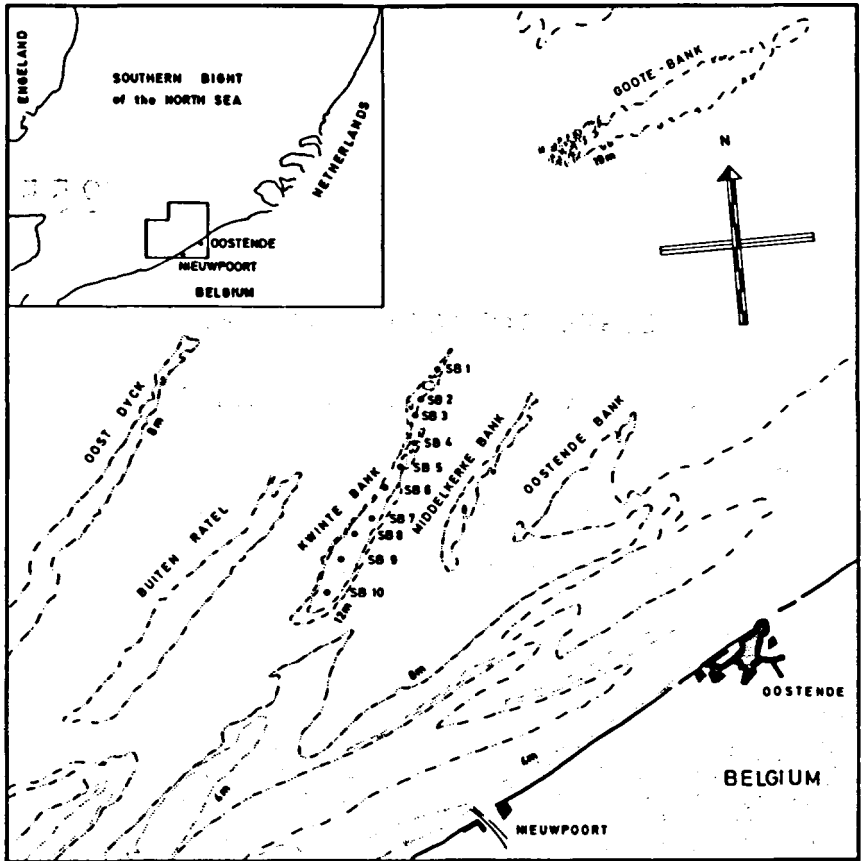
fig. 1.

Map of the study area
(After Vanosmael et al., 1982)

    In spite of intense research on the North Sea during the last
decennium, these sandbanks were not investigated until sand and
gravel exploitation started. This necessitated the evaluation of
the impact of human intervention. Therefore a baseline study
started in 1977. Apart from their use in management these data
are useful in an ecological context, particularly because these
sandbanks are very interesting habitats (stressed high-energy en-
vironments which can be considered as islands of coarser sediments
in a region of fine deposits).

An extensive study of macro- and meiofauna of the Kwinte Bank was made and published in three papers : Vanosmael et al. (1982) and two papers of Willems et al. (1982a, b). In this report we will give a synthesis of these papers.

Material and methods.

Study area.

The description is based on observations made by Bastin (1974) on the Kwinte Bank. In cross-section the bank is asymmetric, with a gentle south-eastern and a steep north-western slope. As the other sandbanks in the area, the Kwinte Bank is submitted to strong turbulence and currents. An irregular structure of the upper sediment layer is the result. The sandbank surface is characterised by sandwaves (5-8 m), megaripples (60 cm) and small sandripples (several cm) [fig. 2]. These structures are continuously broken down and rebuild due to strong currents. This process is very intensive during storms.

fig. 2.
Morphology of the Kwinte Bank
Longitudinal view and cross-sections on three different scales
(After Bastin, 1974)

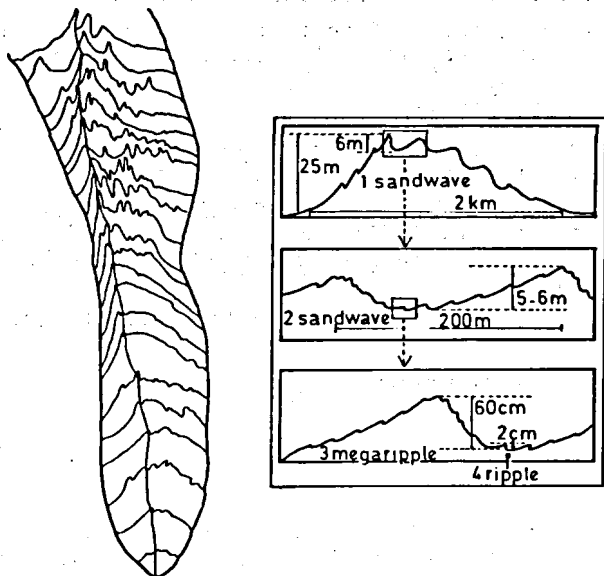In the sediment of the bank exists a gradient from fine (south) to coarse sand (north). In the surrounding depressions turbulence is less important than on the sandbank and some mud and organic matter can deposit. These channels are characterised by an abundant fauna.

Sampling.

In September 1978 ten stations (SB1 to SB10) spread over the whole length of the sandbank were sampled (fig. 1). Per station three macrobenthos samples were taken with a Van Veen grab ($0.1\ m^2$). The meiobenthos samples were obtained by subsampling ($10.17\ cm^2$) a Reineck box-cover ($170\ cm^2$).

The macrobenthos was elutriated using an adaptation of the Barnett-technique, on an horizontal trough (L : 6 m, W : 15 cm, H : 15 cm) closed at one end and open at the other. The samples were washed with a water current over a 250 µm sieve. The meiobenthos samples were decanted over a 38 µm sieve.

For macrobenthos, copepods and nematodes three, two and one sample respectively were examined. For grain size analysis of the sand fraction the method of Buchanan & Kain (1971) was used.

Affinities among the different stations were examined with the Czekanowski qualitative similarity coefficient (Sørensen, 1948) :

$$(1) \qquad S = \frac{2a}{2a + b + c}$$

where a is the number of species present in both stations, b and c the number of species present in station 1 or 2 only.

The stations were clustered by Flexible Sorting (β = - 0.25) [Clifford & Stephenson, 1975] and arranged in an affinity dendrogram [Ordana program of Bloom et al. (1975) adapted by Govaere (1978)].

Species diversity was measured by the Brillouin index H :

$$(2) \qquad H = \frac{1}{N}\ log_2 \frac{N!}{N_1!\ N_2!\ ...\ N_n!}$$

Statistical tests.

It was examined whether a linear trend in the data exists. This was done by correlating the value of the parameter with the series of natural numbers 1 to 10. Differences between the northern part (stations SB1 to SB5) and the southern part (stations SB6 to SB10) were tested with Wilcoxon's U-test. Correlation of the data with the median grain size of the sand fraction was calculated using Spearman's rank correlation coefficient.

Results.

Sediment analysis.

The results are given in table 1. The median particle size of the sand fraction varies between 185 μm and 654 μm . The stations can be arranged according to the Wentworth scale (Buchanan & Kain, 1971) :
— coarse sand : SB3 and SB5;
— medium sand : SB2, SB4 and SB6;
— fine sans : SB1, SB7, SB8, SB9 and SB10.

Mud content and organic material are generally low (less than 5 % except SB2 : 7.2 %). The gravel content varies between 0 and 11 %.

Macrobenthos.

37 species of Polychaeta, 4 species of Archiannelida, 12 species of Mollusca, 17 species of Crustacea and 3 species of Echinodermata have been found (Vanosmael et al., 1982).

Densities.

Densities of the macrofauna range from 500 ind./m^2 (SB8) to 15,330 ind./m^2 (SB4) [table 2]. The mean value is 4,190 ind./m^2. In all stations the small polychaete *Hesionura augeneri* is the most dominant species (about 55 % of the macrofauna).

Total macrofauna density shows a significant linear increasing trend to the north. There is a significant correlation with the median grain size of the sand fraction as well. The same is found for the densities of Polychaeta and Mollusca. For the macro-crustaceans on the other hand, there is a significant linear increasing trend to the southern part of the Kwinte Bank. There is also a difference between north and south in general and a significant negative correlation with the median grain size (fig. 3, 4, 5).

Numeric analysis.

Normal (Q) analysis applied to all stations using all species (except Oligochaeta) Nemertinea and those recorded in one station only) gives us two major station groups (fig. 6).

Group I (SB2, SB3, SB4) is characterized by high density, low diversity (dominance of *Hesionura augeneri*) and more species per station than in group II. The sediment is coarser.

Group II (SB1, SB5, SB6, SB7, SB8, SB9, SB10) has finer sands, lower densities, higher diversity but less species per station than in group I.

Table 1

Coordinates and sediment characteristics of the ten stations on the Kwinte Bank

Station	Coordinates		Depth (m)	Median particle diameter		Sorting		Mud (%) < 63 µm	Gravel (%) > 2000 µm	Organic matter %
	N. lat.	E. long.		(∅)	(µm)	QD ∅	Sk ∅			
SB$_1$	51°20'30"	2°41'40"	15.0	2.09	234	0.38	-0.19	1.61	6.84	3.94
SB$_2$	51°19'45"	2°41'00"	16.0	1.41	375	0.38	+0.25	0	10.62	7.16
SB$_3$	51°19'20"	2°40'45"	15.0	0.61	654	0.28	+0.07	0.30	3.42	3.51
SB$_4$	51°18'40"	2°40'45"	16.0	1.32	402	0.30	+0.02	0.05	1.13	1.81
SB$_5$	51°18'00"	2°40'10"	13.5	0.95	517	0.24	-0.11	0	0.24	2.92
SB$_6$	51°17'30"	2°39'30"	15.0	1.83	281	0.36	+0.25	0.14	2.21	1.69
SB$_7$	51°16'42"	2°38'57"	10.0	2.41	188	0.41	+0.37	0.12	0	4.64
SB$_8$	51°16'20"	2°38'15"	14.0	2.28	205	0.40	+0.36	0	0	1.00
SB$_9$	51°15'35"	2°37'35"	14.0	2.24	211	0.39	+0.32	0.15	0	1.99
SB$_{10}$	51°14'48"	2°37'08"	14.0	2.12	230	0.38	+0.33	0.26	0	1.39

Table 2

Density (N) and relative abundance (A) of macrofauna on ten stations
Mean and standard error of three observations
* : < one individual

	SB1		SB2		SB3		SB4		SB5	
	N	A	N	A	N	A	N	A	N	A
Polychaeta	230± 99	69.3	542± 55	72.1	682±329	80.7	1047±151	68.3	602±64	82.8
Archiannelida	18± 14	5.5	160± 58	21.3	104± 34	12.3	87± 28	5.7	68±14	9.4
Oligochaeta	26± 13	7.7	16± 8	2.1	15± 3	1.8	80± 42	5.2	20± 6	2.8
Nemertini	21± 10	6.3	20± 10	2.6	24± 6	2.9	62± 25	4.0	26± 4	3.6
Mollusca	34± 31	10.3	13± 4	1.8	19± 1	2.2	165± 67	10.8	2± 1	0.3
Crustacea	3± 1	0.8	*	0	*	0.1	5± 2	0.3	8± 2	1.2
Echinodermata	*	0.1	*	0.1	0	0	86± 69	5.6	0	0
Total	332±132		751±119		845±358		1533± 60		727±94	

	SB6		SB7		SB8		SB9		SB10	
	N	A	N	A	N	A	N	A	N	A
Polychaeta	181± 87	64.8	22±5	42.8	14± 3	27.7	67± 24	50.8	105± 49	50.6
Archiannelida	31± 11	11.1	*	1.9	0	0	27± 19	20.4	61± 46	29.5
Oligochaeta	15± 3	5.3	0	0	*	0.6	*	0.5	*	0.3
Nemertini	42± 19	15.0	8±0.3	15.9	4± 2	7.4	23± 5	17.8	27± 15	12.9
Mollusca	6± 3	2.2	*	1.9	0	0	2± 1	1.2	*	0.5
Crustacea	5± 1	1.6	10±6	37.4	32± 7	64.3	12± 4	9.4	13± 6	6.1
Echinodermata	*	0.1	0	0	0	0	0	0	0	0
Total	280± 69		52±8		50± 9		131± 41		207±104	

	Mean	
	N	A
Polychaeta	349± 70	71.1
Archiannelida	56± 12	11.4
Oligochaeta	17± 6	3.5
Nemertini	26± 4	5.3
Mollusca	24± 11	4.9
Crustacea	10± 2	2.0
Echinodermata	9± 7	1.8
Total	491± 91	
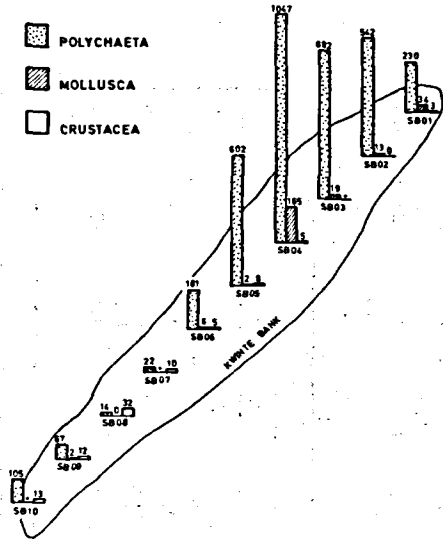
fig. 3.

Density of macrobenthos (N/0.1 m^2)
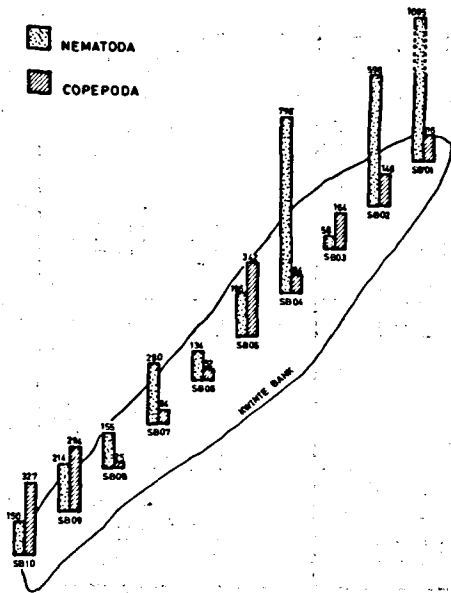
fig. 4.

Density of meiobenthos (N/10 cm^2)
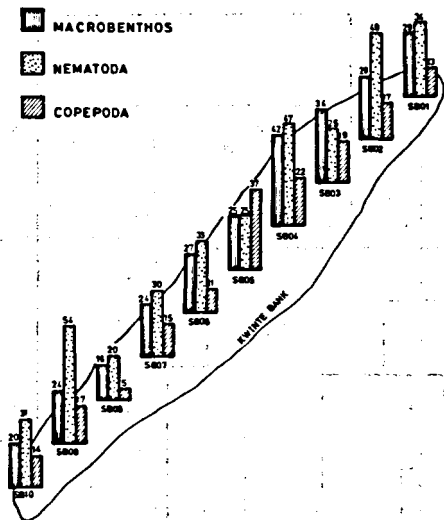
fig. 5.

Number of species per station

Conclusion.

The occurrence of two different macrofaunal communities is reflected in two station groups. Both groups have a different sediment composition.

Geographically the Kwinte Bank is located in the Transition Zone of the Southern Bight of the North Sea described by Govaere et al. (1980). Yet its fauna ressembles that of the Open Sea Zone which holds for sediment composition as well. This sandbank can be seen as a biogeographical island in the Transition Zone, with a fauna resembling that of many other sand biotopes described in literature.

Mobile, quickly moving organisms such as *Hesionura*, *Nephtys*, *Glycera* and others are better adapted to the continuously changing conditions in this turbulent area than sesile tube-builders.
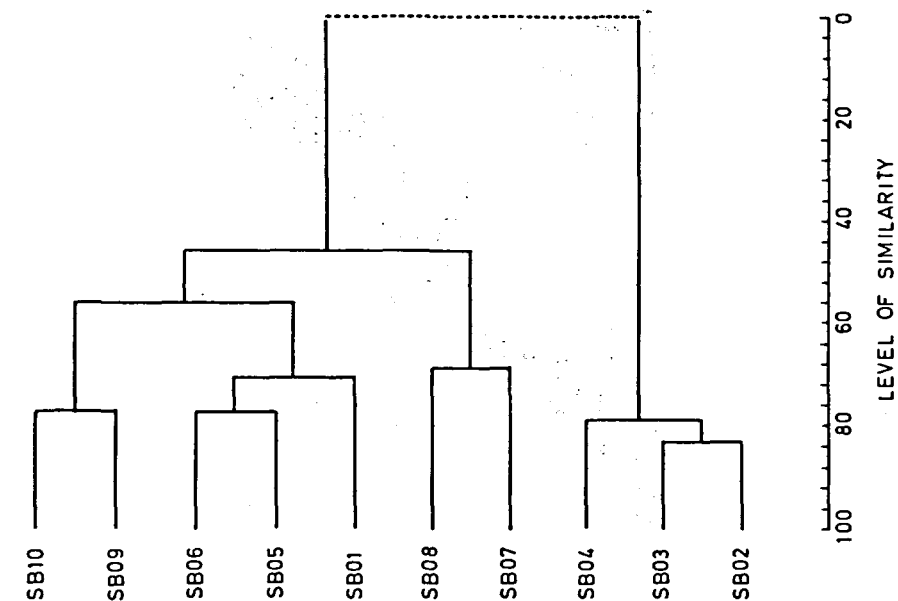
	Group II	Group I
Median particle diameter	188-517 μm	375-654 μm
Mud < 63 μm (%)	0-1.6	0-0.3
Gravel > 2 mm (%)	0-6.8	1.1-10.6
Density (N/0.1 m^2)(SE)	175 ± 46	757 ± 130
Polychaeta	175 ± 46	757 ± 130
Archiannalida	29 ± 10	117 ± 24
Oligochaeta	9 ± 3	37 ± 16
Nemertinea	22 ± 4	35 ± 10
Mollucca	7 ± 5	66 ± 32
Crustacea	13 ± 3	2 ± 1
Echinodermata	0.1 ± 0.1	29 ± 24
Total macrofauna	254 ± 55	1043 ± 165
Species number		
Polychaeta	24	28
Archiannelida	4	4
Mollusca	8	12
Crustacea	16	10
Echinodermata	2	2

fig. 6.

Clustering (Q-mode, Czekanowski-index)
of ten macrobenthos stations
(After Vanosmael et al., 1982)

Table 3

Density (N/10 cm²) and relative abundance (A) of meiofauna on ten stations
Mean and standard error of two observations
* : < one individual

	SB1		SB2		SB3		SB4		SB5	
	N	A	N	A	N	A	N	A	N	A
Nematoda	1095±234	88.7	596±126	75.0	58± 8	21.7	796± 4	80.8	196±10	33.6
Copepoda	116± 28	9.3	146± 6	18.9	164±31	61.4	84±12	8.5	342± 2	58.8
Annelida	21± 8	1.7	22± 4	2.8	5± 1	1.9	54± 9	5.5	11± 2	1.9
Ostracoda	*	0.1	14± 2	1.9	19± 8	7.2	32± 8	3.2	20± 2	3.4
Halacarida	*	0.1	6± 1	0.8	19± 1	7.2	18± 4	1.8	11± 4	1.9
Hydrozoa	0	0	4± 4	0.5	2± 2	0.8	*	0.1	2± 1	0.3
Total	1234±272		771±126		266±29		983±35		581±22	

	SB6		SB7		SB8		SB9		SB10	
	N	A	N	A	N	A	N	A	N	A
Nematoda	134± 62	69.9	280± 6	77.9	155± 3	83.3	214± 7	39.2	150±16	29.8
Copepoda	52± 20	26.9	64±10	17.8	25± 6	13.4	294± 6	54.0	327±67	64.9
Annelida	4± 1	2.3	11± 3	3.1	4± 2	2.4	30± 6	5.5	14± 2	2.8
Ostracoda	0	0	0	0	0	0	0	0	0	0
Halacarida	*	0.2	0	0	0	0	0	0	6± 3	1.2
Hydrozoa	*	0.2	4± 4	1.1	2± 2	1.1	6± 6	1.3	7± 2	1.4
Total	192± 43		358± 17		186± 9		544±12		503±96	

	Mean	
	N	A
Nematoda	366± 77	65.1
Copepoda	161± 26	28.6
Annelida	17± 3	3.0
Ostracoda	9± 3	1.6
Halacarida	6± 2	1.1
Hydrozoa	3± 1	0.5
Total	562± 77	

Meiobenthos.

(Willems et al., 1982a,b).

Densities.

Nematodes represent more than 50 % of the total meiofauna in stations SB1, SB3, SB4, SB6, SB7 and SB8, whereas copepods are dominant in SB3, SB5, SB9 and SB10 (table3, fig. 4).

Mean nematode density is 366 ind./10 cm^2 for the whole sandbank, for copepods it is 161 ind./10 cm^2.

	Group III	Group II	Group I
Median particle size (µm)	188-230	234-281	375-654
Mud < 63 µm (%)	0.0-0.3	0.1-1.6	0.0-0.3
Gravel > 2 mm (%)	0.0	2.2-6.8	0.2-10.6
Density (N/10 cm^2; SE)	200 ± 20	615 ± 295	407 ± 114
Species number	78	52	76
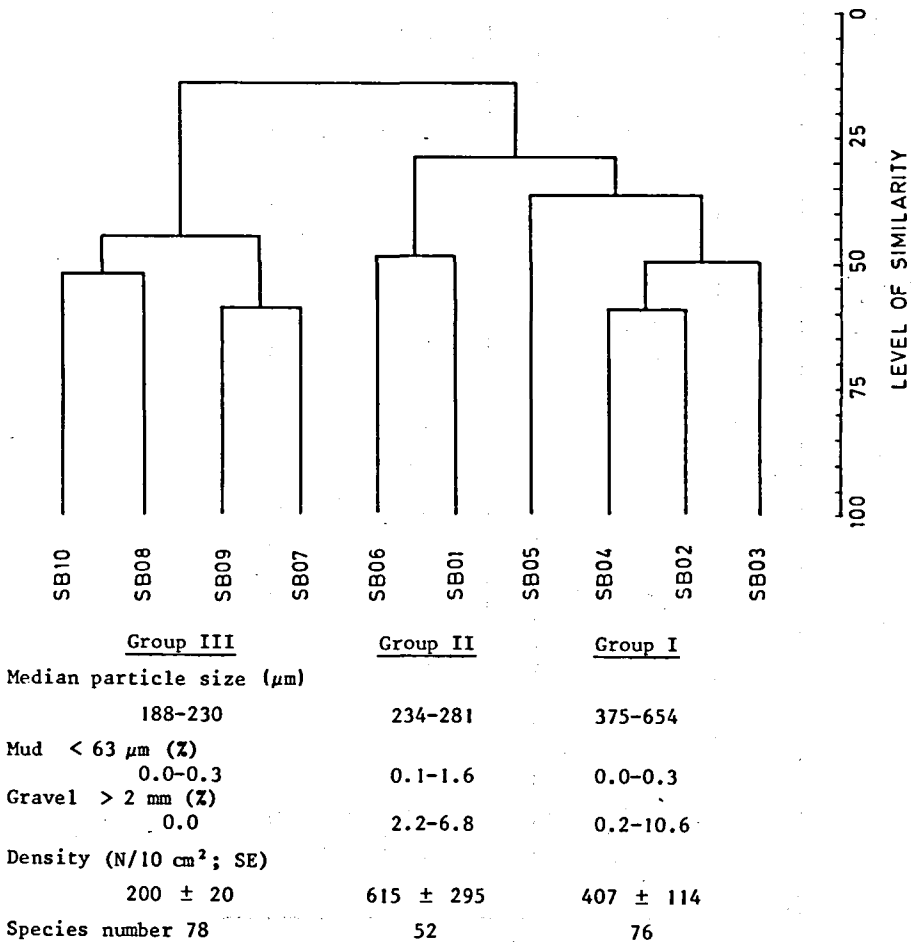
fig. 7.

Clustering (Q-mode, Czekanowski-index) of nematodes

Nematoda.

136 species belonging to 28 families were found in the ten stations of the Kwinte Bank. The ten stations can be arranged in three clusters (fig. 7).

Group I (SB2, SB3, SB4, SB5) : the sediment ranges from medium to coarse sand, densities are intermediate between groups II and III. Low dominance.

Group II (SB1, SB6) : stations are characterized by fine to medium sand. Density is highest.

Group III (SB7, SB8, SB9, SB10) : with fine sands. Density is intermediate between I and II.

Conclusion.

The existence of three nematode associations can be attributed to different sediment types. Species found on the Kwinte Bank are also found in the sandy bottoms of the Southern Bight. The composition of the nematodes on genus level is very comparable to that of other clean sand known from literature.

Diversity is higher than elsewhere in the Southern Bight. This is probably due to the larger number of microhabitats.

Copepoda.

On the Kwinte Bank 65 species belonging to 8 families were found. Clustering yields to station groups (fig. 8).

Group I (SB2, SB3, SB4, SB5) : clusters the medium to coarse sand stations and is characterized by high densities. The mean number of species per station is higher than in group II.

Group II (SB1, SB6, SB7, SB8, SB9, SB10) : contains the fine to medium sand stations. Density is lower, the mean number of species as well.

These two associations correspond to the sediment types as well. The fauna of the Kwinte Bank again resembles that of other pure sand biotopes in the Open Sea Zone described by Govaere et al. (1980). Copepod diversity is significantly higher in the northern part of the sandbank and is positively correlated with the median grain size of the sand fraction.

Number of species.

For macrobenthos, nematodes and copepods we can conclude (fig. 6) : there is a significant linear increase to the north for macrobenthos only; for macrobenthos and copepods there is a significant difference between northern and southern part of the bank and a significant correlation with the median grain size of the sand fraction. This is not true for the nematodes. The number of nematode species in the fine and coarse sand stations does not

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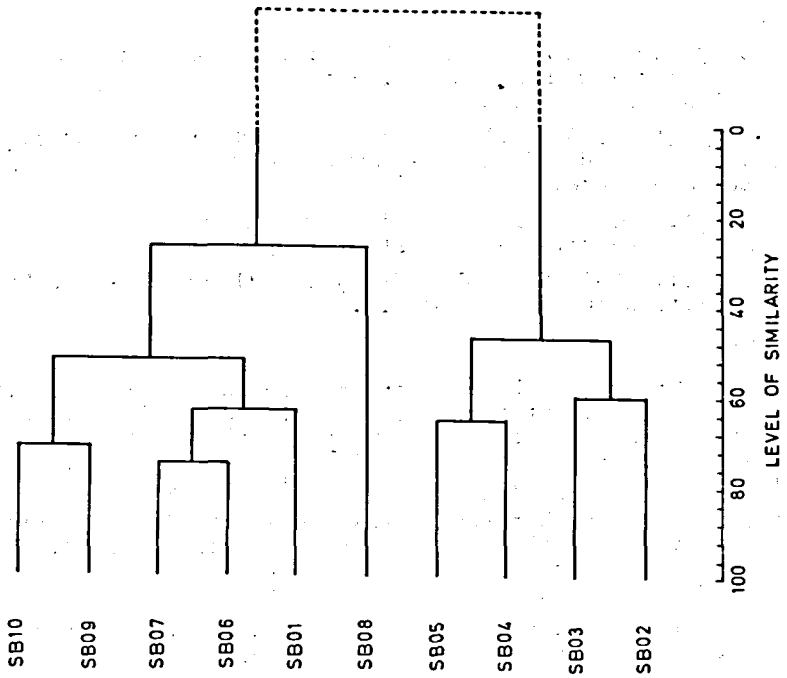
	Group II	Group I
Median particle diameter (μm)	188-281	375-654
Mud < 63 μm	0-1.6	0-0.3
Gravel > 2 mm	0-6.8	0.2-10.6
Density (N/10 cm^2; SE)	146 ± 37	184 ± 37
Total species number	29	52

fig. 8.
Clustering (Q-mode, Czekanowski-index) of harpacticoid species
on ten stations of the Kwinte Bank

differ much. This group therefore shows a higher specialisation
and adaptation. Its high and relatively constant diversity over
the whole sandbank also confirms this.

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VI

MACROBENTHOS IN THE WESTERN SCHELDE ESTUARY

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Abstract.

Species richness, density and diversity of macrobenthos of the Western Scheldt river were measured in September 1980 over four transects. Muddy sands over the whole salinity range contain representative of the *Macoma balthica* community, whereas some species of the *Abra alba* community are present in the more saline parts of the river. Diversity and species richness decrease with decreasing salinity.

Introduction.

The Western Scheldt is the most southern estuary of the Delta area connecting the river Scheldt with the North Sea. It is a coastal plain estuary with partial mixing (Nihoul and Wollast, 1977).

In two previous reports (Heip et al., 1979; Van Damme et al., 1980) quantitative data about Nematoda, Harpacticoida and fluctuations of the meiobenthic communities in the Western Scheldt were presented. This report treats the macrobentic data

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of the same stations in the Western Scheldt. It is an analysis of
the relationship between the distribution of the macrobenthic
infauna and certain environmental parameters.

Material and methods.

Twenty stations distributed over four transects were sampled
on September 27th and 28th, 1978 (figure 1). The sublittoral
stations were sampled with a 0.1 m² van Veen grab, littoral
stations were hand-collected with a plastic core (5 cores of
77.8 cm² per sample), three samples on each station. The
contents of the grabs or cores were fixed in buffered formalde-
hyde and sieved in the laboratory through a stainless steel
screen with 1 mm mesh size. All specimens were determined to
species level and counted. Oligochaetes and nemerteans were only
counted.
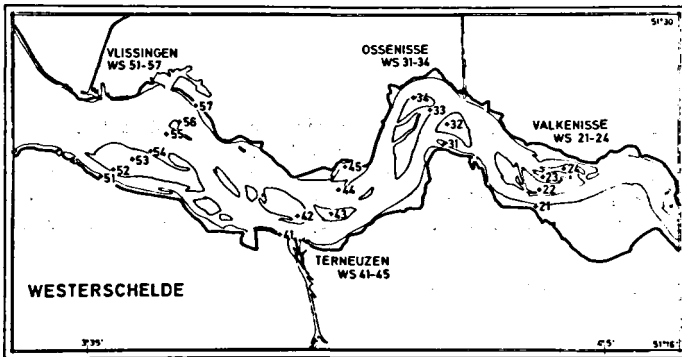
fig. 1.

Location of the 20 sampling sites
distributed over 4 transects in the Western Scheldt

The particle size distribution of the sand-fraction was
determined by dry sieving and classified according to the
Wentworth scale.

The organic matter content was determined according to the
method of El Wakeel and Riley (1957).

Species occuring in more than 5 % of the stations were used
for cluster analysis, based on matrices of similarity between all
possible pairs of stations in the Q-mode and between all possible
pairs of species in the R-mode. In the Q-mode the Sørensen index
for binary data was used and the Canberra metric (Lance and

Williams, 1967) for continuous data. The obtained matrices of similarity were subjected to group average sorting (Sokal and Sneath, 1963).

A Spearman rank correlation coefficient was calculated between the different parameters.

To measure the species diversity the Brillouin index (in bits/ind.) was used :

$$H = \frac{1}{N} (\log_2 N! - \log_2 N_1! - \ldots - \log_2 N_n!)$$

in which N is the total number of individuals and N_i the number of individuals of the ith species (Brillouin, 1956).

Results and discussion.

Sedimentology.

The results are shown in table 1. The sediments consists mainly of fine sand. At WS41 and WS51 the substrate is muddy sand, medium sand is found at WS44, WS55 and WS57. The silt-clay and organic carbon content were generally low. A high percentage of mud was found at WS21, WS41 and WS54.

Table 1

Depth and sediment measures

Station	Median size (phi)	Sorting coeff.	Sand %	Silt-clay %	Gravel %	Organic carbon %	Depth (m)
WS21	2.951	0.490	85.68	14.32	0.00	0.769	-2.0
WS22	2.936	0.382	97.36	2.64	0.00	0.305	+1.0
WS23	2.316	0.266	99.99	0.01	0.00	0.021	-2.5
WS24	2.445	0.391	96.95	3.05	0.00	0.215	+0.5
WS31	2.206	0.496	99.92	0.08	0.00	0.062	-2.5
WS32	2.290	0.324	99.96	0.04	0.00	0.018	-0.5
WS33	2.267	0.346	99.73	0.27	0.00	0.044	-2.0
WS34	2.529	0.419	99.19	0.81	0.00	0.100	-0.5
WS41	3.013	0.533	86.67	13.33	0.00	0.343	-2.5
WS42	2.845	0.375	96.84	2.07	1.09	0.166	+0.5
WS43	2.391	0.354	99.89	0.11	0.00	0.021	0.0
WS44	1.808	0.585	97.88	0.02	2.10	0.037	-5.0
WS45	2.294	0.375	98.27	1.73	0.00	0.169	-0.4
WS51	3.316	0.471	95.30	4.70	0.00	0.334	+0.5
WS52	2.678	0.497	94.41	5.59	0.00	0.172	+0.5
WS53	2.730	0.456	89.33	4.12	6.55	0.159	+0.5
WS54	2.444	0.663	74.60	25.40	0.00	0.226	-3.0
WS55	1.614	0.379	99.81	0.00	0.19	0.032	-3.5
WS56	2.078	0.464	99.96	0.04	0.00	0.022	-3.0
WS57	1.895	0.420	96.04	0.46	3.50	0.153	-1.0

Benthos.

Forty different species were identified in this study. The *number of species* per station (3×0.1 m^2) ranged from two in WS24 to 22 in WS53 and WS41 (Table 2).

Table 2

Total number of species, mean density and diversity per station

Station	Number of species	Density (N/0.1 m^2)	Diversity H in bits/ind.
WS21	10	390	2.17
WS22	13	3929	1.48
WS23	7	13	1.94
WS24	2	1	0.50
WS31	7	5	1.94
WS32	5	57	1.27
WS33	11	15	2.53
WS34	16	703	1.44
WS41	22	1336	1.78
WS42	17	2490	1.69
WS43	7	8	1.80
WS44	3	3	1.02
WS45	3	1	0.86
WS51	17	2708	3.15
WS52	20	1355	2.77
WS53	22	2686	2.31
WS54	10	30	2.10
WS55	5	7	1.46
WS56	4	2	1.33
WS57	6	26	0.83

Density of the macrobenthos is highest in the stations WS22, WS42, WS41, WS53, WS51 and WS52 (Table 2). Both parameters show an increase with increasing particle size (Spearman's rank correlation $r = 0.72$ and $r = 0.74$, $p < 0.01$ respectively).

The hierarchical *clustering* shows the existence of several station- and species groupings.

The first cluster groups eight stations (WS53, WS52, WS51, WS42, WS41, WS34, WS22 and WS21) situated over different transects and located in the shallow parts of the estuary (figure 2). They are characterized by well sorted, muddy and fine sands. Abundant in this cluster is a group of estuarine species corresponding to the first species group of the R-analysis (figure 3). These species are *Corophium volutator, Macoma balthica, Heteromastus filiformis, Hydrobia ulvae, Cerastoderma edule, Nereis diversicolor, Eteone longa, Capitella capitata, Tharyx marioni* and *Pygospio elegans* reaching densities of respectively 1291, 176, 192, 1335, 104, 21, 36, 113, 1020 and 872 individuals
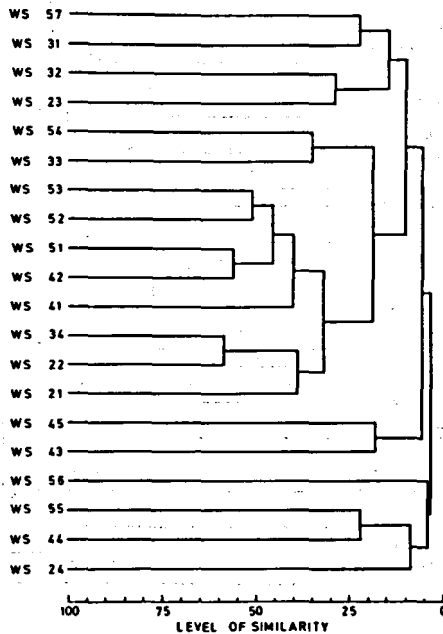
fig. 2.

Dendrogram resulting from clustering the stations
by the Canberra metric

per square meter calculated for the whole estuary. They can
be considered as belonging to the *Macoma balthica* community
(Petersen, 1914) or the "boreal shallow mud association" of Jones
(1950).

A second species cluster is also present in this station group
(WS53, WS52, WS51, WS42, WS41) but confined to the more saline
parts of the estuary, namely *Nephtys hombergii, Anaitides* spp.
(*groenlandica* group), *Scoloplos armiger* and *Crangon crangon*. The
same can be said of a few less frequently observed species as
Tellina tenuis, Cumopsis goodsiri, Magellona papillicornis and
Mysella bidentata. The distribution of these last taxa links up
with the *Abra alba* community of the Belgian coast (Govaere et
al., 1980).

A second smaller station group is composed of two deeper lying
stations in the euhaline part of the Western Scheldt (WS44,
WS55), characterized by coarser sand and less mud, which may be
explained by a stronger current. Species confined to these
stations are *Nephtys cirrosa, Ophelia borealis* and *Gastrosaccus
spinifer*, all preferring a coarser sediment type.
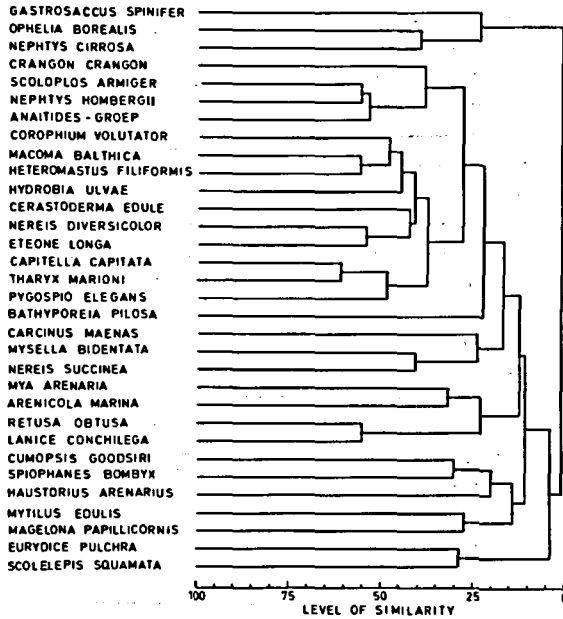
fig. 3.

Dendrogram resulting from clustering the species
by the Sørensen index

Highest diversity values are noted in stations of the first
station   cluster :   WS51   (H = 3.15),   WS52   (H = 2.77),   WS53
(H = 2.31)   and   WS21   (H = 2.17).   Lower   values   as   in   WS22
(H = 1.48), WS34 (H = 1.44), WS42 (H = 1.69) and WS41 (H = 1.78)
are due to dominance of *Corophium volutator*, *Hydrobia ulvae* and
Tharyx marioni.

The mean diversity per transect showed a significant corre-
lation with salinity ($r = 0.97$, $p < 0.01$) and increased towards
the mouth of the river (Table 3).

Table 3

Mean diversity, total number of species
and mean salinity per transect

	transect Vlissingen	transect Terneuzen	transect Ossenisse	transect Valkenisse
H	3.10	2.41	1.90	1.81
n	33	28	20	16
salinity (‰)	30.8 (N) 28.0 (S)	24.9	20.3	15.3

   This decline from the polyhaline parts (transect Vlissingen)
towards the mesohaline reach of the estuary (transect Valkenisse)
has also been demonstrated by Wolff (1973) for the macrobenthos
and by Heip et al. (1979) and Van Damme et al. (1980) for the
meiobenthos. Last authors noted 4.3 taxa of meiobenthos on the
average at Vlissingen and only 1.5 at Doel; the same trend was
shown in the annual mean density, respectively 2.2 million and
0.16 million individuals per square meter.

   In conclusion the distribution of the infauna is roughly
determined by a salinity gradient, although the small scale
distribution of the species and their abundance are correlated
with the sediment characteristics.

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VII

A SUMMARY OF BENTHIC STUDIES

IN THE SLUICE DOCK OF OSTEND DURING 1976-1981

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Abstract.

The Sluice Dock of Ostend (Belgium) has been studied intensively since many years, with early work by Leloup & Miller (1960) and Leloup & Polk (1967). In the seventies ecological investigations were started by the group Podamo. Benthos is studied since 1976.

During these twenty years the Sluice Dock has changed profoundly. In the sixties it was a healthy environment with an important oyster culture. Gradually it became more eutrophic until several dystrophic crises occurred in the early eighties, with pH values exceeding 10 at times.

These high pH values are the result of the enormous primary production by green algae, with *Ulva lactica* especially dominant. Under the *Ulva* beds the sediments become anoxic causing mass mortality of benthos. Some animals are associated with *Ulva* itself, such as the polychaete *Platynereis dumerilli*, which as not been noted from the Sluice Dock before, and the mollusk *Cerastoderma edule*, where the juveniles live attached to *Ulva* but drop to the bottom when they grow too heavy. The total production of the macrofauna inhabiting *Ulva* is considerable and amounts to 160 g C/m^2.year.

Due to repair works the Sluice Dock was drained over a considerable surface in March 1980. The sediments dried out completely. When it was again filled, recolonization of the sediments started rapidly with spionid polychaetes (*Pygospio elegans*), succeeded rapidly by capitellids. Later the mollusk *Mya arenaria* settled heavily, causing the disappearance of the surface inhabiting tube builder *Polydora ciliata*.

Introduction.

The Sluice Dock of Ostend is a 86 ha shallow (mean depth 1.5 m) semi-enclosed poly- to euhalien lagoon (salinity ranges 25-32 %). Polk (1978) gives a more detailed description of the lagoon which has been studied for many years : Leloup and Miller (1960), Leloup and Van Meel (1965) and Leloup and Polk (1967) drew-up an inventory of its flora and fauna completed with some ecological notes.

In the seventies intensive ecological studies were carried out by the group Podamo. These studies resulted in an ecological model of the Sluice Dock in which the planktonic energy fluxes were emphasized (Podamo, 1976). In these studies no particular attention was given to the benthic organisms. We investigated the meio- and macrobenthic populations especially in relation to the eutrophication of the Sluice Dock. At the moment this eutrophication is leading to a total deterioration of the lagoon. The mean reason of the eutrophication is nutrient enrichment caused by the renewal of the water. For scientific purposes (mariculture studies) as well as for non-scientific reasons (recreation, public works) the water of the Sluice Dock is renewed with harbour water, polluted with organic material and nutrients from industrial wastes and sewage. The organic material is remineralised by bacterial activities into soluble nutrients which results within a few days in a plankton bloom. Another part of the nutrients is assimilated by the benthic macroalgae dominated by *Ulva* sp. In 1979 we observed an extremely developed *Ulva*-bed, the thalli covering the bottom of the lagoon totally and locally even the water column was completely filled up with large thalli which were detached from the bottom. The *Ulva* biomass for the whole area estimated to be 250 ton dwt at the maximum of its growth. This immense algal bed influenced both the water column and the sediments in a negative way : during high rates of photosynthesis the pH rises to values of 10 while the oxygen varies strongly with high values during the day and low during the night. On the other hand the *Ulva*-bed suffocates the sediments, the latter becoming totally anoxic and destroying the benthic fauna. In winter the algae decompose, resulting temporally in bad water conditions but the sediments reoxygenate and a recolonization of the sediments by benthic organisms starts. We studied this recolonization process and the associated fauna of the macroalgae and made a comparison with the period just before the disastrous disturbances.

Results and discussion.

Macro- and meiofauna of the sediments before a total destruction.

The meiobenthos is dominated by nematodes and harpacticoid copepods. The mean densities vary from year to year. In 1976 a mean density was noticed of 2,900,000 ind/m^2 and 528,000 ind/m^2

respectively while in 1977 the nematode number increased to 5,600,000 ind/m² and the harpacticoids decreased to 371,000 ind/m². We did not made a detailed study of the nematode community. The benthic harpacticoids are dominated by a single species, *Canuella perplexa*, making up for up to 99 % of the copepod population. This species is found in high numbers throughout the year and reaches a high biomass of 1.75 g dwt/m². Its production was measured with a cohort-analysis and production amounted to 3.43 g/m² in 1976 and 1.73 g/m² in 1977 (Huysseune, 1978).

The macrobenthos is dominated by spionid and capitellid polychaetes and the molluscs *Ceratoderma glaucum* and *Hydrobia ulvae*. Due to their patchy distribution the densities vary strongly from sample to sample and large differences are found between successive years.

Associated fauna and flora of Ulva.

From July 1979 to June 1980 we studied the associated fauna of the *Ulva*-bed (Fredericq, 1980).

On the algal thalli we find some typical macrobenthic organisms which are not living in the sediments or, if they do so, only in different life stages.

Typical algal organisms are the anemone *Metridium senile* and the tunicate *Molgulla manhattensis*. The latter is not found in the lagoon from summer 1980 onwards, probably due to drainage of the Sluice Dock in March 1980.

A dominant polychaete species is *Platynereis dumerilli*, a species not mentioned from the Sluice Dock before (mean density 4,800 ind/m²). In summer a maximum density of 29,000 ind/m² was found but in winter, when the algae died, a small part of the population hibernates in the sediments.

Another dominant species is *Ceratoderma glaucum* (also unmention before). The juvenile stages prefer to settle on macrovegetation enhancing their survival (Brock, 1979). The mean density amount to 3,600 ind/m². In summer we find only juveniles attached to the thalli with small byssus threads. Later in season when they become heavier after a growth period, the byssus threads are not capable to hold and the animal falls into the sediment. This is a critical period in the development of the cockles, involving high mortality.

Further dominant species are two spionid polychaetes, *Polydora ciliata* and *Polydora ligni*. Because of the very similar morphology we considered the two species as one *Polydora ciliata-ligni* group. The mean density was 17,500 ind/m², with a maximum of 96,000 ind/m² just after settling of young individuals from the plankton.

To complete the list of species we mention *Hydrobia ulvae*, *Nassarius reticulatus*, *Mytilus edulis* and *Gammarus locusta*.

A production estimation was calculated using P/B ratio's published in literature. The total macrobenthic community living on the *Ulva*-bed has a mean yearly production of 300 g dwt/m^2.year or 120 g C/m^2.year.

The meiobenthic community on the algae was dominated by nematodes (52 %, mean density 322,000 ind/m^2) and harpacticoid copepods (28 %, mean density 241,000 ind/m^2). Other small groups are Turbellarians, Archiannelids and Halacarids. A production estimate gave 26 g dwt/m^2.year or 10 g C/m^2.year.

The recolonization of a destroyed sediment.

The recolonization of a sandy sediment after the *Ulva* bloom has been studied from January to December 1980 (Burtheel, 1981). Drainage in March 1980 (due to works to repair the dikes) interrupted recolonization and caused a supplementary destruction of the benthos.

Recolonization started with spionid polychates : first appeared *Pygospio elegans*, but it is rapidly replaced by *Polydora ciliata-ligni*. In their turn these species disappear in favour of capitellid polychaetes *Capitella capitata* and *Heteromastus filiformis*. In August we noted an important settling of *Mya arenaria*. Meanwhile *Polydora* disappeared almost because of spatial competition with *Mya* : *Polydora* lives in the top cm of the sediment, *Mya* on the contrary in deep burrows with protuted in- and exhalant sipho's, occupying an important part of the sediment. The capitellids on the contrary live deeper (*Heteromastus* is found between 4 and 20 cm depth) and meet no difficulties of the siphonal activity. This evolution continued until June 1981, when a new *Ulva*-bed destroyed the benthic community again.

A production estimation during the recolonization process gives a mean production of 727 g dwt/m^2.year or 261 g C/m^2.year.

The recolonization by meiobenthos has not yet been studied in detail. We can only state that the mean density of nematodes diminished from 4.4 million to 425,000 and for harpacticoids from 525,000 to 320 ind/m^2. In the first months of the recolonization the dominant harpacticoid *Canuella perplexa* is replaced by *Paronychocamptus proximus*.

Conclusion.

The absence of an efficient management of the lagoon in the past has led to a hypereutrofication, with an accumulation of organic material : the algal biomass and the intensity of plankton blooms increased, the productivity of macro- and meiobenthos is high.

Remineralisation of the organic material in the sediments increases the pool of nutrient which is further increased by the periodical renewal of the water. The benthic community of the Sluice Dock consists of a small group of opportunistic species, with low diversity but high density and productivity.

The recent disturbance decreases the stability of the system. Recolonization after defaunation of the sediments starts with opportunistic species which are succeeded by slower growing and longer living species that should stabilize the system. Due to the continuous disturbances this benthic succession is interrupted and a new recolonization starts. This is a typical phenomenon after organic loading of the environment (Pearson and Rosenberg, 1978).

In the near future a rational management of the lagoon is urgent. In a well developed plan including a benefit/cost analysis, different options to restauration must be considered and their ecological impact studied. The Sluice Dock may become a case study to normalise the hypereutrophic situation to a more olige-trophic.

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CARBON CYCLING IN THE BELGIAN COASTAL ZONE

AND ADJACENT AREAS

Workgroup "Organic Matter"

SUMMARY

Part 1

GENERAL STRUCTURE OF THE ECOSYSTEM

Part 2

NATURE OF THE PRIMARY PRODUCTS

Part 3

UTILIZATION OF PRIMARY PRODUCTS BY ZOOPLANKTON

Part 4

UTILIZATION OF PRIMARY PRODUCTS BY PLANKTONIC AND BENTHIC BACTERIA

Part 1

GENERAL STRUCTURE OF THE ECOSYSTEM

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Studies by the workgroup "Organic matter" are concerned with the description and the understanding of the carbon cycling in the Southern Bight of the North Sea, and more particularly in the zone 1S which includes the belgian coastal zone.

This ecosystem is defined on a hydrological basis (fig.1), the zone being dominated by the residual current entering from the Channel and directed to the North-East. The presence of the Scheldt estuary, however, seems to induce a gyre in front of the Belgian coast, where the freshwater from the Scheldt resides for some times (Nihoul & Ronday, 1975). On basis of this general circulation pattern, the Belgian coastal zone is defined as the region in front of Zeeland and Belgium limited by a current velocity of 200.10^3 $m^3.s^{-1}$ (fig.1). This zone extends to about 40 km offshore over an area of 5.370 km^2, has a mean depth of 15m and is strongly influenced by terrestrial inputs from the Scheldt, the derivation channel of the Lys and the river Yser. Salinity is generally less than 33 ‰. Turbidity is generally high, with mean values above 5 $mg.\ell^{-1}$ and maxima of 15 $mg.\ell^{-1}$ (Moens, 1974).

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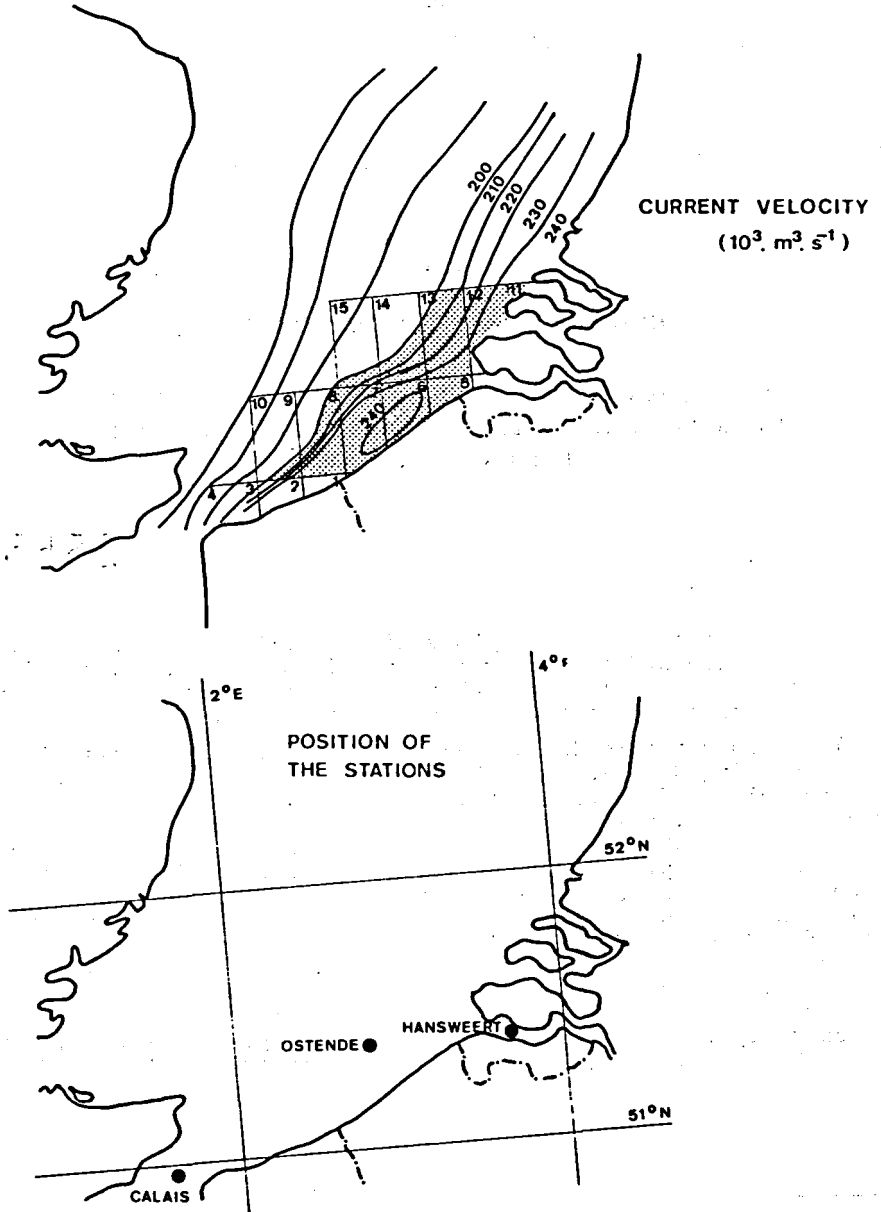
fig. 1.
Position of the stations

The grid points used for establishing the mathematical model of the North Sea have been investigated. However, "Ostend" and "Westhinder" stations have been considered to be representative of the belgian coastal zone and have been more carefully sampled (fig.1).

Moreover, in order to understand the effects of terrestrial inputs on the ecosystem, four other stations have been considered : stations "Calais" and "Boulogne", waters with "atlantic" characteristics in the English Channel ; station "Hansweert", highly eutrophied water of the Scheldt estuary (35 km from the open sea) and the Fladdenground (Northern atlantic water).

The ecometabolism of the zone 1S (Ostende-Westhinder) will be first described and secondly, it will be compared to other stations.

1.— The carbon cycling in the zone 1S.

The general picture of the mean annual carbon standing stocks and fluxes of the ecosystem is described in fig.2. We will examine the successive compartments of the ecosystem with their spatial and seasonal variations, each of them (except inorganic carbon) beeing otherwise more precisely studied in the following chapters of this work.

1.1.— Inorganic carbon.

Inorganic carbon concentration has been estimated to be 390 g C/m^2. Carbonated alkalinity is about 2.35 mM and the mean pH of the zone is about 8.4 (note that is more acidic just near the shore). The partial pressure of CO_2 calculated from these data is about 210 µatm, which suggests an input from air of about 60 g C/m^2.year calculated on the basis of the constant rate of transfer of CO_2 through the air-sea interface of Sugiura et al. (1963) [0.007 mmol $cm^{-2}atm^{-1}min^{-1}$]. This input controls the inorganic carbon decrease resulting from the fact that net photosynthesis (320 g C/m^2.year) is greater than the non-phytoplanktonic global respiration in the ecosystem

$$(155 + 80 + 26) \text{ g } C/m^2 \text{.year.}$$

However, pH shows nycthemeral variations due either to diurnal photosynthetic and respiration activities and nocturnal respiration activity or to the water movements of the tides (fig. 3).
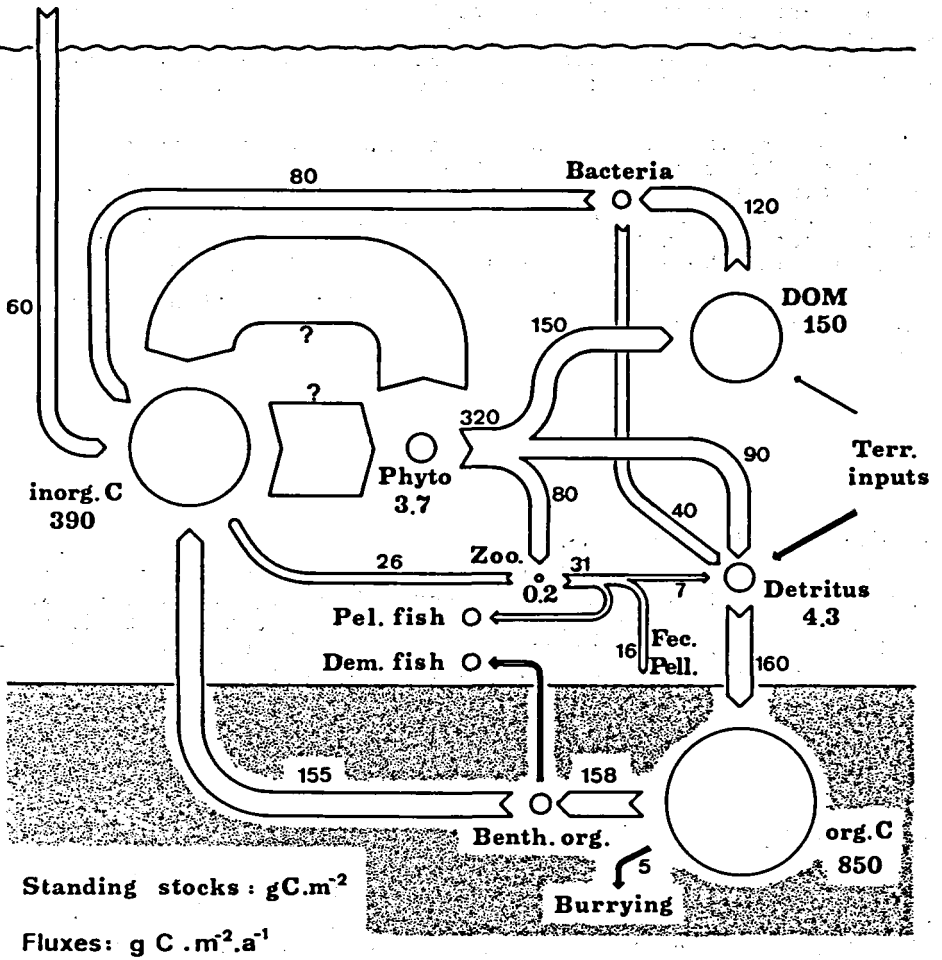
fig. 2.

Mean annual carbon standing stocks and fluxes
in the zone 1S of the Southern Bight of the North Sea

pH

WESTHINDER

june 6-7 1981
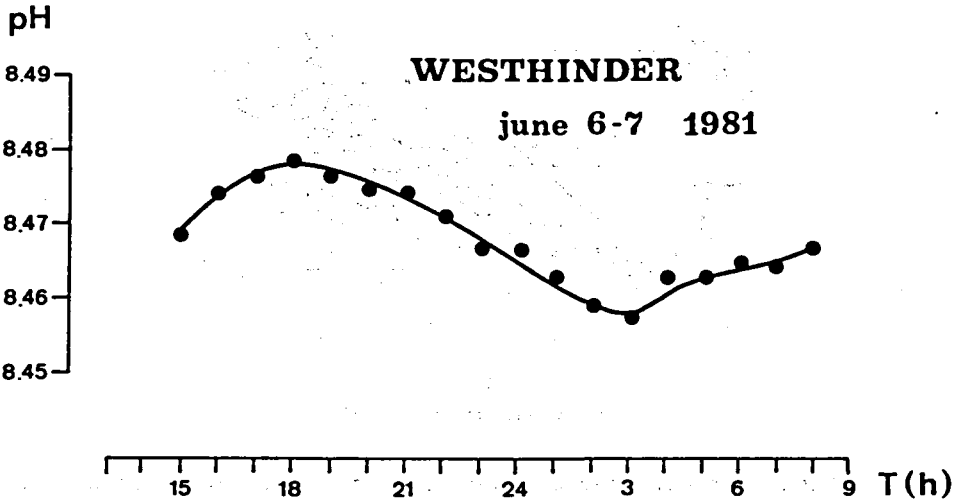
fig. 3.

Nycthemeral variation of pH

1.2.– *Primary production.*

The phytoplankton biomass as well as bacteria and detritus are determined by measuring the chlorophyll a and the total particulate organic carbon (Σ proteins, carbohydrates and lipids). The part of the organic carbon content of phytoplanktonic cells was estimated from the chlorophyll a concentrations, using a C-organic/chlor.a ratio own to each season. These specific ratios were determined by the linear regression of organic carbon on chlorophyll a, both measured on the total particulate organic matter (Lancelot-Van Beveren, 1980).

Chlorophyll a concentrations show a decrease from the shore to sea and from the Scheldt estuary to the French border (fig.4). This gradient is particularly sharp in the spring, when concentrations in the coastal zone are at least one order of magnitude higher than in the offshore zone.

Potential primary production, expressed per unit water volume, follows the same distribution as chlorophyll, but as the coastal water is more turbid, the photic layer is much shallower there (about 5m) than in open sea (up to 25m), so that the integrated primary production is rather uniform in the whole zone (Mommaerts, 1973b). The phytoplanktonic communities are however different in the coastal and the offshore zone : the ratio net-/nannoplankton in primary production shows a prominent role of
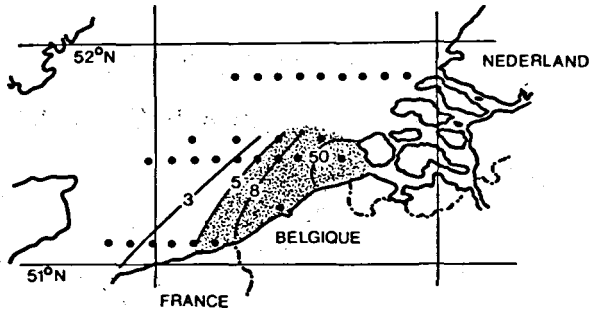
fig. 4.

Distribution of chlorophyll *a*

netplankton nearshore and micro-flagellates offshore, during the
spring bloom (Mommaerts, 1973a). Netplankton cannot be too
strickly assimilated to diatoms, because it is largely dominated
by the colony forming micro-flagellate *Phaeocystis poucheti* during
the spring bloom.

Chlorophyll measurements (fig.5a), estimations of phytoplank-
ton biomass and of detritus concentrations (fig.5b), obtained du-
ring several years in the area show a clear spring bloom, during
the second half of April and the beginning of May, immediately
followed by a peak in detritus. An autumn bloom was sometimes
detected, but not found each year. Particulate primary produc-
tion measurements (fig.5c) again show a clear spring bloom now
lasting from mid-March to June, while no second bloom clearly
appears - but the existence of an August bloom, sometimes detec-
ted in an adjacent area, cannot be excluded. In situ and semi-
situ measurements of dissolved primary production show a relation-
ship between extracellular release, expressed as a percentage of
the total primary production, and the concentration of mineral
nitrogen : excretion decreases with increasing nitrogen concen-
tration (fig.6). This empirical relation was used for calcula-
ting the curve of seasonal evolution of dissolved primary produc-
tion (fig.5d).

The gross primary production however reveals uneasy to be
estimated. indeed, whilst it is generally accepted that the
phytoplanktonic respiration should be about 50 % of the gross
primary production, the loosing of labelled CO_2 by phytoplankton
in the dark suggests that phytoplanktonic respiration is very
high (about 10 % of the biomass/hour) [fig. 7]. Moreover, the
measurements of the nycthemeral variations of oxygen, CO_2 and
particulate carbon concentrations in the water column suggest
gross primary production and phytoplanktonic respiration much
higher than expected. However, it is necessary to point out the
fact that till now it has not been possible to differentiate the
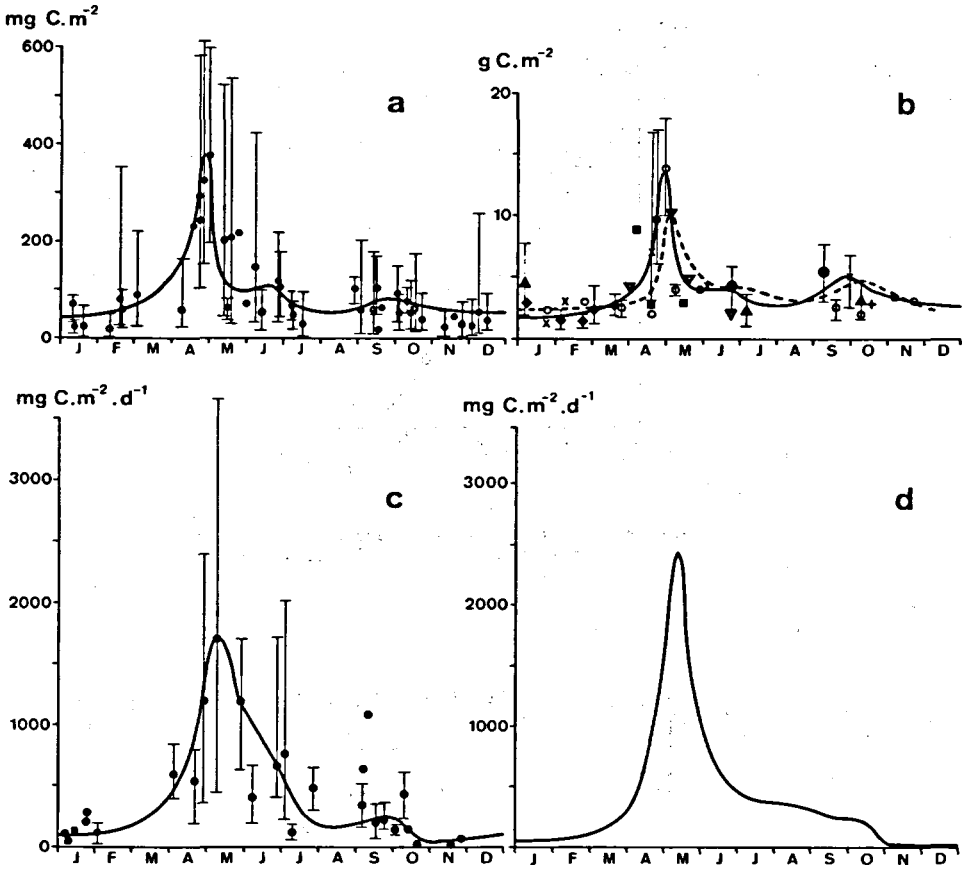
fig. 5.

(a) Annual variations of chlorophyll *a* concentrations
(b) Phytoplankton biomass (———) and detritus concentrations (------)
(c) Particulate primary production
(d) Dissolved primary production

part of the variations resulting from the nycthemeral variations of the biological activities and from the water movements of the tides.

Whatever the case, the estimation of the phytoplanktonic respiration does not affect the general picture of the carbon cycle since only the net primary production is used in the food web. The mean annual phytoplankton biomass has been estimated to be 3.7 g C/m² and the net primary production 320 g C/m².year.
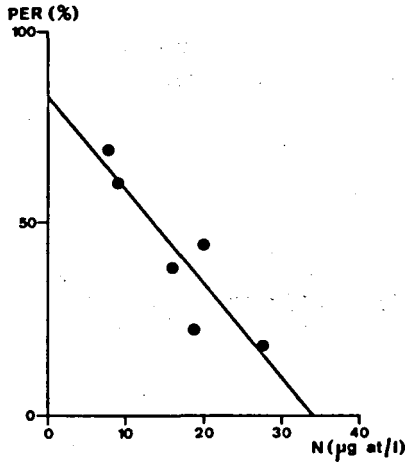
fig. 6.

Relationship between extracellular release
and concentration of mineral nitrogen
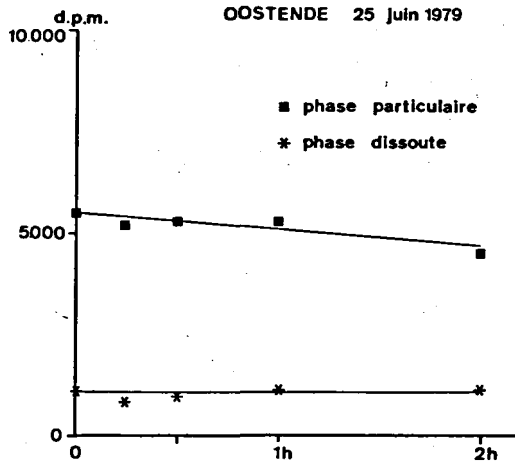
fig. 7.

Loosing of labelled CO_2 in the dark by phytoplankton

The fate of the net primary production is threefold :

1°) the dissolved production (150 g C/m^2.year) contributes to the enrichment of the pool of *dissolved organic matter* (see § 1.3 and part 4 of this work);

2°) one part (80 g C/m^2.year) of the net particulate primary production is grazed by *zooplankton* (see § 1.4 and part 3 of this work);

3°) the ungrazed part (90 g C/m^2.year) of the net particulate primary production contributes to the enrichment of the pool of *detritus* which are recycled by benthic organisms (see § 1.5 and part 4 of this work).

1.3.– *The dissolved organic matter and its utilization by planktonic microheterotrophs.*

The total dissolved organic carbon concentration is quite important (150 g C/m^2) but a large part of this pool seems to be unused by bacteria. Indeed, the BOD$_5$ is only 15 g C/m^2 , i.e. 10 % of the total, and the directly usable substrates of low molecular weight account for 2 g C/m^2 . Exoenzymatic hydrolysis of macromolecules is therefore required to explain the hetero-trophic activity (see part 4). However, it is worth noticing that previously carried out experiments about the comparative study of initial rates of organic matter consumption measured on total dis-solved organic matter and the pool of small metabolites suggests that the low molecular weight fraction could account for most of the directly usable organic matter and that a part of that low molecular weight substances is not quickly used by micro-hetero-trophs (see Lancelot et al, 1980).

Plate counts of bacteria present a distinct decrease from shore to sea in spring time (fig.8) ; at other periods, their distribution is homogeneous (Joiris, 1974). Glucose utilization rates show the same distribution (fig.9), higher rates characte-rizing the Belgian coastal zone compared with the more "Atlantic" water masses. The station "Ostend", for which more data on hete-rotrophic activities are available, seems reasonably representa-tive of the whole area. Planktonic respiration rates, on the other hand, do not display any clear pattern of spatial distribu-tion.

In order to determine the planktonic heterotrophic activity, the utilization of a given substrate was calculated as the product of its natural concentration in seawater and the relative utili-zation rate (reciprocal of the turnover time) obtained from the incorporation kinetic of the same radioactive substrate. The na-tural concentration of small substrates was shown to vary little around its equilibrium value set by the affinity of the microorga-nisms utilizing them (Billen et al, 1980). Most surveys of the concentration of amino acids or monosaccharides do indeed not re-flect any significant seasonal evolution (e.g. Andrews & Williams,
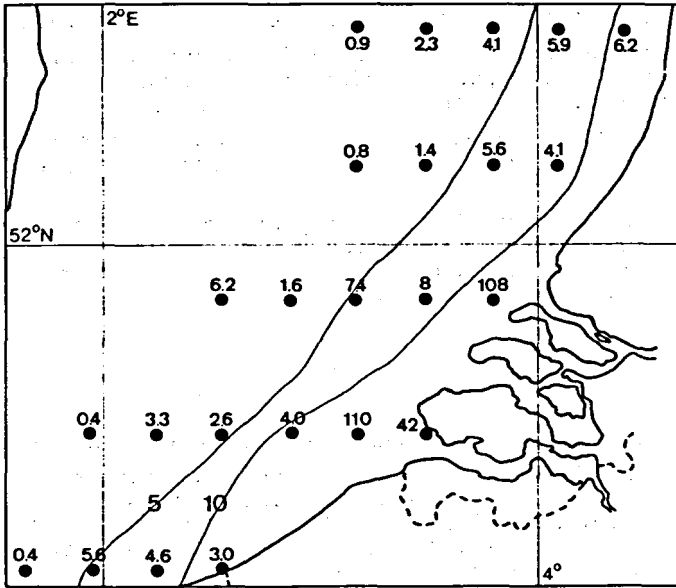
fig. 8.

Distribution of plate counts of bacteria in springtime
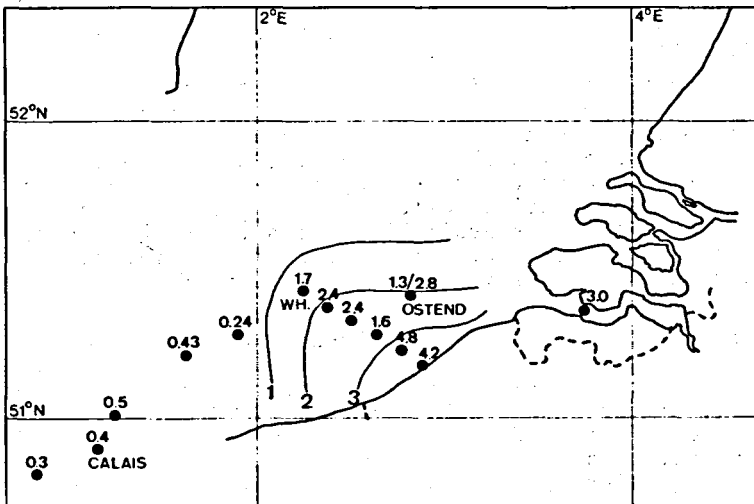
fig. 9.

Distribution of glucose utilization rates ($\% \ h^{-1}$)
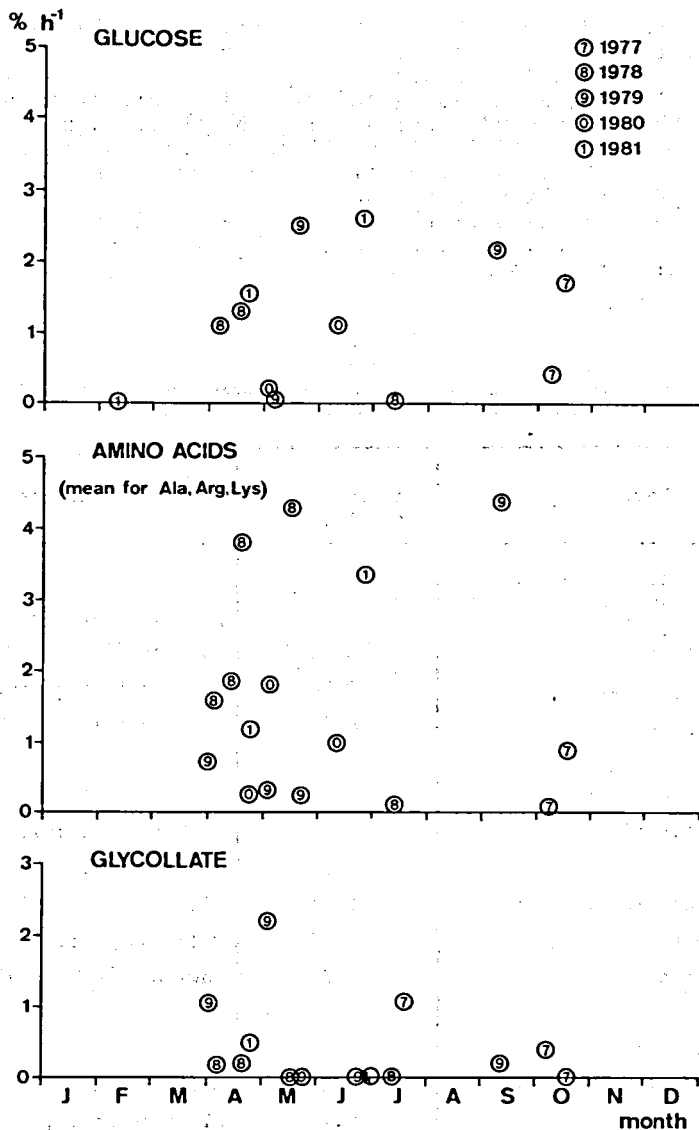
fig. 10.

Annual variation of relative utilization rates
for glucose, amino acids and glycollate

1971 ; Crawford et al, 1974) : the variations of the absolute uti-
lization rates are thus reflected by the variations of the rela-
tive rates. Available measurements of the relative utilization
rates for glucose, amino acids and glycollate are plotted in fig.
10, these three compounds representing the three main classes of
substrates directly utilizable by microheterotrophs : free mono-
(and oligo) saccharides, free amino acids and organic acids resul-
ting from phytoplankton excretion. The similarity between most
values of determination of these three classes in various marine
environments (table 1) reflects again the efficiency of the con-
trol by microheterotrophs.

<div align="center">

Table 1

Determination of the main classes of substrates directly utilizable
by microheterotrophs in various marine environments

</div>

Environment	Mean concentration (mole.ℓ^{-1})	Authors
Free monosaccharides :		
North Atlantic	0.90	Burney et al, 1979
Gullmarfjord	0.86	Josefson, 1970
Narragansett Bay	0.88	Johnson & Sieburth, 1977
Sargasso Sea	0.58	Liebezeit et al, 1980
Free amino acods :		
North Atlantic	0.26	Pocklington, 1971
English Channel	0.26	Andrews & Williams, 1971
North Sea (Southern Bight)	0.51	Billen et al, 1980
Irish Sea	0.15	Riley & Segar, 1970
Baltic Sea	0.26	Dawson & Gocke, 1978
Glycollate :		
North Sea (Southern Bight)	2.25	Billen et al, 1980
Irish Sea	0.7	Al Hasan et al, 1975
Ipswich Bay	1	Shah & Wright, 1974
Ipswich Bay	0.5	Wright & Shah 1975
Essex River Estuary	0.26	Wright & Shah, 1975

For calculating the heterotrophic activity in our area, following mean values of concentration were used : free mono-saccharides (0.8 $\mu mol.\ell^{-1}$, 60 μg $C.\ell^{-1}$); free amino acids (0.5 $\mu mol.\ell^{-1}$, 25 μg $C.\ell^{-1}$); glycollate (2 $\mu mol.\ell^{-1}$, 50 μg $C.\ell^{-1}$). The obtained values are considered as provisional, since not all possible substrates were determined on the one hand, and on the other hand, since some substrates measured as "free" could, in some circumstances, not be available to the microheterotrophs (Gocke et al., 1981).

It is worth noticing that the total dissolved primary production (150 g C/m^2.year) is able to account for the total heterotrophic activity of microorganisms (120 g C/m^2.year). Even if less of 15 % of this dissolved production is a low molecular weight one, the existence of an exoenzymatic hydrolyzing activity in water could explain the utilization of this production by bacteria (see part 4).

1.4.– *Utilization of primary products by zooplankton.*

Zooplankton numbers and biomass, expressed as concentrations, are rather uniformely distributed, even if a slight increase from coast to open sea can sometimes be detected (fig.11). Owing to the greater depth of offshore stations, an increasing gradient appears when the biomass is expressed per area unit.
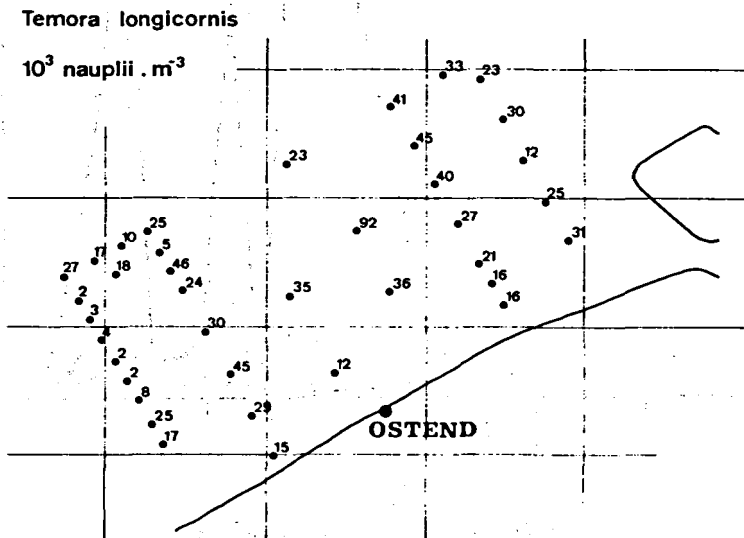
fig. 11.
Distribution of *Temora longicornis*

   It has been observed that the various stages of zooplanktonic
populations develop simultaneously in the whole studied area.
At the station "West-Hinder" (51°23'00"N, 02°26'20"E), where most
of the samples for zooplankton counts were obtained, zooplankton
concentrations are very close to the mean value of the whole area.

   Zooplankton counts obtained daily in 1978 at the station
"West-Hinder" show three main peaks from April to July, mainly
formed by copepods (fig.12). From one year to another, however,
the peaks can be quantitatively different (Bossicart, 1980).
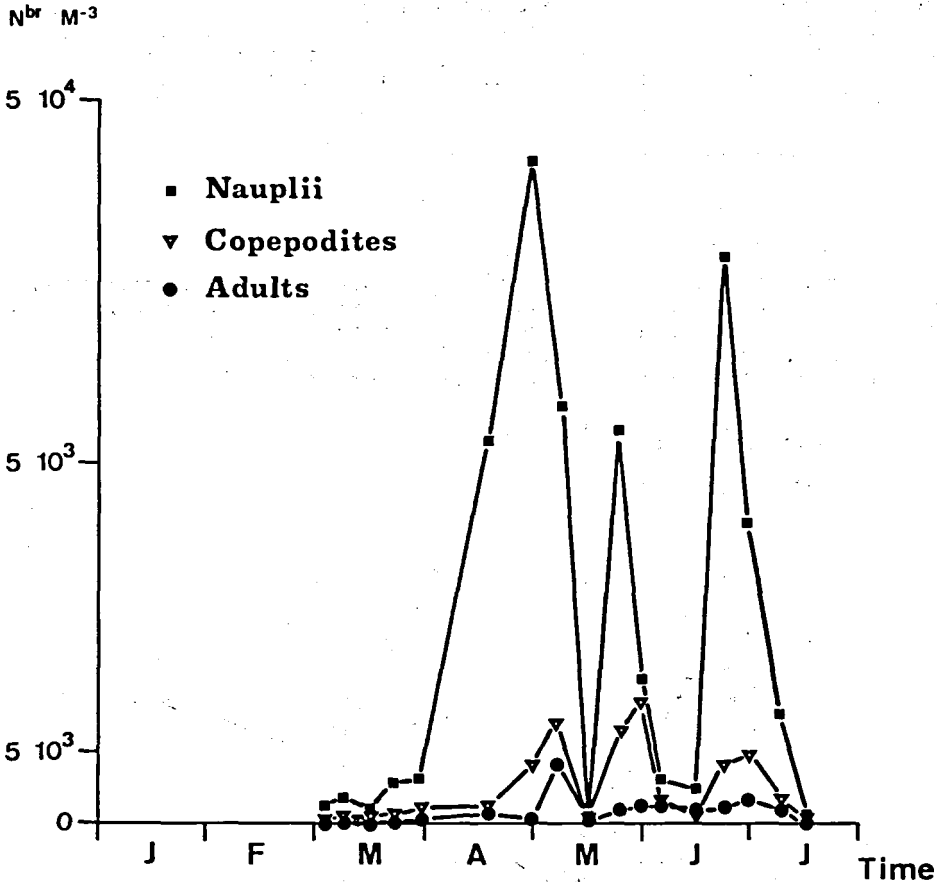
fig. 12.

Annual variation of Copepods' biomass

Zooplankton organic content was determined at 20 stations, on samples taken monthly (table 2) [Hecq et al. (1980)]. The mean annual value of zooplankton biomass is 0.2 g C/m^2 . The data on respiration activity of zooplankton, determined by oxygen consumption rate (table 3) show a peak in zooplankton respiration during the period April-May and also important variations of respiration rate (per unit of biomass), with high rates corresponding with the growing phase of zooplankton (Hecq, unpublished results). The mean value of zooplanktonic respiration is 26 g C/m^2.year. Data on grazing activity of herbivorous zooplankton on living phytoplankton (fig. 13) show a peak in mid-May. An October peak was found in experiments carried out during a short period in 1977; these results still have to be confirmed.

Table 2

Mean zooplankton organic content (proteins + carbohydrates + lipids) in monthly samples taken in 1979 and 1980 at 20 stations of the Belgian coastal zone

Month		n	Zooplankton organic matter mg.m^{-3} ± S.D.
1979	February	1	0[*]
	May	1	250
	June	16	490 ± 80
	July	18	60 ± 15
	September	12	10 ± 5
	October	16	0
	November	13	15 ± 5
1980	January	20	0
	February	16	0
	March	13	0
	May	19	0
	June	11	40 ± 7
	July	11	0
	September	16	45 ± 6
	October	19	30 ± 5
	November	20	30 ± 7

n : number of samples.
* 0 : not detectable (< 10)

Table 3

Mean value of zooplankton respiration rate
in the Belgian coastal zone for the period 1973-1975

Month	n	Respiration rate per biomass unit mg C.(mg C)$^{-1}$.d^{-1} ± S.D.	Zooplankton respiration mg C.m^{-3}.d^{-1}
January 1973	3	0.07 ± 0.04	0.28
February 1975	7	0.03 ± 0.01	0.03
April 1973[1]	3	0.43 ± 0.07	5.59
April 1973[2]	8	0.60 ± 0.10	4.02
April 1974[1]	3	0.68 ± 0.16	17.10
April 1974[2]	6	0.30 ± 0.18	1.17
April + May 1975	11	0.24 ± 0.07	12.0
May 1973[3]	12	0.84 ± 0.06	4.79
May 1974[4]	7	0.12 ± 0.04	6.72
May 1974[5]	4	0.06 ± 0.03	1.86
June 1975	9	0.26 ± 0.04	5.20
July 1975	9	0.17 ± 0.04	2.89
September 1975	3	0.58 ± 0.13	9.28
Sept. + Oct. 1974	7	0.18 ± 0.05	3.42
October 1975	9	0.10 ± 0.01	1.08
Nov. + Dec. 1974	3	0.10	0.2
Integrated annual mean		0.21	4.0

[1] Second decade   [2] Third decade
[3] First week   [4] Second week   [5] Last week

It appears therefore that the first peak of zooplankton is related to the phytoplankton bloom in the spring but that the two other ones are not related to phytoplankton biomass. It is moreover worth noticing that only 40 % of the net particulate primary production (80 g C/m^2.year; 20 % of the total net) is grazed by zooplankton. This can be explained by the fact that grazing reveals to occur only on the 25-100 μ size classes of phytoplankton which are the less abundant during the period of grazing activity (see part 4).
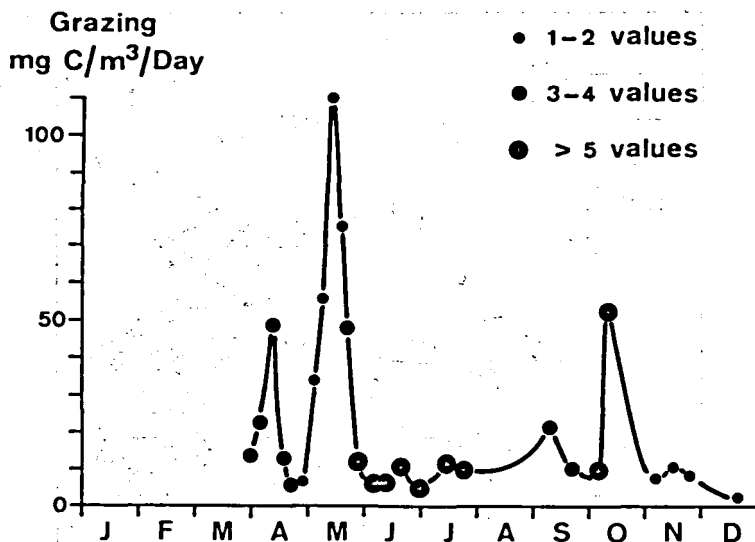
fig. 13.

Annual variation of grazing activity of zooplankton

1.5.— *The detritus — Benthic organisms relations.*

Most of the particulate primary production remaining ungrazed
is converted in detritus which settles down to the sediments
(fig.2).

The composition and distribution of sediments in the Eastern
Southern Bight of the North Sea have been described in detail by
Gullentops (1974) and Wollast (1976). The greater part of the
bottom consists of rather coarse sandy deposits, with much gravels
and shell fragments, particularly in the Southwestern part. The
Belgian coastal zone, on the other hand, is characterized by finer
sediments, with a higher content in organic matter. Organic mat-
ter content of the upper 1 cm of the sediment was used as an in-
dex of the importance of the flux of depositing organic carbon
(see e.g. Billen, 1982). Thus, the geographic distribution of
ignition loss of the bottom sediments (fig.14) indicates a higher
flux of sediment organic matter in the coastal zone than in the
offshore zones. This is particularly true in a region of mud ac-
cumulation just in front of the Belgian coast.

The quantitative importance of the benthos in recycling or-
ganic matter in the Belgian coastal zone shows that an important
part of primary production settles down on the sediments. Faecal
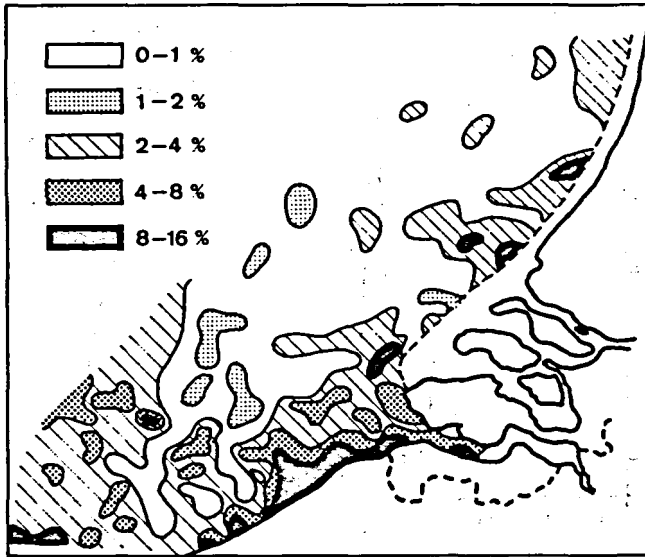
fig. 14.

Geographic distribution of ignition loss of the bottom sediments
(After Wollast, 1976)
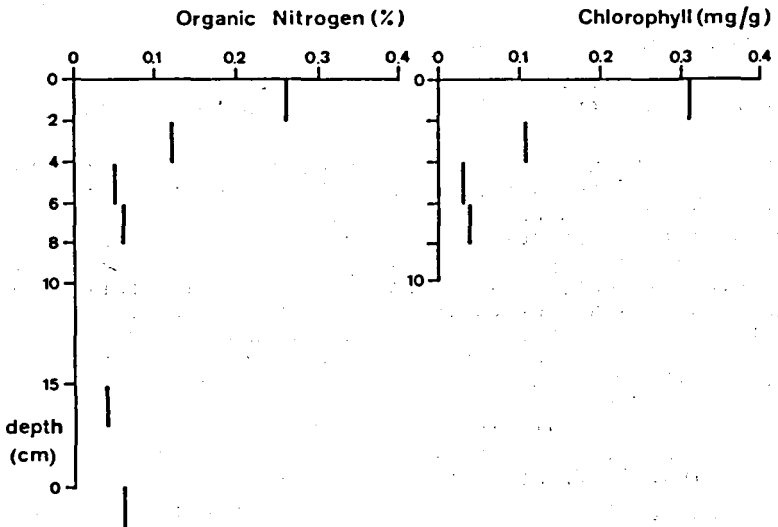
fig. 15.

Vertical distribution of chlorophyll and particulate nitrogen in the sediment

pellets and zooplankton corpses can only make up a small fraction of this flux : it is therefore likely that phytoplankton cells and - detritus constitute the bulk of the organic matter flux to the sediments.

A direct confirmation was obtained in the area of mud accumulation off the coast, where vertical distribution of chlorophyll and of particulate nitrogen in the sediment was determined (fig. 15), showing the importance of benthos in the recycling of the organic phytoplanktonic matter.

1.6.– Conclusions.

Accumulation of data on the carbon budget in the Belgian coastal zone clearly shows that zooplankton grazing is not the main cause of phytoplankton mortality, as it was generally expected for marine ecosystems : planktonic and benthic microheterotrophs play the predominant role in recycling primary production. From literature data, this could be the situation in all coastal seas, as opposed to open sea systems.

This is confirmed by the comparison data from "Ostend" and "Westhinder" compared to open sea and estuarian ecosystems as shown in § 2 hereafter.

From the results presented in the previous section, the integrated mean fluxes of carbon between the main compartments were calculated, in order to build up a budget of carbon cycling within the ecosystem. This was done for a complete year, on the one hand, and on the other hand for the vernal period (15 March – 15 July), including the main peaks of activity : spring phytoplankton and main zooplankton blooms. These results are summarized in table 4; the annual budget is diagramatically summarized in figure 2.

As seen in this figure, the annual budget is fairly balanced, as far as the fate of net primary production is concerned. It must firts be noted that exogenous – i.e. terrestrial – imports of organic matter are negligible with respect to engogenous production : domestic and industrial discharges from the Belgian coast represent a maximum of 4000 tons C per year, and the import by the Scheldt estuary has been estimated as 9000 tons C per year (Wollast, 1976). This amounts to about 2.5 g C/m^2.year for the whole coastal zone, representing less than 1 % of the primary production.

Of the total net primary production, only 20 % (40 % of the net particulate production) is grazed by zooplankton, while 40 % (80 % of the dissolved production) is consumed by planktonic microheterotrophs, the rest (about 40 %) being degraded by the benthic microorganisms.

Table 4

Annual and vernal budget of carbon cycling in the Belgian coastal zone

	Annual budget g C/m²	Vernal budget (15 March - 15 July) g C/m²
Mean biomass		
Phytoplankton	3.7	5.5
Detritus	4.3	4.9
Zooplankton	0.3	0.4
Mean fluxes		
Primary production :		
• net particulate	170	110
• dissolved	150	105
• total net	320	215
Zooplankton grazing	80	40
Zooplankton respiration	22	11
Microheterotrophic activity	120	70
Benthic mineralization	155	55
Benthic fossilization	5	–
Planktonic oxygen consumption	2000	1000

A major unbalance appears, however, when the estimation of the total planktonic oxygen consumption is converted into carbon flux and compared with the figures of primary production, the former being much higher than the latter : this unbalance was already detected a few years ago (Joiris, 1977).

This total planktonic oxygen consumption comprises respiration by microheterotrophs, by phytoplankton and by zooplankton.

If our estimates of microheterotrophic activity and of zooplankton respiration are accepted, they clearly cannot explain the high values of total respiration. On the other hand, some preliminary determinations of phytoplanktonic respiration, measured after incubation at maximal light intensity, show decrease of incorporated radioactivity at a rate of about 10 % of phytoplankton biomass per hour. Extrapolating this rate to the whole water column and to the 24 hours-day considering that day and night phytoplanktonic respirations are identical, one can calculate a value of phytoplankton respiration of about 3000 g C/m².year, in reasonable agreement with the measured planktonic oxygen consumption rate : this implies that the phytoplankton respiration could be, by far, the main element of the total plankton respiration.

This unexpected interpretation is in contradiction with most published budgets of aquatic ecosystems. But, in the frame of the contradiction between gross primary production and much higher consumption rates in various marine systems (Sorokin, 1973; Sieburth, 1976; Joiris, 1977), it offers however an explanation : in most cases, phytoplankton respiration was calculated as a percentage of primary production (Steemann Nielsen, 1952), which leads to much lower estimations than in our proposition, and could roughly underestimate the gross primary production, especially in deeper water masses.

2.– Comparison of carbon cycling in the Belgian coastal zone with other marine systems.

Although classical textbooks have presented marine systems in general as grazing ecosystems, as opposed to terrestrial eco-systems where detritus food chains dominate (Cushing, 1958; Crisp, 1964; Odum, 1972; Steele, 1974), this conception seems to hold only for oligotrophic open ocean systems. Table 5 summarizes the results of recently published studies, from which it has been possible to calculate the part of net primary production consumed by zooplankton and bacterioplankton respectively. In table 6, the same kind of comparison is made with data collected in the Central North Sea (Fladenground, FLEX 76) with the same kind of techniques as in the Belgian coastal zone. Table 7 compares some carbon fluxes in stations "Calais-Boulogne", "Ostend", "Westhinder" and "Hansweert". All these data quite clearly show that in coastal and upwelling systems zooplankton grazing usually represents less than 40-30 % of the net particulate primary production, while it consumes more than 40 %, and often up to 100 % of it in open sea systems.

On the other hand, the part of primary production being degraded in the benthos is known to be inversely related to the depth of the water column (Suess and Müller, 1980).

These different remarks lead to the following general picture : the food chain initiated by zooplankton grazing and leading to pelagic fish is more efficient when primary production is diluted in a deep euphotic zone, i.e. when predators actively hunting their food have an advantage. On the contrary, when important primary production is concentrated in shallow eco-systems, most of it is recycled by microheterotrophs, in the water and in the sediments.

Table 5

Survey of the literature on the relative role of zooplankton
and bacterioplankton in recycling primary production

Environment	Authors	Zooplankton grazing Z particulate primary production	Bacterioplankton activity Z particu- late primary	Z dissol- ved production
Coastal systems				
Belgian coastal zone	1	40	–	80
Cochin estuary (India)	2	15	–	–
Long Island sound	3	26	–	–
Soanish Inlet (Canada)	4	–	68	–
English Channel	5	–	100	–
Akkeshi Bay	6	10	–	–
Gulf of Mexico	7	3	–	–
Texas coastal zone	7	33	–	–
Behring sea	7	10	–	–
La Jolla (California)	4	–	56	–
Baltic Sea	8	–	–	91
Southern California Bight	9	7.5	101	–
Saonish Inlet (Canada)	9	11	91	–
Black Sea	10	5 – 25	–	–
Washington coastal zone	11	3	–	–
Upwelling area				
Peru	12	6	78	–
Open sea systems				
Fladenground (North Sea)	13	100	–	–
Tropical Pacific	14	75	–	–
	15	90	–	–
Sargasso Sea	16	100	–	–
Pacific off Oregon	11	36	–	–
Sub tropical Pacific	17	40	–	–
		110	–	–

1. This work. 2. Oasin (1970). 3. Riley (1956). 4. Furhman and Azam (1979).
5. Andrews and Williams (1971).   6. Hogetsu (1979).   7. Walsh et al. (1981).
8. Larsson and Azam (1979).   9. Harrison (1978).   10. Grefe (1970).
11. Jawed (1973).   12. Walsh (1981) & Sorokin (1978).   13. Daro (1980).
14. Steemann Nielsen (1972).   15. Shuskina and Vinigradov (1979).
16. Menzel and Ryther (1961).   17. Eppley et al. (1973).

Table 6

Comparison between two biotopes of the North Sea :
the Belgian coastal zone and the Fladenground (Flex 76)
[Both data sets concern the period May-June]

	Southern Bight (a)	Fladenground (b)	
Phytoplankton biomass $(g\ C.m^{-2})$	10	5 - 10	(1)
Particulate primary production $(g\ C.m^{-2}\ d^{-1})$	1	0.5 - 1.5	(2)
Zooplankton biomass $(g\ C.m^{-2})$	0.3	3.6	(3)
Grazing $(g\ C.m^{-2}\ d^{-1})$	0.3	1 - 2	(4)

(a) Present study.
(b) Results from : 1. Brockmann et al., unpubl. results;
2. Weigel and Hagmeier, unpubl. results;
3. Krause and Radach (1980);
4. Daro (1980).

Table 7

Comparison between some carbon fluxes
in stations "Calais-Boulogne", "Ostend-Westhinder" and "Hansweert"

	Calais-Boulogne	Ostend-Westhinder	Hansweert
BOD_5	$0.6g\ C.m^{-3}$	$1.0g\ C.m^{-3}$	$1.3g\ C.m^{-3}$
Heterotrophic activity	$6g\ C.m^{-3}.y^{-1}$	$8g\ C.m^{-3}.y^{-1}$	$38g\ C.m^{-3}.y^{-1}$
Total planktonic respiration	$61g\ C.m^{-3}.y^{-1}$	$133g\ C.m^{-3}.y^{-1}$	$78g\ C.m^{-3}.y^{-1}$
Particulate net production :			
April 1981	$25\ mg\ C.m^{-3}d^{-1}$	$67mg\ C.m^{-3}d^{-1}$	
September 1981	$7\ mg\ C.m^{-3}d^{-1}$	$17mg\ C.m^{-3}d^{-1}$	
Grazing : May 1979 (-3m)	$7.0mg\ C.m^{-3}d^{-1}$	$40\ mg\ C.m^{-3}d^{-1}$	
September 1979 (-3m)	$0.4mg\ C.m^{-3}d^{-1}$	$10\ mg\ C.m^{-3}d^{-1}$	

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Part 2

NATURE OF THE PRIMARY PRODUCTS

Ch. LANCELOT[1]

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1.— Introduction.

As pointed out by the general pattern of organic carbon cir-
culation at the first trophic levels of the Belgian coastal area,
phytoplankton net primary production is used with a higher
efficiency by bacterioplankton than zooplankton (see Bouquegneau
et al., this volume).

Understanding of this particular trophodynamic structure
requires a more subtle knowledge of the factors affecting the two
trophic relationships :

> phytoplankton —➤ zooplankton

> phytoplankton —➤ bacterioplankton

One important step in this study concerns the nature of the
primary products (intra- and extracellular) and their changes
with reference to simultaneous changes in the environmental
conditions, namely light intensity and dissolved mineral nitro-
gen, the likely limiting factor of the primary production in the
Southern Bight of the North Sea.

Indeed, growth and breeding of the filtering herbivorous
zooplankton are dependent on the dietetical qualities of the
phytoplankton cells. These nutritive qualities are defined by the
cell content in essential metabolites : proteins, carbohydrates
and lipids.

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In a similar way, utilization of the primary products by
bacterioplankton is dependent on the nature of phytoplankton
excreted and/or degraded products. Among the molecules originated
from these two sources of dissolved organic matter, only a part
— *the directly usable organic compounds* (DUDOM) — can be rapidly
used by the microheterotrophs. This pool includes molecules of
molecular weight inferior to 500 d such as : organic acids,
amino acids, monosaccharides and their small oligomers (Billen et
al., 1980). Macromolecules, on the other hand must be first
hydrolysed into small metabolites before being used by bacteria.
Exoenzymatic hydrolysis constitutes one among the possible mecha-
nisms of proteins hydrolysis in the Southern Bight of the North
Sea (see Billen and Somville, this volume).

This paper presents thus some results about the intra- and
extracellular metabolism of the phytoplankton cells of the
Belgian coastal area, especially of the species *Phaeocystis
poucheti*, the most important species of the spring period, in
term of biomass, as indicated on figure 1.

One aspect of this study will concern the cellular content in
proteins, carbohydrates and lipids of phytoplankton and their
production rates with reference to light intensity and dissolved
mineral nitrogen.

The second aspect will develop more specifically the relation-
ship phytoplankton bacteria and will discuss the extracellular
release of small (MW < 500 d) and large (MW > 500 d) molecules by
phytoplankton and their utilization by bacteria.

Finally, a discussion will be presented about the whole
metabolism of the spring species *Phaeocystis poucheti* which
probably accounts for the weak efficiency of zooplankton grazing
in the spring period (see Bouquegneau et al., this volume).

2.— Methods.

2.1.— *Biochemical composition of the phytoplankton cells.*

Biochemical composition of the phytoplankton cells was sta-
tistically estimated by the linear regression of each biochemical
compound (proteins, carbohydrates, lipids, on the chlorophyll *a*,
characteristics of the only phytoplankton, both measured on the
total particulate organic matter (Lancelot-Van Beveren, 1980).
This provides an indirect method to eliminate the interference of
non phytoplanktonic material (microheterotrophs and detrital
matter, the bacterio-detritus). This can be seen on figure 2 :
the zero ordinate of the regression line gives an average of the
non phytoplanktonic material although the regression coefficient
gives a ratio biochemical compound/chlorophyll *a*, characteristic
of the only phytoplankton. The absolute value of this ratio is
bound to the physiological state of the phytoplankton that
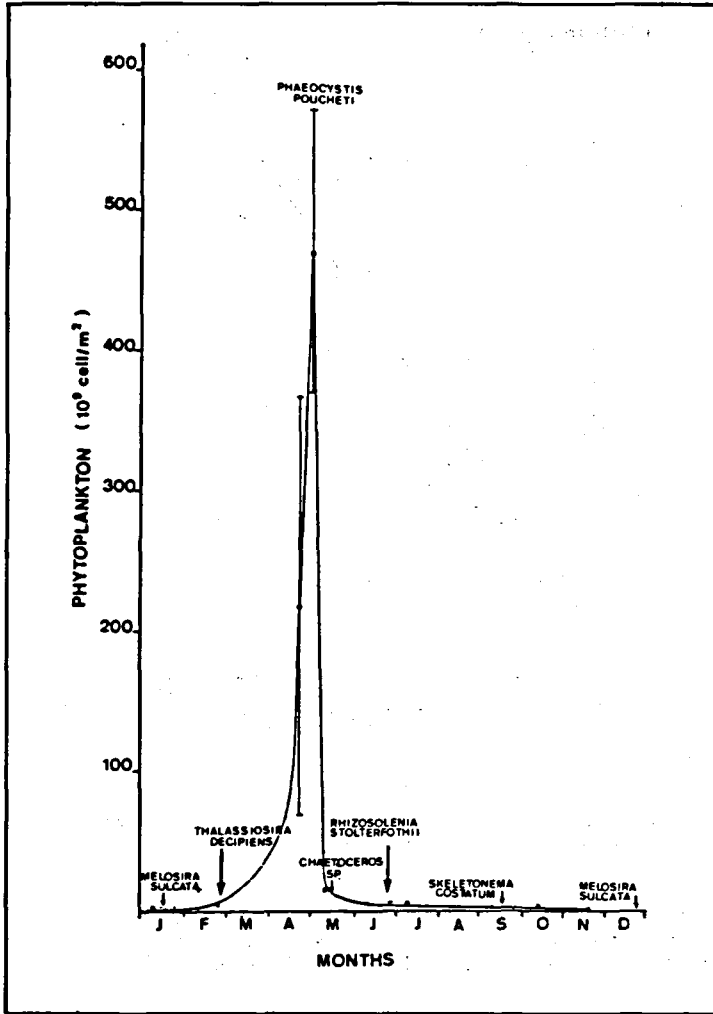
fig. 1.

Seasonal variations of phytoplankton cells
and relative abundance of some dominant species

depends on the growth conditions of the surrounding medium.
Consequently, this calculation was accomplished for four growth
periods defined from the annual cycle of primary production (see
Bouquegneau et al., this volume) : one active phytoplankton
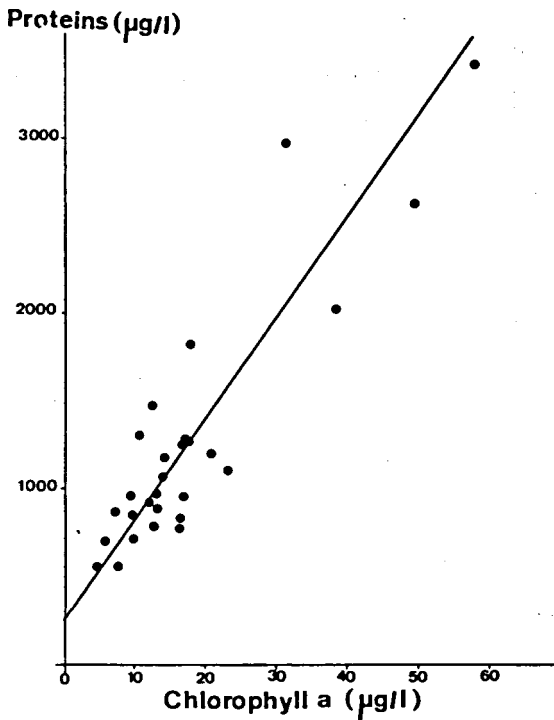
fig. 2.

Regression curve of proteins on chlorophyll a both measured
on the particulate matter during the Spring period

growth period (Spring), two periods of more reduced activity
(Summer and Autumn) and a *pause* (Winter). Biochemical analysis
were described earlier (Lancelot-Van Beveren, 1980).

2.2.– *Speciation of net primary production (particulate + dissolved).*

A whole fractionation procedure (fig. 3) was developed to
measure the production rate of intracellular macromolecules
(proteins, polysaccharides, lipids) together with the extra-
cellular release of oligomers (MW < 500 d) and polymers
(MW > 500 d), from the classical experiment of Steemann Nielsen
(1952).
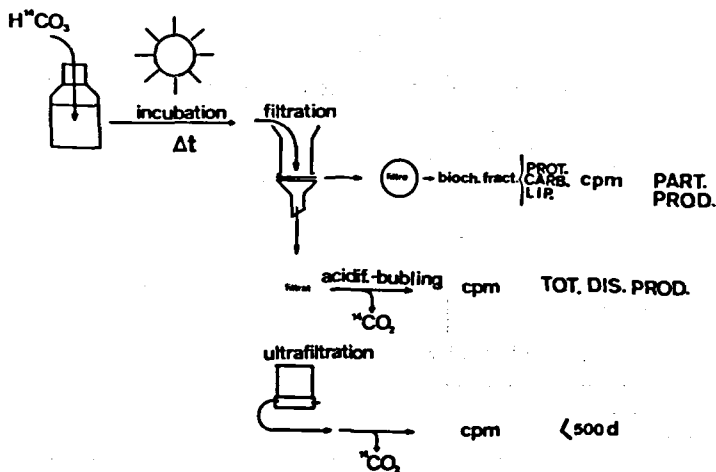
fig. 3.
Experimental procedure for the measure
of the intra- and extracellular primary production

Production rate of each class of intracellular macromolecules was determined by counting the radioactivity into different fractions of the cellular C^{14} biochemically isolated by successive extractions with appropriate solvants, as indicated on figure 4.

Extracellular release of small and large molecules was defined by the radioactivity contained in the two fractions of the total excreted, separated by the ultrafiltration procedure using a diaflo membrane UMO5 (nominal porosity 500 d).

On basis of kinetical analysis, it was shown that the classical $H^{14}CO_3^-$ experiment of Steemann Nielsen, when used during 2-6 hours incubation time provides an evaluation of net particulate primary production (Lancelot, 1982).

Diurnal net primary productions were then calculated using Vollenweider's model as adapted by Mommaerts for the phytoplankton of the Southern Bight of the North Sea (see Bouquegneau et al., this volume).

FILTER

+

1 mℓ CHCl₃ +   3 mℓ CH₃OH

10 min at 4 °C

Evaporation to dryness

+

3 mℓ CHCl₃

Filtration

Washing (2 mℓ CHCl₃)

supernatant
= lipids

Filter + 3 mℓ EtOH

Boiling 1 h

Filtration

Washing (2 mℓ EtOH)

supernatant
organic molecules of
weak molecular weight :
amino acids, organic acids,
monosaccharides

filter

+

3 mℓ TCA 5 %

Washing 2 mℓ TCA 5 %

supernatant
= polysaccharides
(nuclear acids)

filter
= proteins

fig. 4.
Biochemical fractionation of the cellular content

3.— Results and discussion.

3.1.— *Intracellular metabolites : proteins, carbohydrates, lipids.*

3.1.1.— Biochemical composition of the phytoplankton cells.

For each chosen growth period, table 1 gives the statistical parameters of the regression biochemical compound/chlorophyll a. Correlations between the two variables are significative at the 95 % level for each period and for each biochemical compound. This mean that although the phytoplankton populations which follow each other are composed of different taxonomic species (fig. 1), they are characterized inside each season by an identical biochemical composition.

Table 1

Statistical parameters of the regression biochemical compound/chlorophyll a

Season	Proteins	Carbohydrates	Lipids
Winter	$r = 0.90$ $a = 147 \pm 65$ $b = 272 \pm 308$	$r = 0.86$ $a = 63 \pm 34$ $b = 300 \pm 163$	$r = 0.90$ $a = 27 \pm 14$ $b = 8 \pm 77$
Spring	$r = 0.89$ $a = 49 \pm 4$ $b = 412 \pm 87$	$r = 0.78$ $a = 27 \pm 6$ $b = 327 \pm 168$	$r = 0.76$ $a = 8 \pm 3$ $b = 166 \pm 81$
Summer	$r = 0.89$ $a = 60 \pm 18$ $b = 411 \pm 126$	$r = 0.78$ $a = 44 \pm 21$ $b = 218 \pm 65$	$r = 0.53$ $a = 29 \pm 11$ $b = 65 \pm 66$
Autumn	$r = 0.84$ $a = 62 \pm 26$ $b = 326 \pm 64$	$r = 0.78$ $a = 32 \pm 15$ $b = 223 \pm 162$	$r = 0.79$ $a = 24 \pm 11$ $b = 63 \pm 66$

r : correlation coefficient.
a : angular coefficient of the regression line ($\mu g/\mu g$).
b : zero ordinate of the regression line ($\mu g/\ell$).

The test of egality of two regression coefficient shows that the biochemical compositions of the phytoplankton populations are statistically different from one season to the other at the level 90 % except for the summer and autumn populations which are statistically identical although prevailing taxonomic species are different.

Table 2

Biochemical composition of phytoplankton cells of the belgian coastal area

Season	Phytoplanktonic species	Proteins			Carbohydrates			Lipids			N mineral
		(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)	µg at/ℓ
Winter	*Melosira sulcata* *Dimerogramma minor* *Sceletonema costatum*	62	1.3	56	27	0.5	22	11	0.2	17	30
Spring	*Thalassiosira deciplens* *Phaeocystis poucheti*	58	1.2.	53	32	0.7	27	10	0.2	15	18
Summer	*Rhizosolenia stolterfotii* *Leptocylindrus danicus*	45	0.9	38	33	0.6	26	21	0.4	31	5
Autumn	*Sceletonema costatum* *Chaetoceros sp.*	42	1	45	27	0.5	21	20	0.4	30	10

(1) % metabolite/weight.
(2) metabolite/cellular carbon (w/w).
(3) metabolite-C/cellular carbon (%).

This can be seen from table 2 which gives, for each growth period, an average of the biochemical composition of the phytoplankton cells, expressed in % of total metabolites (weight), of cellular carbon, in % of cellular organic carbon. This table shows that proteins are the main cellular component of phytoplankton cells, whatever the season is. The percentage of cellular proteins ranges from 42 % to 62 %. The highest values are found during Spring and Winter, when dissolved mineral nitrogen is most abundant. The decrease of the cellular load in proteins, observed after the Spring period, is balanced by an increase in the weight of lipids that double their value from Spring to Summer.

Carbohydrates, on the other hand, show little variations during the seasons. Extreme values range from 27 % to 33 %, the highest being measured in Spring and Summer.

These results, although agreeing with some of the literature (Collyer and Fogg, 1955; Strickland et al., 1969; Werner, 1970 and Conover, 1975) stand yet in contrast with others indicating that phytoplankton cells of Summer are characterized by higher contents of carbohydrates than proteins. This corresponds in fact to the building of the reserve polyssacharides β 1-3 glucan occuring when N is depleted (Myklestad and Haug, 1972; Haug et al., 1973; Myklestad, 1974).
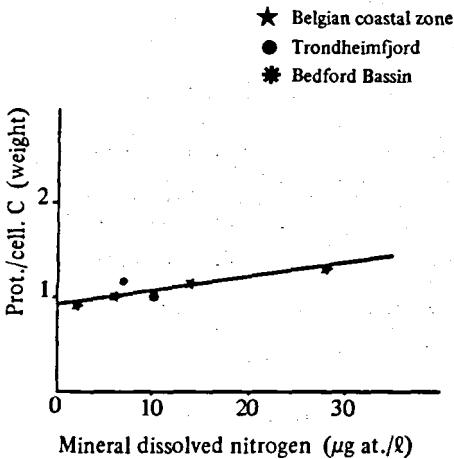
fig. 5.

Linear relation between the cellular proteins of phytoplankton and the mineral nitrogen for different biotopes.
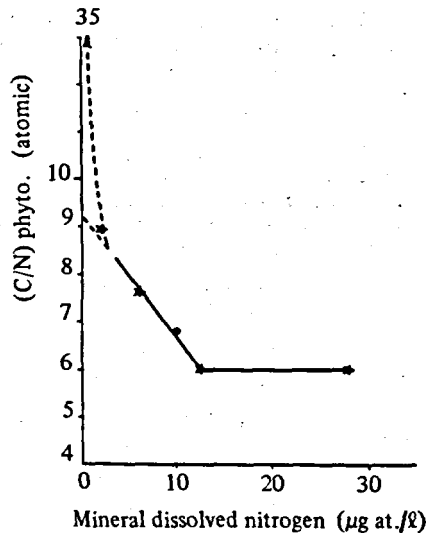
fig. 6.

Relation between the C/N ratio of the phytoplankton and the mineral nitrogen of the surrounding medium.

In any case, our results clearly indicate that the biochemical composition of the phytoplankton cells, shows, during the year, significant variations mainly bound to the availability of the dissolved mineral nitrogen of the surrounding medium. This can be generalizes to other biotopes as indicated by figure 5 which shows a positive linear correlation (r = + 0.94) between the protein content of phytoplankton and mineral nitrogen concentration.

The decrease in the protein content, as a consequence of the decrease in mineral nitrogen, is balanced by an increase of carbohydrates or lipids content, following the biotopes. This can be clearly seen on figure 6 which shows a linear relation between the atomic C/N ratio of phytoplankton and the mineral nitrogen when nitrogen concentrations range between 5 and 18 µg at N/ℓ. When the nitrogen content is inferior to 5 µg at N/ℓ, C/N tends hyperbolically to a maximum of 35 (Saskhaug and Holm-Hansen, 1977). On the other hand, the value of the C/N ratio is uniformly 6 when the concentrations of the mineral nitrogen is above 18 µg at N/ℓ. This value of 6 seems to be general for the C/N ratio of phytoplankton when growing in balanced nutritive conditions.

3.1.2.— Biosynthesis rates and their regulation by nitrogen.

Previous results show that the biochemical composition of phytoplankton depends on growing conditions. A better understanding of these physiological changes will be effective if one knows more finely how the changes in the growth conditions operate on the rate of synthesis of these macromolecules.

Macromolecules synthesis by phytoplankton (protein, polysaccharide, lipid) was thus measured in three biotopes of very different nitrogen richness (stations Ostend, Calais, Hansweert : see Bouquegneau et al., this volume), during the Spring bloom when large ranges of nitrogen concentrations are encountered.

Results, expressed in % of the total ^{14}C fixed into the cells, indicate that the relative rate of macromolecules synthesis varies independently of the light intensity (fig. 7) but is strongly bound to the content of mineral dissolved nitrogen of the surrounding medium, as indicated by figure 8a, 8b and 8c.

So, the percentage of newly synthetized proteins is linearly correlated to the mineral nitrogen (r = + 0.86), following the relation :

$$\% \, ^{14}C - protein = 1 \times N + 21 \quad if \quad N \leqslant 20 \ \mu g \ at \ N/\ell$$

The decrease of the proteosynthesis which proceeds simultaneously to the decrease of nitrogen is balanced by an increase of the synthesis rate of polysaccharides which is negatively correlated to nitrogen (fig. 8b). The relation is

$$\% \, ^{14}C \ polys. = - 0.6 \ N + 39 \quad if \quad N \leqslant 20 \ \mu g \ at \ N/\ell$$
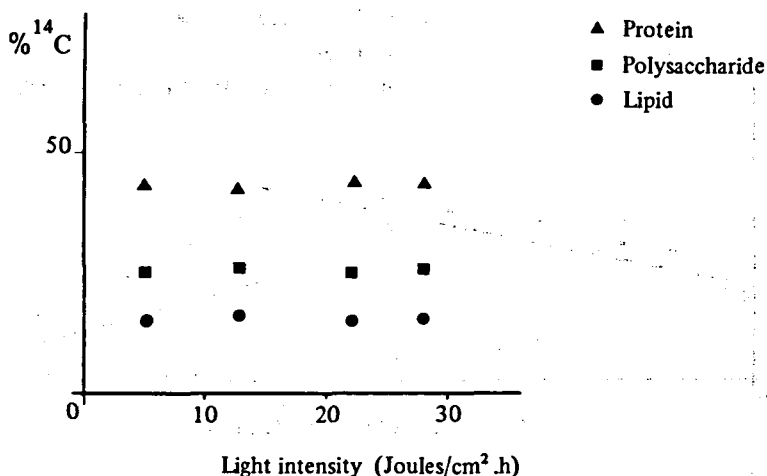
fig. 7.

Incidence of light intensity on the relative proportions
of ^{14}C incorporation into three classes of macromolecules
Station Ostend : 02-04-1979

    The relative synthesis of lipids, on the other hand, seems to
be independent of nitrogen concentration (fig. 8c). It takes a
part of about   15 %   of the total fixed carbon, except when the
phytoplankton   population   is   mainly   composed   of   *Phaeocystis*
poucheti. In this particulate case, the lipid synthesis presents
more than   20 %   of the photosynthetized carbon.

    These results apparently contrast with those of the cellular
content where the polysaccharide part seems to be less important
than the one produced during the light period. This can be
explained only if the polysaccharides newly synthetized during
the light period are catabolized during the night period in
preference   to   the   other   metabolites   (proteins   and   lipids),
according to the results of Handa (1969) and Rickett (1966).

3.2.– *Extracellular metabolites : polymers and oligomers.*

    Extracellular release of dissolved organic matter by phyto-
plankton will be discuss in this section. As a first attempt to
the determination of the nature of this material, separation into
small molecular weight and high molecular weight fractions has
been achieved.
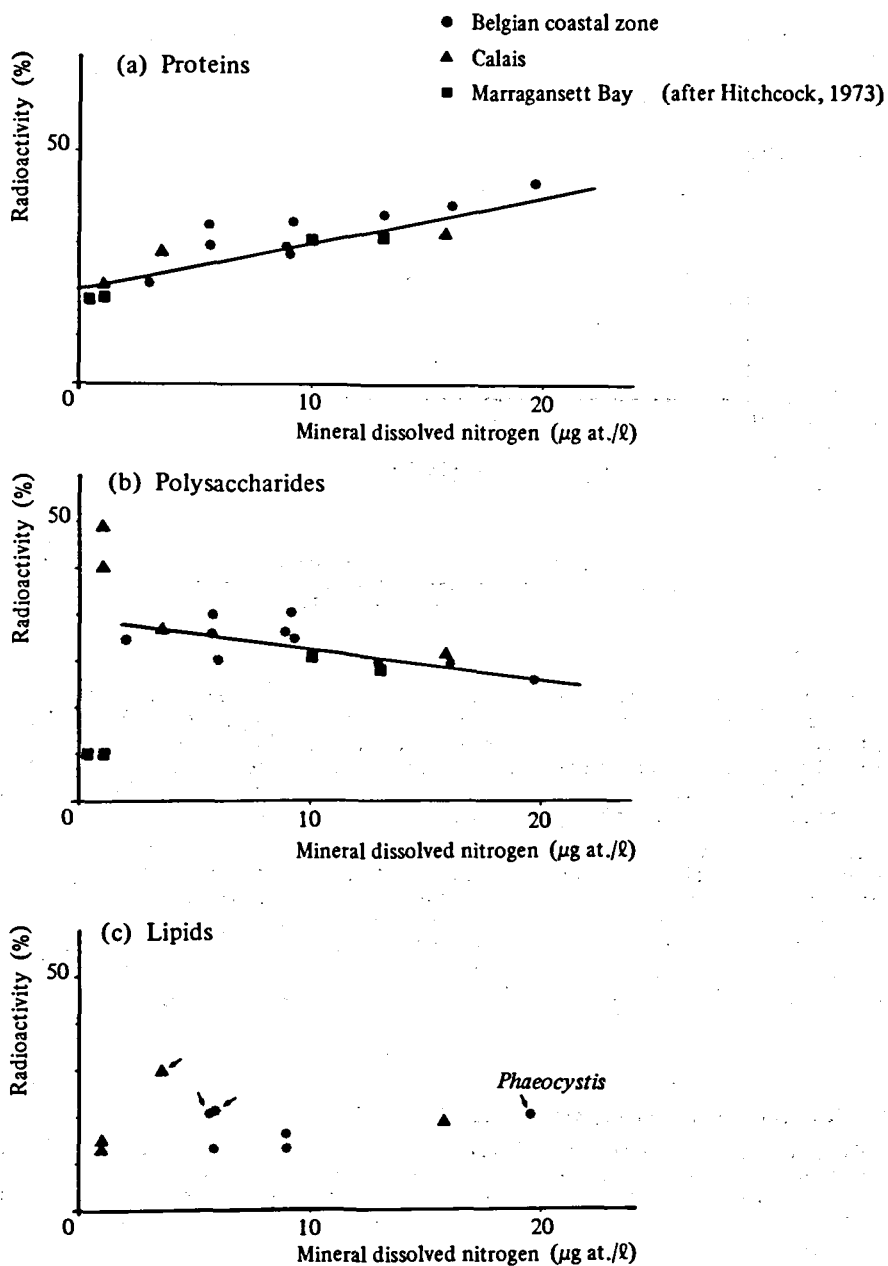
fig. 8.

Relation between the concentration of mineral dissolved nitrogen
and the ^{14}C content of each biochemical fraction
expressed in percent of the total ^{14}C fixed
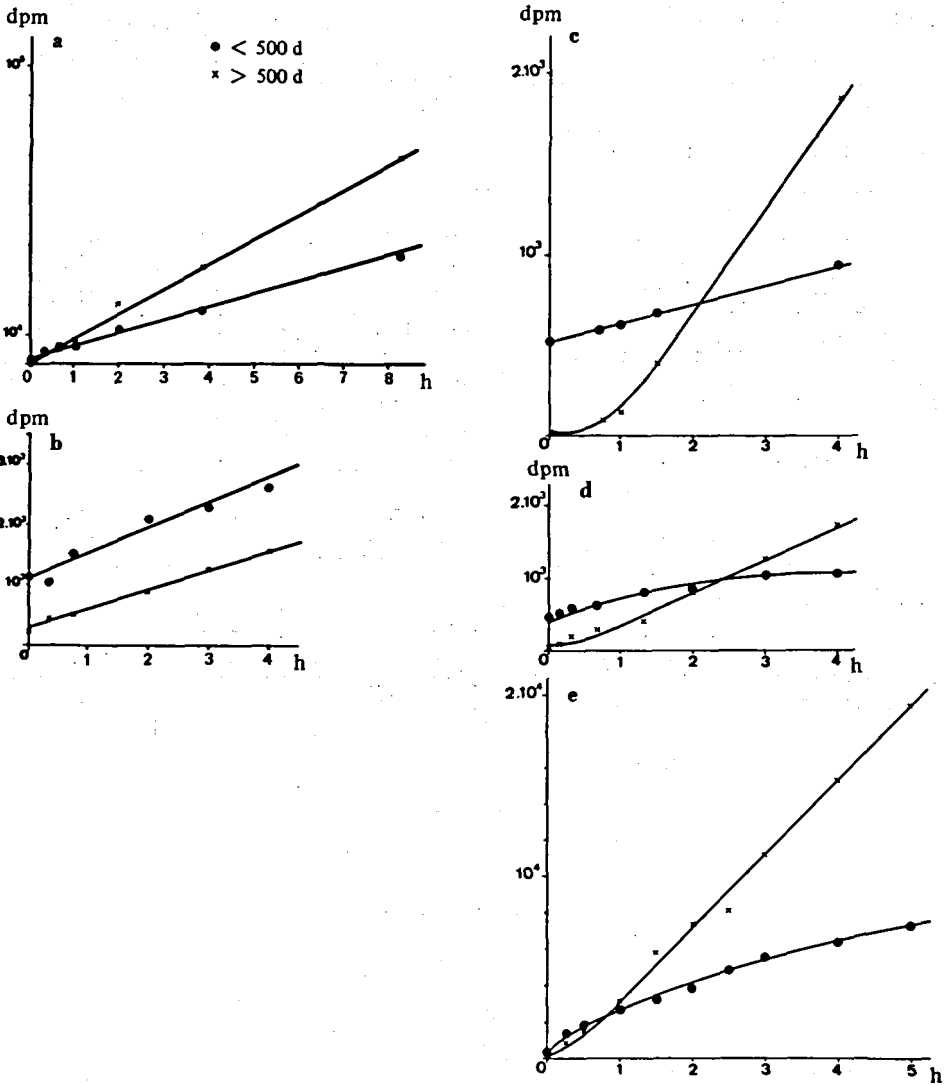
fig. 9.

Extracellular release of small (< 500 d) and large (> 500 d) molecules
by the phytoplankton of the Southern Bight of the North Sea
a) St. Hansweert (3-5-79); b) St. Hansweert (23-5-79)
c) St. Hansweert (27-6-79); d) St. Calais (26-6-79)
e) St. Ostend (21-5-79)

3.2.1.– Extracellular metabolites as energy source for microheterotrophs : kinetic study.

Extracellular production rate of oligomers (M.W. < 500 d) and polymers (M.W. > 500 d) were measured kinetically during the spring period in the Southern Bight of the North Sea. The duration of the incubation was four hours but sometimes more (up to maximum eight hours).

Such a kinetic study was useful for two reasons. First it gives the possibility of estimating, when necessary, the relative heterotrophic activity of bacterioplankton, unavoidably present with phytoplankton cells in the samples. Bacteria can effectively use some of the excreted primary products from the first minutes of the ^{14}C inoculation (Nalewajko et al., 1975; Lancelot, 1979). This leads to significant underestimates of the gross phytoplankton excretion rate which can reach 30 % at some occasions in the Belgian coastal area (Lancelot, 1979). Secondly, it can provide some hypothesis concerning the nature of the excreted products.

Two different kinetics of small metabolites extracellular release were observed, as can be seen from figure 9 : linear kinetics (fig. 9a, b, c) and non linear kinetics (fig. 9d, e). These last include most of the time a linear phase following by a significant decrease of the apparent excretion rate of small metabolites after 30 to 60 minutes from the beginning of the incubation. This decrease can be attributed to the uptake by bacteria of some of the phytoplankton excreted products, as indicated by figure 10 which compares the time evolution of radioactivity in two filtrates differing in the porosity of the filtrations membranes. Filtration with the 0.2 μm membrane
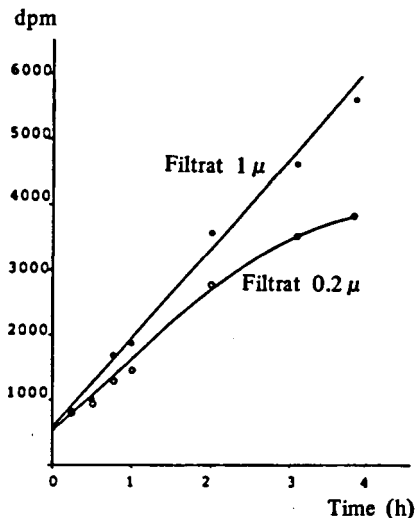
fig. 10.

Comparative study of the time evolution of the radioactivity
contained in two different filtrates

retains bacteria although the 1 μm filtration although re-
taining phytoplankton cells, does not retain free bacteria. This
experiment shows clearly that the decrease of - the apparent
heterotrophic activity of free bacteria.

In these conditions, the relative heterotrophic uptake of
phytoplankton extracellular products can be calculated easily
from the time evolution curve of ^{14}C phytoplankton excreted
products, using a simple mathematical model (Lancelot, 1979).

Results (table 3) indicate that some of the small metabolites
excreted by phytoplankton can be sometimes quickly used by the
microheterotrophs in the Southern Bight of the North Sea. As can
be seen from table 3, the relative heterotrophic uptake can reach
sometimes 20 %/h, according perfectly with results of Iturriaga
and Hoppe (1977) in the Kiel Bight. The range of values measured
by these authors lies between 8 and 17.5 %/h. These results
show thus that metabolites with high turnover rate can effecti-
vely be actively excreted by phytoplankton in the Southern Bight
of the North Sea. It is now essential to determine the chemical
nature of these metabolites. This would be done in the future.

Table 3

Relative heterotrophic activity 1/r
of phytoplankton small extracellular products
in the Belgian coastal area

Datum	$1/r$ %/h
02-04-79	0
03-05-79	20
21-05-79	?
25-06-79	7

Also two different kinetics of extracellular polymers were
observed : first linear kinetics from the beginning of the [14]C
incubation (fig. 9a, b) and secondly non linear kinetics charac-
terized by a lag phase reaching sometimes more than one hour
(fig. 9c, d, e).

In some cases, this lag phase was identical to the time
necessary to the phytoplankton cells to have marked pools of
proteins, lipids and polysaccharides (fig. 11). This experiment
suggest that such complexe molecules might thus be actively
excreted by natural phytoplankton cells.

Comparatively to the kinetics of small metabolites release,
kinetics of polymers extracellular production do never show any
decrease of the apparent excretion rate, at least during the used
time incubation (4 to maximum 8 h). This corresponds perfectly to
the fact that polymers are not *directly usable by microhetero-
trophs* (see Billen and Somville, this volume) and have a longer
residence time in sea-water.

26-06-79
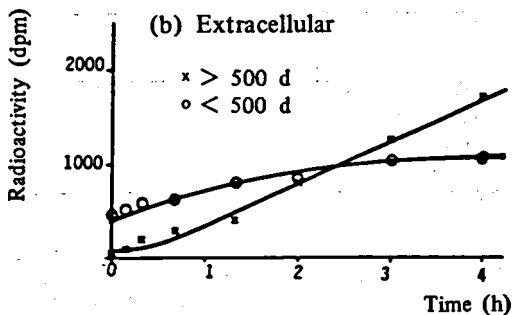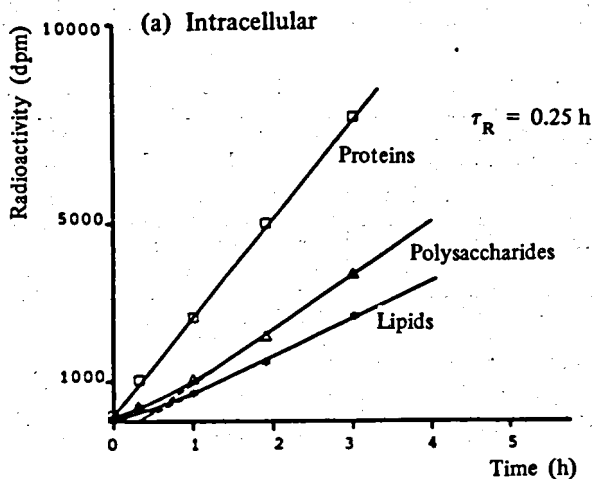
fig. 11.
Kinetical study of the intracellular production of proteins,
polysaccharides, lipids and extracellular release
of small and large molecules at the station Calais

3.2.2.— Relative proportions of small and large extracellular products.

Early studies (Lancelot, 1982) indicate that the relative
proportions of small and large metabolites vary independently of
the light intensity. On a similar way, no clear trend can be seen
from the geographical and seasonal variations (table 4). This
table shows that the amount of excreted oligomers is always
weaker than polymers, whatever the biotope and the season are.

Table 4

Geographical and seasonal variations of the relative proportions
of small and large extracellular products

Sample	Dissolved Primary Production		Dissolved nutriments		
	small	large	$NO_3^- + NO_2^-$	NH_4^+	PO_4^{3-}
		%		µg at./l	
OSTEND					
020479	22	78	19.6	0.6	15
030579	35	65	5.6	0.6	09
210579	16	84	5.7	0.3	13
250679	15	85	9.1	0	2.6
CALAIS					
220579	12	88	3	0.6	17
260679	20	80	1	0	17
HANSWEERT					
040579	9	91	288	16	7.3
230579	15	85	291	20	9.5
270679	17	83	211	15	9.4

As can be seen from table 4, the percentage of small metabolites
ranges from 10 to 35 %. These results agree perfectly with
those of Guillard and Hellebust (1971) on the species *Phaeocystis
poucheti* and Watanabe (1980) on freshwater phytoplankton. The
formers show that only 20 % of the excreted molecules have a
molecular weight inferior to 700 d. The last indicates that
polysaccharides are the main excreted products by freshwater
phytoplankton.

Yet some other results of the literature suggest on the
contrary that small metabolites would be the main extracellular
products of natural phytoplankton (Al-Hasan and Coughlan, 1975;
Nalewajko et al., 1976; Wiebe and Smith, 1977).

3.3.— *Whole metabolism of the phytoplankton cells of the Belgian coastal area.*

3.3.1.— Intra- and extracellular metabolism of the Spring species *Phaeocystis poucheti.*

As indicated on figure 1 the spring bloom is characterized in
the Belgian coastal area, by the proliferation of the microfla-
gellate *Phaeocystis poucheti* which dominates the phytoplankton

population in terms of biomass and represents more than 65 % of
the annual primary production. The knownledge of the metabolism
of this single species is therefore very important for the
understanding of the trophic relationships during spring when
biological activities are the highest. Seasonal changes, observed
during the Spring period, in the metabolism of the phytoplankton
cells correspond thus to a physiological adaptation of the
Phaeocystis species to simultaneous changes in the nutritive
conditions of the surrounding medium. These physiological changes
of the species *Phaeocystis. poucheti* can be observed from figure
12 which gives the seasonal evolution of the relative proportions
of ^{14}C into the intra- and extracellular chosen classes of
metabolites. Results are expressed in % of total ^{14}C fixed.
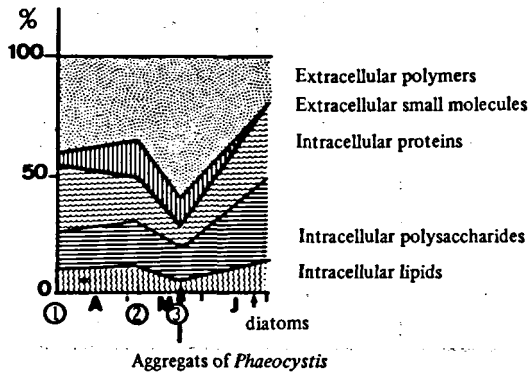
fig. 12.

Seasonal evolution of the ^{14}C distribution
between the different classes of intra- and extracellular metabolites
(expressed in percent of the total fixed carbon)
Station Ostend

As can be seen from figure 12, at least three different stages
can be distinguished for *Phaeocystis poucheti*. During the growth
period (stages 1 and 2), the relative intracellular production
is greater than the extracellular one. At the early beginning of
the growth (stage 1), the intracellular proteosynthesis is
relatively more important than those of polysaccharides and
lipids. At maximal growth, the relative proteosynthesis is
decrease simultaneously to the decrease in the mineral nitrogen
of the surrounding medium. This decrease is balanced by an
increase of the relative rate of lipids and polysaccharides
intracellular production decrease although the extracellular
production of polymers proceeds to a relatively high rate. These
two fractions occupy respectively 36 and 57 % of the total
fixed carbon.

This important production of extracellular polymers coincides with the presence of numerous colonies of *Phaeocystis poucheti* in the phytoplankton population. This species is effectively well known to be able to produce when in the stationary stage, a gelatinous substance of high molecular weight leading to the formation of large aggregates where the cells are agglutinated (Guillard and Hellebust, 1971). Our results suggests thus that the *Phaeocystis poucheti* species of the Belgian coastal area can produce actively the envelope which encloses themselves. Moreover, this property seems to belong especially to the species *Phaeocystis poucheti*. Effectively, the diatom population (*Chaetoceros sp.*) which succeeds to *Phaeocystis* in June is characterized by a weak rate of polymers extracellular production comparatively to the intracellular production, as can be seen on figure 12.

3.3.2.— Yearly balance of intra- and extracellular metabolism of phytoplankton.

Tables 5 gives the yearly estimates of intra- and extracellular productions of phytoplankton cells and their mean biochemical composition. These values were estimated from the yearly cycle of the diurnal production rate of the different classes of metabolites on the one hand and their concentration on the other (Lancelot, 1982).

Table 5

Yearly balance of intra- and extracellular metabolims of phytoplankton of the Belgian coastal area

Biochemical compounds	Concentration g C/m^2	%	Production intracellular g C/m^2.y	%	Production extracellular g C/m^2.y
POLYMERS	3.7		170		128
Proteins	1.8	48	70	41	?
Carbohydrates	1	27	70	41	?
Lipids	0.9	25	30	18	?
OLIGOMERS					22

As said previously, the apparent discordance between the concentrations and the productions of the intracellular metabolites can be explained only if the newly synthetized polysaccharides during the light period are catabolized preferentially to the proteins and the lipids during the night. If one assumes that proteins are not catabolized during the night, one may estimates the nightly respiration from the data of table 5.

Indeed suppose that P_N is the diurnal net particulate primary production, R_N the nightly phytoplankton respiration, G_B the dayly growth of biomass. The carbon balance is :

$$G_B = P_N - R_N$$

The protein balance is :

$$G_B \times 0.48 = (P_X \times 0.41) - (R_N \times 0)$$

Then $R_N/P_N = 0.15$

These results contrasts with those of Bouquegneau et al. (this volume). These authors assume that the daily phytoplankton respiration can be very high at some occasions (about 7 % of the phytoplankton biomass). One has yet to remember that our calculation gives an estimates of the nightly respiration on an annual basis. Nothing is known on the other hand about the diurnal phytoplankton respiration which could be very high. Secondly, we have assume that proteins are not catabolized during the night and this would have to be confirmed in the future.

4.– Concluding remarks.

The study of intra- and extracellular metabolism of the phytoplankton of the Belgian coastal area, with reference to changes in the nutritive conditions of the surrounding medium has shown that the biochemical changes in the primary products are bound to the mineral dissolved nitrogen. Light intensity on the other hand has no incidence. The biochemical changes of the cells are attributable both to changes in the species dominance and to physiological adaptations of a specific species. This last is particularly true for the spring species *Phaeocystis poucheti* for which at least three physiological stages were distinguished.

The protein content, which is always the main biochemical component of the phytoplankton cells is positively correlated to the nitrogen content of the surrounding medium. When nitrogen becomes depleted, the amount of intracellular protein decreases for the benefits of the other intracellular macromolecules (storage products) and secondly of extracellular polymers which represents more than 50 % of the total net primary production at mid-spring. This increase of the extracellular production of polymers leads during spring to the formation of many colonies of *Phaeocystis poucheti* whose shape is not suitable for the zoo-plankton filter-feeders (see Bouquegneau et al., this volume). So, the metabolism of the spring species *Phaeocystis poucheti* which is accountable for more than 65 % of the annual primary production, can explain the particular trophodynamic structure of the Belgian coastal area where the zooplankton efficiency is very weak. Most of the primary products are consequently available to the microheterotrophs either directly by the extracellular release or indirectly after lysis of the non-consumed phyto-plankton cells. These two sources of dissolved organic matter

include mainly polymers. Consequently they are not directly
usable by microheterotrophs and chemical hydrolysis must be an
important process in the Belgian coastal area.

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Part 3

UTILIZATION OF PRIMARY PRODUCTS BY ZOOPLANKTON

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1.– Introduction.

Expressing our conclusion at the end of a synthesis report on "Environment National Program — Sea Project", we showed that the zooplankton plays only an unimportant part in the recycling of organic phytoplanktonic matter. During the following years, zooplanktonologists, wishing to get a more accurate view on the share of zooplankton in this process of recycling, approached the question in different ways. They also tried to detect the mechanisms which can be considered as rules for the zooplankton's nutritional behaviour.

The first aim was to specify the relation between nutrition and the different phytoplanktonic species. Number of authors, indeed, have shown that zooplankton, especially copepods, do not feed on any size-class of particles. Even, within a given range, they select or neglect some species.

Our second target was to get an accurate evaluation of the zooplanktonic production. Such calculation could only be achieved by a detailed survey on long series, where it was necessary to follow all developmental stages day by day. Knowing that the development of a copepod, from egg to adult takes three weeks to one month, in temperate areas, one understands the necessity of gathering a dense sampling, the more so as mortality varies at each stage, according to age and environmental conditions.

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Zooplankton biomass had to be expressed on basis of the number of individuals. So a specific study has been made on difference of weight presented by the animals in function of the seasons.

A proper evaluation of zooplanktonic production and biomass within a marine ecosystem requires knowledge of the spatial distribution of the zooplanktonic communities : campaigns were carried out with this unique aim. The evaluation is still complicated by the fact that the zooplanktonic organisms have their own way to move themselves, obeying to a night/day rhythm of feeding, according to the seasons and their developmental stage.

Consequently, one observe night/day rhythms, consisting in migration towards upperwater during the night while the animals remain in deeper layers during the day.

Therefore it is obvious that time and depth of sampling will be of great importance for the evaluation of biomass and acti-vities. Preference has been given these last years to sampling along vertical profiles at different moments of the night and day.

Finally, considering that an analysis of the numbers of animals is part of an integrated survey on the cycles of the organic matter, this work assumes all its force only if its different components are separately valued. A first approach was to analyse the main biochemical components of zooplankton (proteins, carbohydrates and lipids). In the same option, the nutritional potentiality of these organisms can be inferred from digestive enzymes (amylase, protease). This method, applied to a large spatial scale, may practically instantaneously reveal the trophic structure of the whole planktonic ecosystem. The juxta-position of the different time-sections makes it possible to apprehend the trophic structure of the zooplankton, as to its seasonal evolution within the ecosystem Southern Bight.

2.— Results.

2.1.— *Biomass and zooplanktonic production.*

Besides the facts already stated in the synthesis Report on National Program "Sea Project", it has to be mentioned, firstly, that most of the zooplanktonic activities take place during the spring peak and, secondly, that copepods, during the spring zooplankton bloom, represent 80 % of the zooplankton biomass. The copepods and the description of their spring life cycles have then been submitted to comprehensive surveys.

Samples have been collected every day on board "West-Hinder" (2°86'20" E 51°23'00" N). Counting of the copepod's three main stages (nauplii, copepodites, adults) has shown a sequence of three peaks (fig. 1a) spreading from April to July. The figure 1b
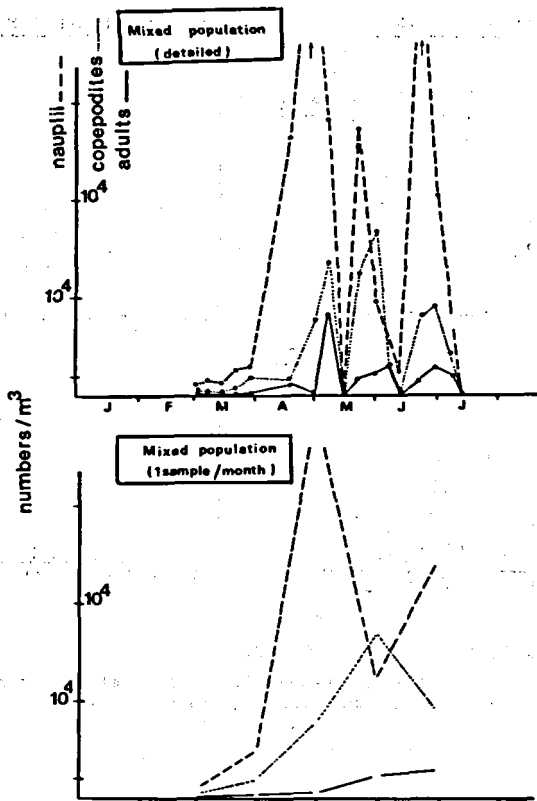
fig. 1.

Seasonal evolution of the numbers of different stages
(nauplii, copepodites and adults) of copepods, all species together
(a) Detailed data with one sample a week.
(b) Detailed data with one sample a month.

discloses that counting based on a unique sampling per month lets
only appear an important spring peak culminating at the beginning
of May. The comparison between both figures clearly indicates the
danger of simplified, if not wrong conclusions, when sampling is
not regularly enough.

Still determination of species shows more accurately that five
copepod species are dominating : *Temora longicornis*, *Pseudocalanus
elongatus*, *Paracalanus parvus*, *Acartia clausi*, *Centropages ha-
matus*, with a definite prepotence of the first two.

Temora longicornis and *Pseudocalanus elongatus* are always
present together, *Temora* dominating in April, *Pseudocalanus* in
June (fig. 2a, b).
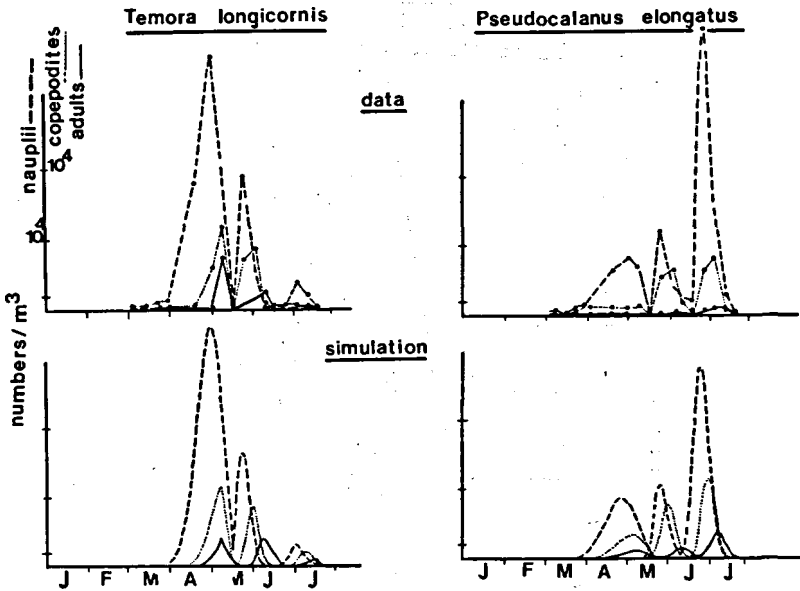
fig. 2.

Seasonal evolution (rough data and simulation model) of the numbers
of different stages (nauplii, copepodites and adults) of
(a) *Temora longicornis*
(b) *Pseudocalanus elongatus*

A simulation model, designed by Mommaerts and Bossicart (1979)
and applied to the sequence of the different peaks, has shown
that three generations of the two dominant species follow one
another from April to July with respective duration indicated in
table 1.

Table 1

Development duration (in days) of the three main stages
(nauplius, copepodite and adult) at the three spring generations
of *Temora longicornis* and *Pseudocalanus elongatus*

	Temora longicornis			*Pseudocalanus elongatus*		
	1st gen.	2nd gen.	3rd gen.	1st gen.	2nd gen.	3rd gen.
Nauplius	9 d.	9 d.	8 d.	8 d.	6 d.	6 d.
Copepodite	8 d.	9 d.	6 d.	7 d.	7 d.	6 d.
Adult	12 d.	10 d.	10 d.	10 d.	9 d.	8 d.
Total	29 d.	29 d.	24 d.	25 d.	22 d.	20 d.

'Between April and June-July, there is a shortening of about five days in the development period of the two species. A well-known situation, to be attributed to the raise of temperature (Kurada, 1962; Miller et al., 1977) and with the consequence that the animals, growing faster, show smaller size and lower weight when reaching the adult stage.

Figure 3 shows the seasonal evolution in weight for the five main species of copepods. All species reach their maximal weight in April, followed by a progressive weight-decrease of all adults of the following generations, the minimum being situated in July.
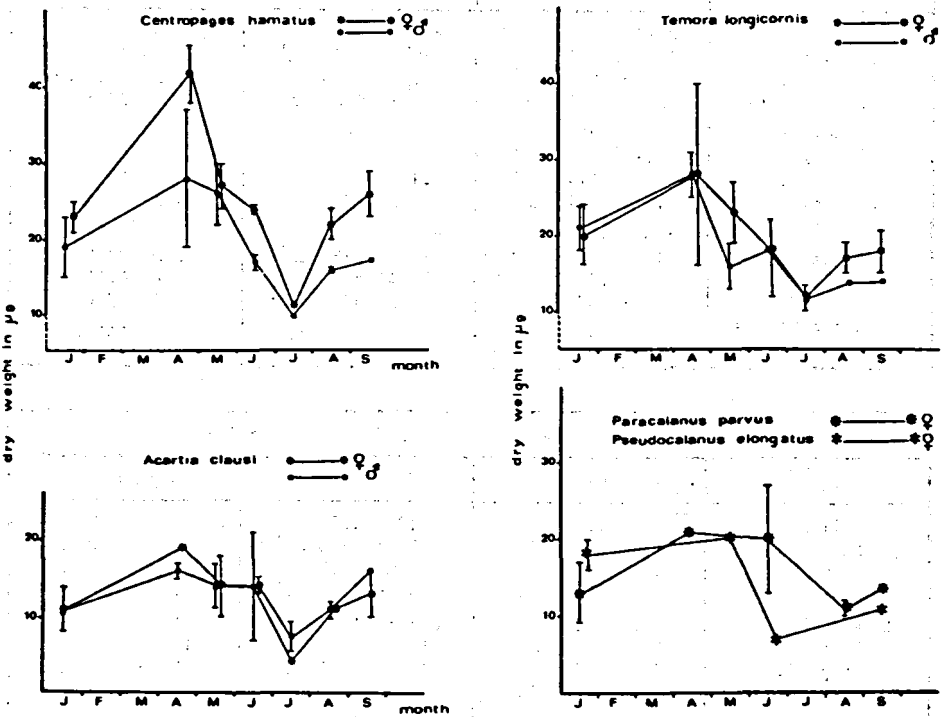
fig. 3.

Seasonal variation of adult copepods' dry weights
already corrected taking into account a 43 % loss due to formaldehyde

We have been able to indicate a correlation between development temperature and final weight of the adult (fig. 4). This was also found by several authors (Deevey, 1960, 1964, 1966; Marshall and Orr, 1966) and must be taken into account when expressing numbers in terms of biomass.

If all data are taken in consideration, we can now use the simulation model for the calculation of the different biomasses and productions. Table 2 is summing them up, with the year 1978 as an example.

Table 2

Pseudocalanus elongatus			
1978	1st generation	2nd generation	3rd generation

Nauplii
  biomass
  net prod.
  dead biomass
	d 85 135
	144 mg C/50 dm³
	28 mg C/50 dm³
	21 mg C/50 dm³
		d 135 160
		66 mg C/25 dm³
		22 mg C/25 dm³
		9 mg C/25 dm³
			d 155 190
			145 mg C/35 dm³
			49 mg C/35 dm³
			32 mg C/35 dm³

Copepodites
  biomass
  net prod.
  dead biomass
	d 90 135
	91 mg C/45 dm³
	20 mg C/45 dm³
	17 mg C/45 dm³
		d 135 165
		124 mg C/30 dm³
		27 mg C/30 dm³
		21 mg C/30 dm³
			d 160 195
			167 mg C/35 dm³
			45 mg C/35 dm³
			34 mg C/35 dm³

Adults
  biomass
  number
  number ♀
  number viable
  nauplii
	d 80 135
	13 mg C/45 dm³
	2178/45 dm³
	1605/45 dm³
	
	47468/m³
		d 125 155
		24 mg C/30 dm³
		4116/30 dm³
		3033/30 dm³
		
		52722/m³
			d 140 190
			28 mg C/50 dm³
			4810/50 dm³
			3544/50 dm³
			
			86982/m³

Temora longicornis			
1978	1st generation	2nd generation	3rd generation

Nauplii
  biomass
  net prod.
  dead biomass
	d 85 135
	217 mg C/90 dm³
	30 mg C/50 dm³
	43 mg C/50 dm³
		d 135 160
		45 mg C/25 dm³
		6 mg C/25 dm³
		4 mg C/25 dm³
			d 165 200
			12 mg C/35 dm³
			2 mg C/35 dm³
			1 mg C/35 dm³

Copepodites
  biomass
  net prod.
  dead biomass
	d 100 135
	133 mg C/35 dm³
	31 mg C/35 dm³
	15 mg C/35 dm³
		d 135 165
		55 mg C/30 dm³
		11 mg C/30 dm³
		9 mg C/30 dm³
			d 180 200
			10 mg C/20 dm³
			5 mg C/20 dm³
			2 mg C/20 dm³

Adults
  biomass
  number
  number ♀
  number viable
  nauplii
	d 85 135
	42 mg C/50 dm³
	13166/50 dm³
	6558/50 dm³
	
	179108/m³
		d 120 170
		11 mg C/50 dm³
		3298/50 dm³
		1649/50 dm³
		
		23685/m³
			d 150 205
			2 mg C/55 dm³
			632/55 dm³
			316/55 dm³
			
			7133/m³

Figure 5 represents the adding up of all data gathered on the two main species, showing a picture of the zooplanktonic production, so far as 80 % of its component is concerned.

TEMORA LONGICORNIS
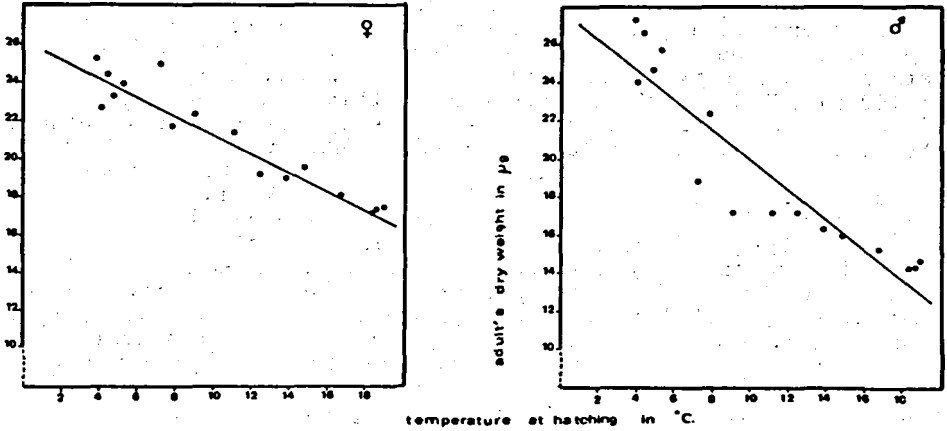
fig. 4.

Correlation between dry weight of males and females
of *Temora longicornis* and temperature at the hatching time

Pseudocalanus elongatus and *Temora longicornis*
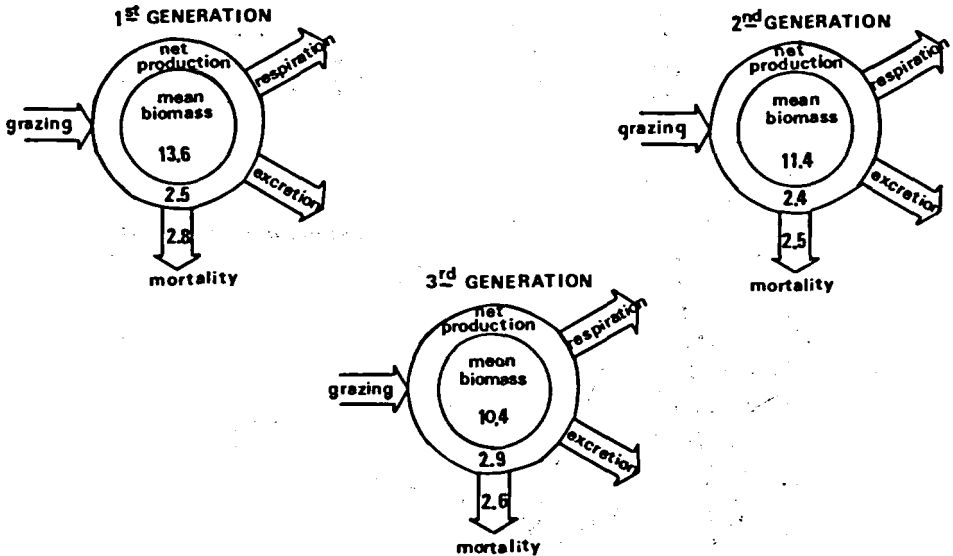
fig. 5.

Budget of the transfer of matter (in mg C/m^3.day)
for the two main spring and summer species together :
Pseudocalanus elongatus and *Temora longicornis*
(detailed for three successive generations)

2.2.— Nutrition of zooplankton.

It is a long-known fact that the filter-feeder copepods, to which belong the two species dominating in our ecosystem (*Temora longicornis* and *Pseudocalanus elongatus*) do not efficiently retain either very small particles (< 5 μ) or very large ones (> 100 μ) [Gauld, 1955]. Moreover, within this spectrum, particles of some sizes are more successfully retained than others (Mullin, 1963). As to us we have, since a couple of years undertaken a study of zooplankton nutrition on three main size classes, i.e. smaller than 25 μ, between 25 and 100 μ and larger than 100 μ. The reason for this choice is that the study takes mainly place during spring time, when the phytoplankton bloom is dominated by the nannoflagellate *Phaeocystis poucheti*, which is to be found, either isolated in the 25 μ fraction, or as a colony nestled in a mucuous envelope in the fraction larger than 100 μ. A further motivation, yet is the unsophistication of our method, which consists in labelling the phytoplankton with radioactive ^{14}C bicarbonate in presence of zooplankton, · a process that is very easy to use on shipboard (Daro, 1978). The more elaborate and comprehensive method of automatic counting of the whole particle-spectrum by the "Coulter Counter" ("cell count method") is unpracticable on board, at least with the present means available on sea.
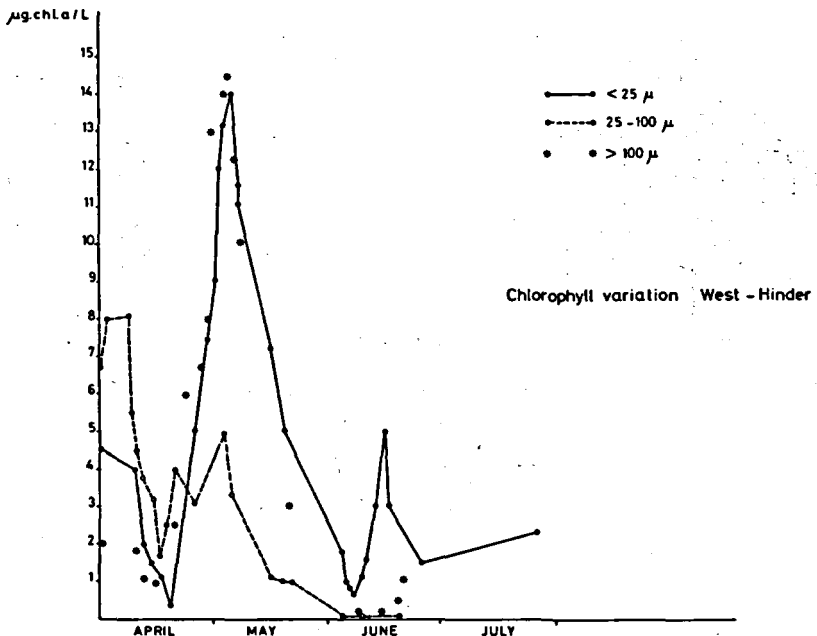
fig. 6.

Seasonal evolution of three chlorophyll fractions

Figure 6 shows the seasonal evolution from April to July, of three phytoplankton fractions, expressed as chlorophyll a. The main fraction 25-100 μ, dominating in April, is rapidly replaced during the bloom of May, by the two others : < 25 μ and > 100 μ. During June-July, only the smallest fraction maintains itself, and with rather weak concentration rates.

So, the studied organisms, *Temora longicornis* and *Pseudocalanus elongatus*, are submitted to temperature variations in the course of their three successive generations (from 7°C to 16°C between April and June), but have also to face important changes affecting their nutritional situation, relating to modifications of size and concentrations of phytoplankton which can reach a factor 20 in three weeks time.

The results of our experiments carried out on the two main species of copepods can be summarized as follows. Concerning *Temora longicornis*, the young stages, from nauplii to copepodites IV cannot be said very selective, in that they feed on the whole spectrum of particles smaller than 100 μ, the older stages, copepodites V and adults showing a clear preference for medium particles (fig. 7). Out of all experiments, only very few have produced a non-null result, as regards nutrition on particles larger than 100 μ.
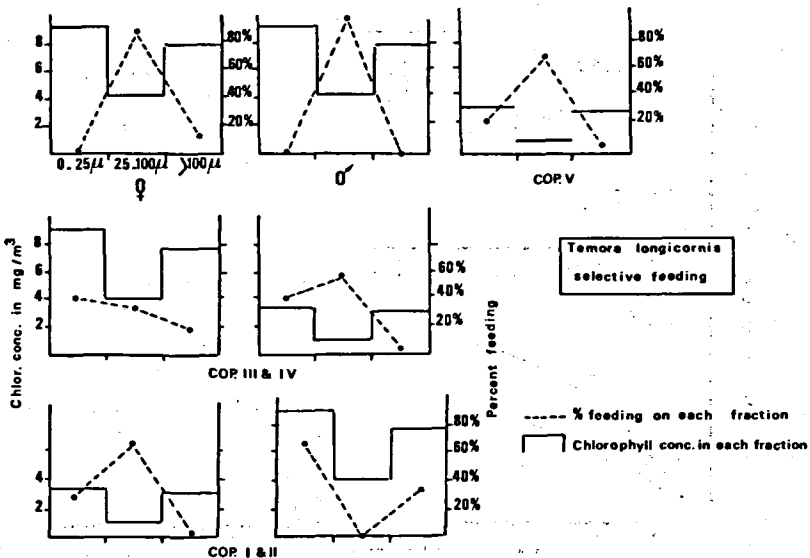
fig. 7.

Example of the selective feeding of copepodites and adults
of *Temora longicornis* on three different phytoplankton fractions
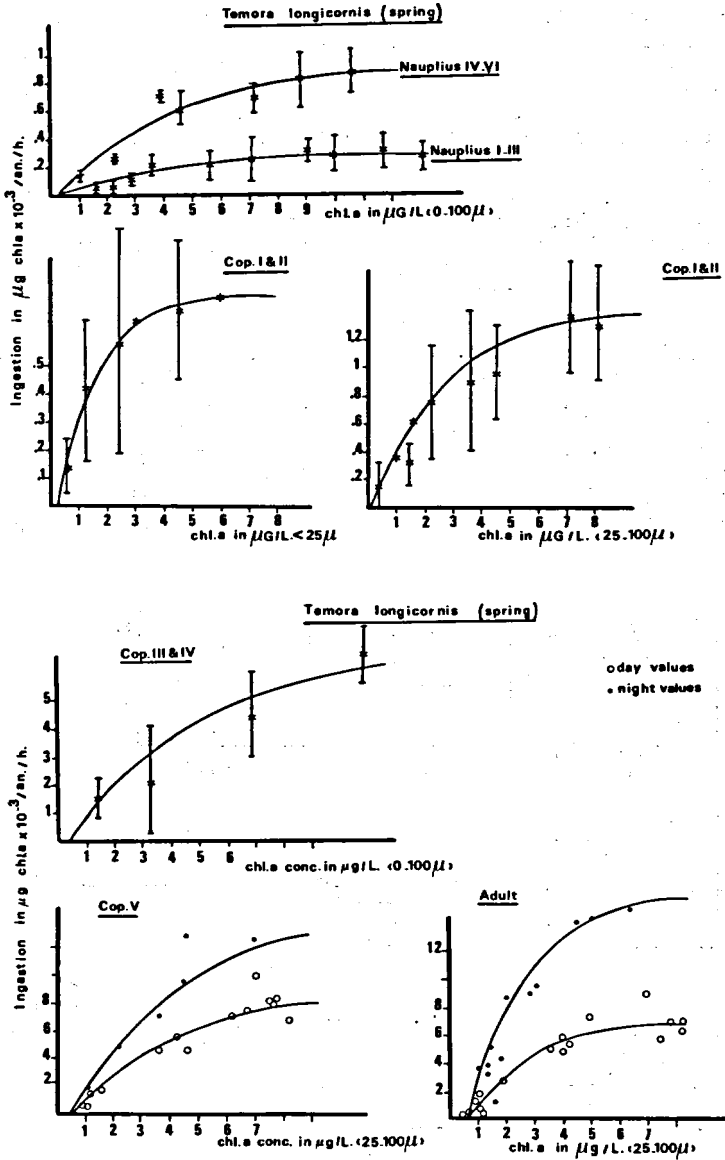
fig. 8.

Spring feeding Ivlev-Parsons relationships between ingestion and concentration
of chlorophyll in different phytoplankton fractions
and for all developmental stages of *Temora longicornis*

For the months April and May, we have been able to form the Ivlev-Parsons relations of ingestion as a function of the phytoplankton concentration for the different developmental stages of *Temora longicornis* (see also fig. 8).

(1) $Ih = .32 [1 - e^{-.22(p - .2)}]$ Nauplii I-III

(2) $Ih = .94 [1 - e^{-.24(p - .2)}]$ Nauplii IV-VI

(3) $Ih = .76 [1 - e^{-.71(p - .3)}]$

(4) $Ih = 1.4 [1 - e^{-.4 (p - .2)}]$ Cop. I-II

(5) $Ih = 7 [1 - e^{-.2 (p - .4)}]$ Cop. III-IV

(6) $Ih = 9 [1 - e^{-.25(p - .5)}]$ Cop. V at day-time

(7) $Ih = 14.65 [1 - e^{-.25(p - .5)}]$ Cop. V at night

(8) $Ih = 6.8 [1 - e^{-.51(p - .5)}]$ Adults at day-time

(9) $Ih = 16 [1 - e^{-.25(p - .5)}]$ Adults at night

Ih being ingestion in µg chl $a \times 10^{-3}$/animal.hour and p the concentration of chlorophyll a in µg/ℓ in the fraction 0-100 µ for the relations (1), (2) and (5), in the fraction < 25 µ for the relation (3), in the fraction 25-100 µ for the relations (4), (6), (7), (8) and (9).

Hereby we are brought to different remarks : at all stages exists a threshold concentration under which the animals do not feed anymore on phytoplankton, this threshold being .2 µg chl a/ℓ for the young organisms going to .7 µg chl a/ℓ for the adults.

Only the oldest animals, displaying at the same time their selectivity, show a nutritional night/day rhythm, the night nutrition being the highest, the older the animals are the more pronounced this rhythms is.

The month of June represents a characteristic feature for these animals. Actually, only nanoplankton is available at this period, and in rather weak concentrations (from .5 to 3 µg chl a/ℓ). Experiments carried out during this period indicate that the way the animals make use of their food is by far less efficient than previously, as proved by the following relations between ingestion and concentration of chlorophyll

          $Ih = .2 p$ copepodites I-II

          $Ih = .20 p$ copepodites III-IV

          $Ih = .46 - .16 p$ copepodites V

          $Ih = .59 - .22 p$ females

where Ih represents the ingestion in µg chl $a \times 10^{-3}$/animal.h and p the concentration of phytoplankton in µg chl a/ℓ (see

also fig. 9). At the same time they indicate the absence of a
night/day rhythm for the oldest animals.
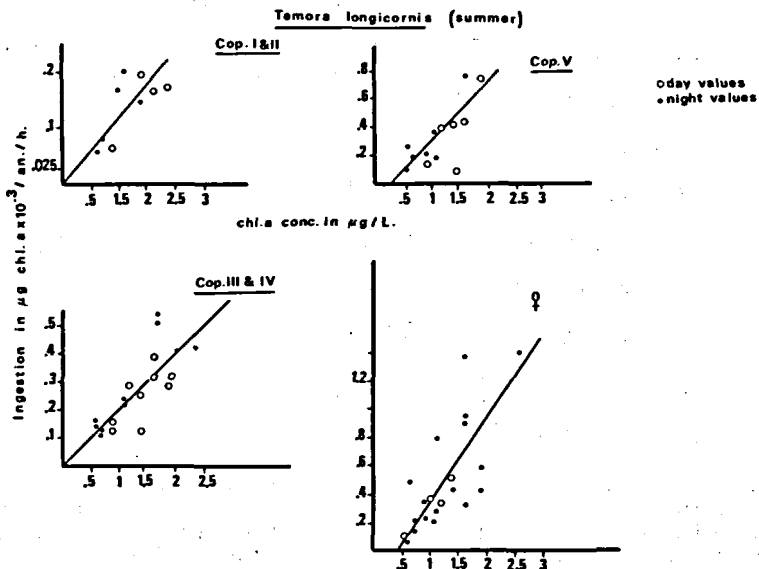
fig. 9.

Summer feeding relationships expressed in ingestion
as a function of chlorophyll concentration for *Temora longicornis*

Comparing Ivlev-Parsons relationship with the linear ones, we
notice that for the same chlorophyll concentration, ingestion is
by far inferior in June than in April-May, even though the
relation chlorophyll/carbon is higher during the month of June.
An example :at a concentration of 2 µg chl a/ℓ, an adult will
ingest 132.6 × 10⁻³ µg chl a or 4.2 µg C in spring, against
23.4 × 10⁻³ µg chl a or 17 µg C in summer.

The equivalences chlorophyll/carbon are given by Lancelot
(1980). At the end of this chapter, we will see that the nu-
trition decrease has negative consequences not only on the
production (as well for what concerns biomass as for egg produc-
tion) but also on the population by means of a decrease of
numbers. The experiments with *Pseudocalanus elongatus*, the other
dominant spring species, produce different relations between
ingestion and chlorophyll concentration. All developmental stages
show a clear preference for the small particles (< 25 µ). The
relations we found are all linear

$$Ih = .7 \ p - .03 \qquad\qquad\qquad \text{copepodites I}$$
$$Ih = .12 \ p - .05 \qquad\qquad\qquad \text{copepodites II}$$

$$Ih = .13\ p \qquad\qquad\qquad\qquad\qquad \text{copepodites III}$$
$$Ih = .29\ p - .17 \qquad\qquad\qquad\qquad \text{copepodites IV}$$
$$Ih = p - .52 \qquad\qquad\qquad\qquad\qquad \text{copepodites V}$$
$$Ih = .48\ p \qquad\qquad\qquad\qquad\qquad \text{copepodites adults}$$

Ih being ingestion in µg chl $a \times 10^{-3}$/animal.hour and p the concentration of chlorophyll a in µg/ℓ in the fraction < 25 µ (see fig. 10). So we may conclude that the two cohabitating species, especially during the phytoplankton bloom share their food in a very efficient way qua particles-sizes.
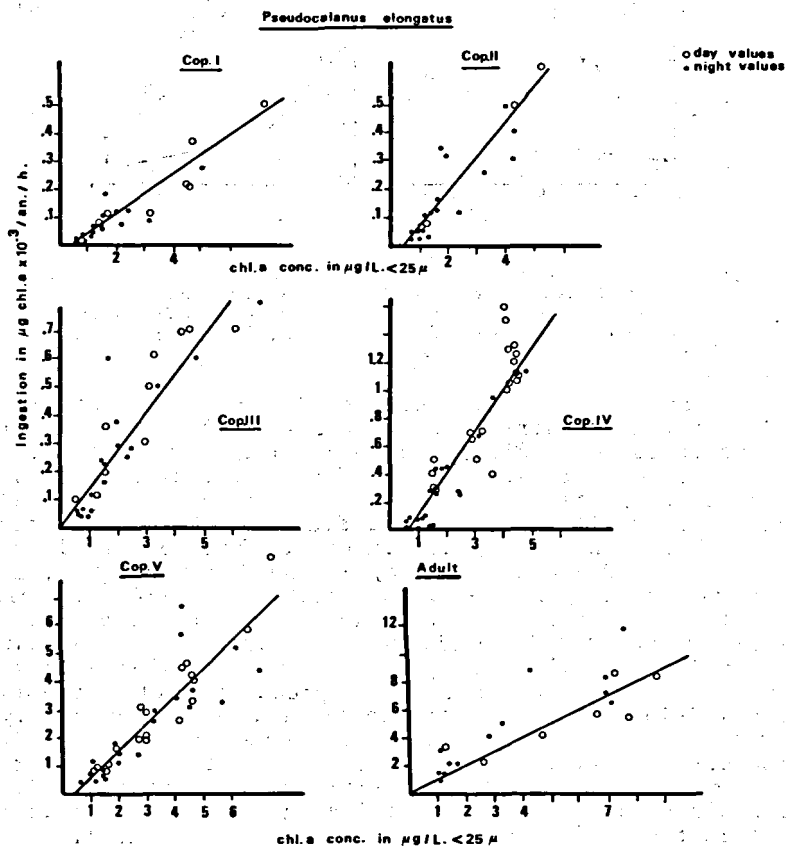
fig. 10.

Feeding relationships expressed in ingestion
as a function of chlorophyll concentration for *Pseudocalanus elongatus*
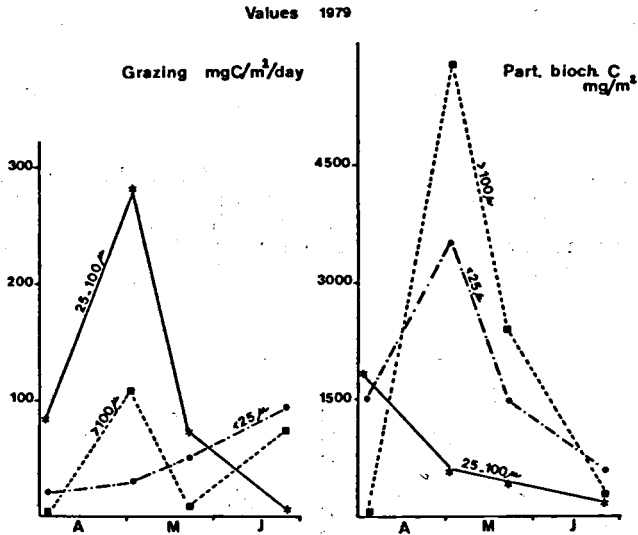
fig. 11.

Total grazing for the whole zooplankton community
expressed in mg C/m².day on the three phytoplankton fractions
and the concentration of biochemical carbon in the same three fractions

On the other hand, *Pseudocalanus elongatus* does not follow a
night-day rhythm of nutrition. We will show that the behaviour of
the two species also differs in the matter of vertical migra-
tions. Subject of a final step will be the summing up of all data
gathered on the grazing by the different species. The results are
covered by figure 11 for the three fractions taken into account.
The same figure shows the biochemical carbon contained in these
three fractions. One can see that, during April and May, most of
the grazing occurs on the mean fraction 25-100 µ, which is
actually the weakest in quantity. In other words the nutritional
behaviour of the total herbivorous zooplankton is dominated by
Temora longicornis, which is precisely showing this selectivity.
The absence of measure of primary production of this definite
fraction (25-100 µ) is regrettable because it is precisely from
those data that the grazing pressure could have been evaluated.
Nevertheless, as first approximation, we still cannot exclude
that the grazing could be responsible of most of this 25-100 µ
fraction disappearing. Cross-checkings indeed allow us to consi-
der that in the beginning of May the biomass of this fraction has
a particulate production of 250 mg C/m².day, while the grazing
represents a higher value (300 mg C/m².day). This remark must
only be taken as an indication, because based on a rough calcu-
lation : the percentage of c.p.m. of the fraction 25-100 µ
resulting from our grazing experiments has been applied to the

total particulate production data measured during the same period, allowing us to infer a value of 250 mg C.

Our data, especially those on the selective behaviour of *Temora longicornis* present some contradictions with the following data found in the literature. Generally speaking, the copepods, within a certain particle-spectrum, tend to feed on those particles with the highest concentrations, and, in the case of a multimodal distribution pattern, grant their preference to the largest particles (Boyd, 1976; Nival and Nival, 1976). The ingested food, expressed in carbon, is more important when the copepods feed on large particles (Mullin, 1963; Frost, 1972; O'Connors et al., 1976; Paffenhöfer and Knowles, 1978). Concerning *Temora longicornis*, O'Connors, Biggs and Ninivaggi (1980) indicate that the maximal ingestion is increasing by a factor 3 to 5, when the particle's diameter increases from 5 to 30 µ.

Poulet (1978) observed in the Bedford Basin that the five dominant copepod species, and among them *Temora longicornis*, feed on particles at the highest concentrations in the range 5-40 µ. In September, as the particle-spectrum showed two peaks, one at 2 µ and the other at 50 µ, the ingestion of the copepods was following exactly this bimodal figure. Poulet's conclusion was that an opportunistic filtration strategy was displayed by those copepods.

Lehman (1976), Lam and Frost (1976) have worked out "energy maximization" models, according to which filter-feeders should feed, within the available spectrum, on particles that furnish most energy relatively to the energy spent during filtering; they propose an "electivity index" which covers the relation between ingested biomass and filtration rate, tending to be so small as possible. Number of practical cases confirm their theory.

As to us, it seems we are confronted with a quite different mechanism, which we may call a reject or an incompatibility. *Temora longicornis* does not seem to be satisfied with *Phaeocystis poucheti*, either because this species should be toxic, or, simply, that the filtering apparatus of *Temora longicornis* should be clogged by the mucus produced by this species. On the other hand Lehman's theory is not always valid, as we showed for another species of copepod, *Acartia tonsa*, at the Sluice Dock, Ostend. The "cell count method" is a procedure used all over the world in feeding experiments. It requires long time incubation (24 hours) and high concentrations of animals.

In our experiments with *Acartia tonsa*, we used short incubation times (4-5 hours) and decreasing concentrations of animals; it resulted in quite different filtration patterns. Figure 12 illustrates these results and makes clear that the highest filtration rate occurs on the smallest particles (1-2 µ) although this requires the highest energy for a finally very feeble profit. This fact is totally overlooked when using long incubation times and high concentrations of animals.

Therefore one can put in doubt, whether numerous results cited in the literature are not submitted to different interpretations
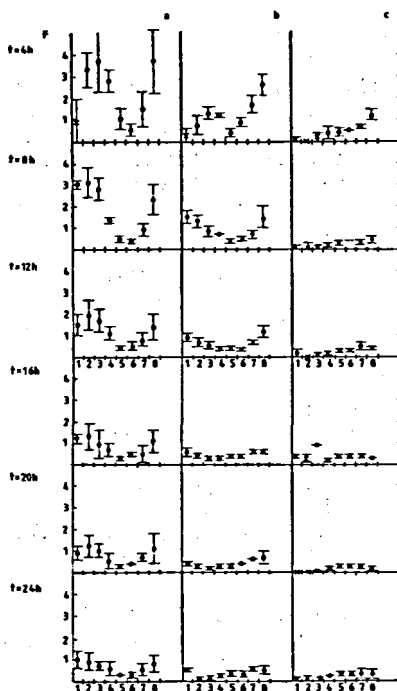
fig. 12.

Filtering rates of *Acartia tonsa* feeding on natural phytoplankton
(a : 25 animals/ℓ, b : 50 animals/ℓ, c : 100 animals/ℓ) as a function of
time and diameter of particles; 1,2,—,8 numbers of the Coulter Counter
canals, 1 being the smallest particles (1.10 diam.), 8 being the largest
ones (5.56 diam.). This diagram shows, that with high concentrations of
animals (b & c) and/or long incubation times, the filtering rate on the
smallest particles disappears from the results.

in the light of the data mentioned here, which imply that the
nutrition on very small particles (mainly bacteria) could play a
more important part than commonly believed.

2.3.— *Relation between growth and nutrition.*

The relation was studied with more emphazis on *Temora longi-
cornis*. Referring to § 2.1, we indicated that all species of
copepods decrease in weight in the course of the seasons, a fact
which found its main explanation in the raise of temperature. We
are able to show that also nutrition has an influence on the low
weights found in summer (Daro and Van Gijsegem, 1982).
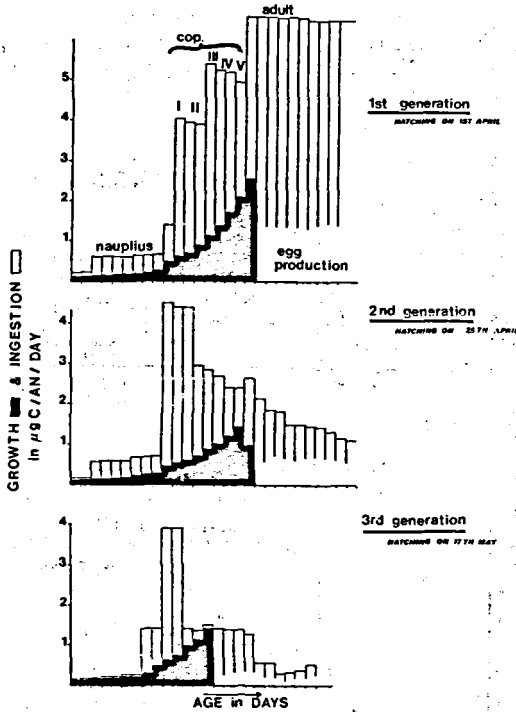
fig. 13.

Daily growth and ingestion expressed in the same units
for *Temora longicornis* at three successive generations

Figure 13 represents the daily growth as well as the daily
ingestion, both expressed in the same units (µg C/animal.day)
throughout the life of an animal of the first, the second and the
third generation. At the first generation, ingestion amply satis-
fies the requirements of growth and egg production. Nutrition
diminishing in a sizeable way in the course of the second
generation, the adults get into real trouble and are only able
to produce a few eggs (an egg weights about .2 to .5 µg C).
Finally, we can reasonably be sure about the third generation
living in a situation of underfeeding, so that the adults are not
only incapable of producing eggs, but also of meeting their
metabolic needs. Our opinion is that such a situation is respon-
sible for the decreasing weights of the adults. Scarcity of food,
leading either to a reduction of egg-production or no production
at all, has another consequence and altogether explains what
figure 2 already illustrated : the decline of the population of
Temora longicornis. *Pseudocalanus elongatus*, for its part, is
also submitted to uneasy nutritional conditions in the course of

the three spring and early summer generations. As we already
pointed out this species takes a satisfying profit of the
Phaeocystis bloom at all stages of development, at least for the
< 25 µ fraction.
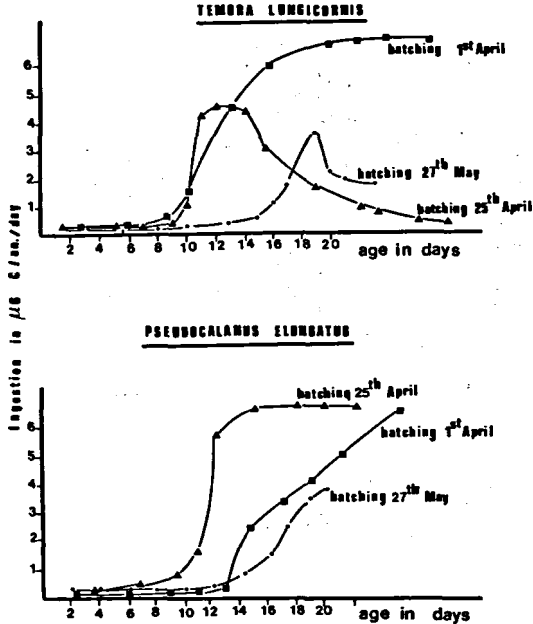
fig. 14.

**Daily ingestion during the life of an animal at three successive generations
for *Temora longicornis* and *Pseudocalanus elongatus***

Figure 14 represents the results of feeding-experiments for
both *Temora longicornis* and *Pseudocalanus elongatus* through their
three spring generations. During the bloom, *Pseudocalanus
elongatus* takes a maximal profit of the food, with the exception
yet that both the first and third generation get in some trouble
by the shortness of appropriate food at the available concen-
trations.

However good feeding conditions prevailing during the spring-
bloom result in a high egg-production, which explains the
increasing numbers of *Pseudocalanus elongatus* between April and
June (*cf.* fig. 2b).

2.4.– *Night day rythms of zooplankton and their relation with phytoplankton.*

Many vertical profiles indicate a heterogenous way of dis-
tribution of the zooplankton along the watercolumn. Moreover,
certain developmental stages tend to move towards the upper water
layer during the night. The water column being not very deep
(max. 20 m), it is extremely difficult to demonstrate night/day
vertical migrations, the more that they are performed mainly by
the oldest organisms and that their amplitude changes from one
season to another. On the other hand, while migrations occur in
close link with light intensity, the euphotic depth has to be
perfectly determined. Well, this layer extends to a maximum of
10 m depth, i.e., half of the water column. Finally, the height
of the water column itself fluctuates about 4.5 m with the
tide. These remarks clearly demonstrate that, at any moment, it
is imperative to sample the whole height of the water column,
down to the bottom, and that is very difficult, by a distance of
5 m, for example, to get an accurate picture of the animals'
movements. In other words, as the bottom is at 150 m depth, it
is easy to demonstrate movements of animals passing from - 100 m
to - 40 m (euphotic zone) without errors. The task is much more
uncomfortable when one has to demonstrate migrations from - 15 m
to - 7 m, movements obeying exactly to the same stimuli (fading
or absence of light). This is the reason why we principally will
show the differences below and above 10 m.
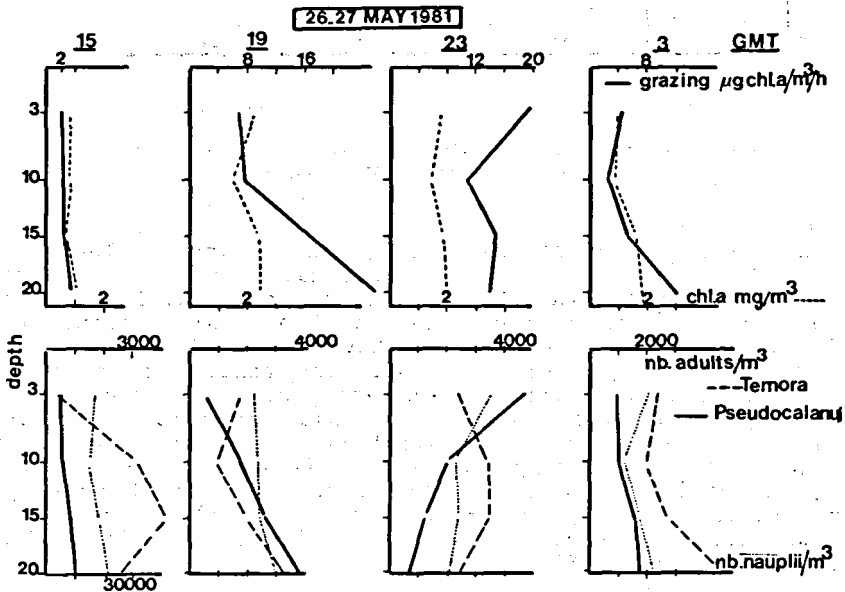
fig. 15.

Grazing and chlorophyll profiles and numbers of animals profiles
at different times of the same day

   In general, we see that the zooplanktonic organisms remain
below   10 m, and even very close to the bottom, practically
during 18 hours out of 24 (see fig. 15, 16, 17).
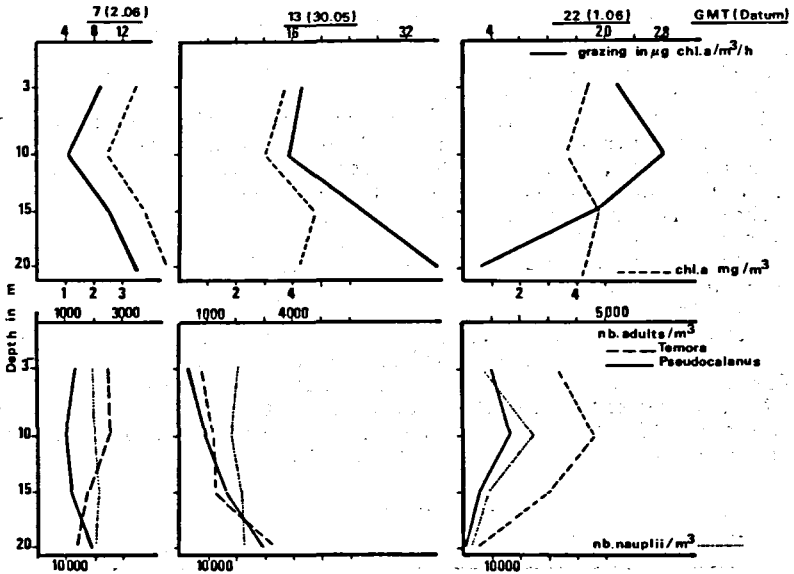
fig. 16.

Grazing and chlorophyll profiles and numbers of animals profiles
at different days at the end of May

   When vertical migrations occur, they vary following the
species. *Temora longicornis*, at least mainly the oldest stages,
begins to swim up towards the surface at sunset. If *Pseudocalanus
elongatus* is performing night movements, they rather occur around
midnight. At sunrise all species have regained their position
below the 10 m zone. Let us underline that, here again, the
migration behaviour of the two cohabiting dominant species differ
from one another. The same can be said for their nutritional
behaviour. *Temora longicornis* displays a day/night nutritional
rhythm, possibly according to the decline of the feeding condi-
tions, and begins to migrate towards the surface at sunset, at
the moment that the phytoplanktonic biomass is the highest of the
day; we already observed a similar behaviour with *Calanus
finmarchicus* (Daro, 1980).

   So we can conclude that most of the zooplankton, particularly
the oldest stages, is permanently staying in the dark. An
occurrence which may have important consequences on — let us
say — an alternative nutrition. Lancelot (this paper, part 2)
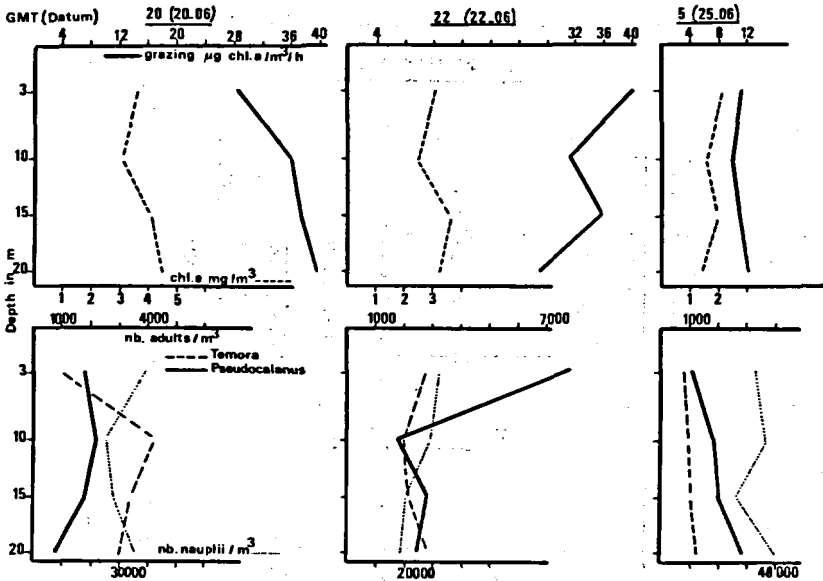
fig. 17.

Grazing and chlorophyll profiles and numbers of animals profiles
at different days at the end of June

found out that detritic phytoplankton sedimentation can be very
important. Numerous vertical profiles of chlorophyll made out
last year allow us to verify this important sedimentation. The
presence of the largest phytoplankton concentrations (alive or
detritic) and the abundance of copepods in the deepest layers
result, during many hours of the day in an intense nutritional
activity.

It is significant that more chlorophyll is to be found below
10 m (so in complete darkness) than above (fig. 18). Taking as a
reference more than thirty profiles, we may say that in 85 % of
the cases, 55 to 60 % of the phytoplankton is found below
10 m, and the proportion of active chlorophyll within the
totality of pigments is the same in the two layers. In other
words the phytoplankton does not die in the upperwater before
migrating or sedimenting, their is a constant rain of phyto-
plankton falling down into the darklayers. By heavy sea (6-8) and
with only four profiles available, the relation is reversed and
most of the chlorophyll (55 to 60 %) will be found in the upper
10 m layer.

In a general way, a calculation consisting in multiplying the
chlorophyll value from - 3 m by the height of the water column
leads to an underestimation (by 0 to 20 % with an average of
10 %) of the integrated value. The greatest differences along the
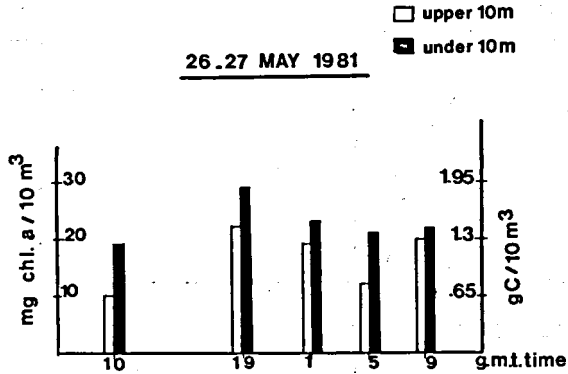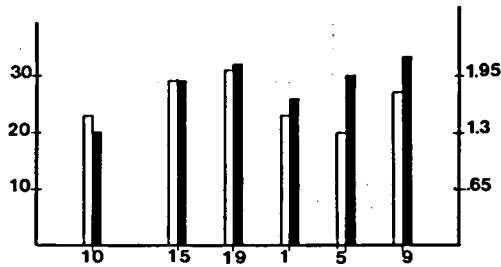
fig. 18.

Particulate organic matter expressed in chl/10 m³ and in g C/10 m³
in the upper and the lower layers, the limit between both being 10 m

water column are to be found between the - 3 m value of
chlorophyll and - 20 m, just above the bottom : on an average
one finds 40 % more chlorophyll at - 20 m than at - 3 m, the
highest differences (80 to 100 % more at - 20 m than at
- 3 m) being observed in the early morning. Yet, at - 20 m,
there is proportionnaly as much active chlorophyll as at - 3 m.

Tables 3 illustrates the different results. As conclusion we
can say that most of the phyto- and zooplankton is located
underneath 10 m. It is obvious, their food source being
available in highest concentrations below 10 m and even close
to the bottom, that the animals take advantage by concentrating
also in the same region. The vertical migrations of the zoo-
plankton towards the surface are phenomena extremely limited in
time within the 24 hours period.

Table 3

Comparison of phytoplankton biomasses
(expressed in total pigments active chlorophyll and phaeophytin)
between the upper 10 m layer and the underneath 10 m layer

Datum	Time (GMT)	Upper 10 m			Under 10 m		
		Total pigm. in mg chl a/10 m³	Phaeo. in mg/10 m³	% active chlor. within the tot. pigm.	Total pigm. in mg chl a/10 m³ and in %	Phaeo. in mg/10 m³ and in %	% active chlor. within the tot. pigm.
22-05	9	15	5	67	24.4 62	6 55	75
24-05	17.30	13.2	6.7	49	15.5 54	6.7 50	54
26-05	10.30	8.2	3.5	57	8.4 51	6 63	51
	15.30	10.3	6.7	35	19 65	9.6 54	49
	19	9.6	3.6	63	10.3 52	5 58	51
	23	22.4	9.8	56	28.7 56	13.7 58	52
27-05	3	19.6	8.3	58	23.6 55	11.9 59	50
	7	12.4	6.1	51	21 63	9 60	57
	7	20.3	11.3	44	22.9 53	10.8 49	53
28-05	17	23.8	13.8	45	28 54	15.4 53	45
30-05	11	40.5	18.8	54	55.6 58	27.9 60	50
31-05	7	18.4	11.8	36	30.4 62	17.4 60	43
	22	44.6	11.9	73	47.9 52	15.9 57	67
1-06	10.30	39	11	72	46.2 54	13.5 55	71
	22	45.1	14.7	67	46.2 51	17.8 55	61
2-06	9	36.2	13.2	64	41.3 53	20 60	52
19-06	14.30	30.2	9.8	68	29.4 49	9.4 49	68
20-06	20	37.5	12.3	67	46.5 55	16.5 57	65
22-06	14	32.7	11	66	44.4 58	16 59	64
	23	33	11.9	64	39.3 54	15.9 57	60
23-06	19	36	17	53	42.8 54	19.8 54	54
25-06	5	22.6	9.4	58	19.9 47	7.2 43	64
	11.30	22.2	7.5	66	27.8 56	14.5 66	48
27-06	17	28.3	13.0	52	30.2 52	12.5 48	59
28-06	17	12.0	6.6	48	12.4 50	5.2 44	58
	6.30	8.7	3.8	56	7.1 45	3.4 47	52

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Part 4

UTILIZATION OF PRIMARY PRODUCTS
BY PLANKTONIC AND BENTHIC BACTERIA

G. BILLEN[1] and M. SOMVILLE[1]

25540

Abstract.

Structural and functional modifications induced by eutrophication of coastal ecosystems are examined from the point of view of planktonic microbial activity.

It is suggested that increasing input of organic matter tends to favour r-strategists among the heterotrophic organisms utilizing organic matter. This implies namely the prevalence of microorganisms food chains and the occurrence of special adaptations of microorganisms in their transport system for substrates.

It is also shown that a high bacterial heterotrophic activity is not necessary linked to a high rate of ammonium regeneration, depending on the C/N ratio of the organic matter used.

Increasing flux of organic material depositing on the bottom results in the establishment of more reduced conditions in the sediments, which affects strongly both the form and the efficiency of benthic nitrogen recycling.

1.— Introduction.

As stressed in other paper of this volume (Bouquegneau et al., Daro et al.), one of the most striking features in the ecological working of coastal ecosystems, and of the Belgian coastal zone in particular, is the importance of the role of bacteria, both in the planktonic and in the benthic phases, in the utilization of primary production. This paper discuss the mechanisms and the

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control of bacterial activity in carbon and nitrogen cycles of coastal ecosystems. We shall suggest that the importance of bacterial activity versus macroorganisms activity is a characteristic of enriched, or concentrated media — i.e. media where the production or input of organic matter is high per unit volume — versus oligotrophic, diluted environments.

Therefore, the whole paper can be read as a discussion of the effect of increasing organic matter input or stimulation of primary production in coastal ecosystems on the structure of their carbon and nitrogen cycle. The underlying question is of course : how do the structure and the working of our coastal zone react in response to progressive eutrophication. We think that the understanding of bacterial activity is a most important key for answering this question.

2.— Role of microorganisms in carbon cycle.

2.1.— *Microorganisms-macroorganisms competition.*

A classical concept in general ecology is the distinction between two kind of organisms according to the strategy they adopt in their interspecific competition for living resources :
- r-strategists, on the one hand, devote most efforts in colonizing as fast as possible all potential niches. These are fast reproducing, fast disseminating organisms;
- K-strategists, on the other hand, reproduce and disseminate themselves more slowly, but develop complex structures allowing a deeper utilization of the resources.

The symbols r- and K- refer to the parameters of the logistic growth curve of population, r representing the specific capacity for growth, K the carrying capacity of the environment for the population.

Table 1

Ecological characteristics of microorganisms versus macroorganisms

	Bacteria	Higher organisms	
		Zooplankton Meiobenthos	Fish Macrobenthos
Generation time	1 day	1 month	1 year
Growth Yield	30 – 50%	25%	10%
Strategy in food capture	Passive	Active	Active

   What characterizes microorganisms (bacteria in particular)
with respect to higher organisms (table 1) is :
• their low generation time,
• their high growth yield,
• their rather passive nutritional behaviour.
While higher organisms have developed complex structure for
actively chasing their food, bacteria generally do not dispose of
such structures and depend to a great extend on passive diffusion
of dissolved substances near to their cells.

   As a group, microorganisms can therefore be considered as
r-strategists with respect to higher organisms, displaying in
general a more K-oriented strategy.
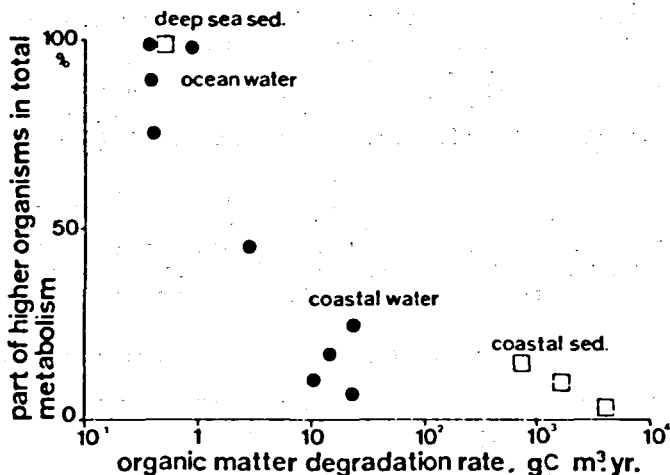
fig. 1.

Relative part of higher organisms versus microorganisms in the use of
organic matter in planktonic and sedimentary systems of various "richness"
("richness" being defined by the total flux of organic matter through the
system). [Data from Billen et al., 1976; Jannasch and Wiersen, 1972;
Lancelot, 1982].

   What is observed as well in sedimentary than in open water
systems (fig. 1) is that microorganisms dominate in the use of
organic matter in environments like coastal waters (where an
intense primary production is concentrated in a shallow water
column) or *a fortiori* like coastal sediments (where an important
part of the primary production of the whole water column is
deposited and therefore concentrated in a 10 cm layer). On the
contrary, higher organisms prevail in the use of the scarce
resources of poor, diluted environments like oceanic waters or
deep-sediments, receiving only a very small flux of organic

matter. In other words, if "richness" is defined as the total flux of organic matter through the system, r-strategy dominates in rich, an K-strategy in poor environments.

This suggests that a general increase of the level of primary production in our coastal zone, as a result of increasing nutrient input, does not necessarily lead to an increase of pelagic or demersal fish production, but could induce a modification of the food web resulting in enhencement of microorganisms activity at the expense of long trophic chains dominated by macroorganisms.

2.2.– Mechanisms of organic matter utilization by bacteria.

Most of the organic matter primarily supplied by primary production in marine systems consists in macromolecules (polysaccharides, proteins, lipids), or small organic acids are directly produced (Billen, 1982). Even the process of phytoplankton excretion, at least in the Belgian coastal zone, leads to liberation of high molecular weight material (see Lancelot et al., this volume).

Such macromolecular compounds, either dissolved or particulate, cannot be directly taken up by bacteria, and have first to be hydrolyzed into smaller units, probably mainly through the action of exoenzymes. Once produced, these low molecular weight, directly usable compounds are rapidly taken up and metabolized by heterotrophic bacteria.

Organic matter utilization through the bacterial food chain therefore involves two successive stages :
• exoenzymatic hydrolysis of macromolecules;
• uptake of small, directly usable substrates.
We shall examine them in turn.

2.2.1.– Exoenzymatic activity in sea-water.

Due to the lack of a convenient and sensitive method, little information is available concerning the occurrence and activity of exoenzymes in natural waters. By use of insoluble synthetic protein-dye, releasing a soluble color upon enzymatic hydrolysis, some authors (Kim and Zobell, 1974; Little et al., 1979; Meyer-Riel, 1981) demonstrated the occurrence of free exoproteasic activity in lake- or sea-water samples. This method, however, is not sensitive enough for rapid measurements and requires either very long incubation times (a few days) or preconcentration of the samples by dialysis of ultrafiltration.

Somville and Billen (in press) have reported a very sensitive and reliable method allowing determination of exoproteasic activity in a few minutes without any concentration of the sample even in oligotrophic waters.

The method, adapted from the procedure of Roth (1965) in clinical analysis, is based on the use, as substrate for proteo-lytic exoenzymes, of amino-acyl derivatives of β-Naphtylamine, which give rise to a fluorescent product after hydrolysis of their peptidic-like bond.

The specificity of the method toward exoproteases was indi-rectly tested by studying the effect of varying concentrations of serum-albumine on the rate of hydrolysis of LLβN (fig. 2). A strong competitive inhibition was found. The inhibition constant of albumin (which in the case of competitive inhibition is equal to its Km for the enzymes) is about 50 µmoles/ℓ.
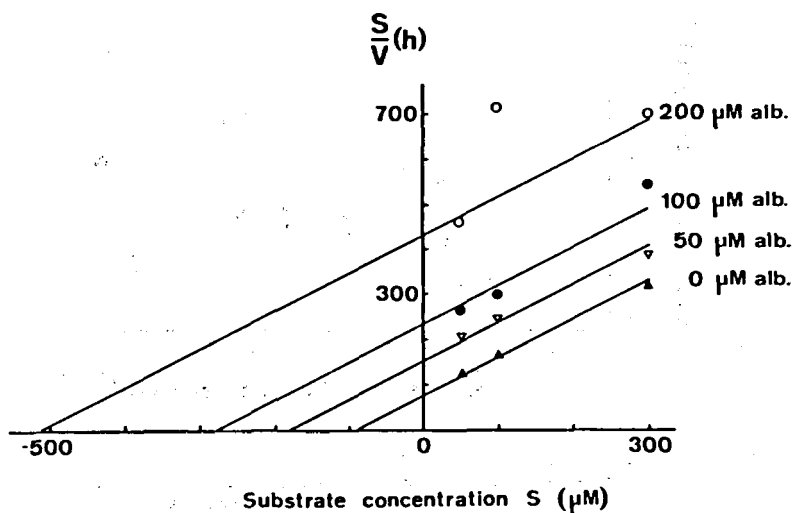
fig. 2.

Inhibitory effect of serumalbumine on the hydrolysis
of L-Leuctl-β-Naphtylamide (reciprocal plot)

This indicates that the enzymes responsible for LLβN hydro-lysis have a higher but similar affinity for proteins. On the other hand, free amino acids have only a smaller inhibitory effect on LLβN hydrolysis, the Ki of L-Leucine being about 200-300 µmoles/ℓ.

Up to now, about 50 determinations of potential exoproteasic activity are available in various aquatic environments, from rather oligotrophic, as in the English Channel, to hypereutro-phic, as in the heavily polluted Scheldt estuary. In some cases, the relative rate of amino-acid utilization has been simulta-neously determined on a parallel sample, by means of a ^{14}C labelled amino acid mixture (protein hydrolysate, Amersham) [Williams et al., 1976].

· As will be shown below, this relative rate can be considered as a good measurement of the total rate of amino acids heterotrophic utilization. Figure 3 shows that a good correlation is obtained between potential exoproteasic activity and the rate of amino acid uptake. This observation confirms the obligate role of exoenzymatic activity in the bacterial utilization of organic nitrogen in aquatic environments.
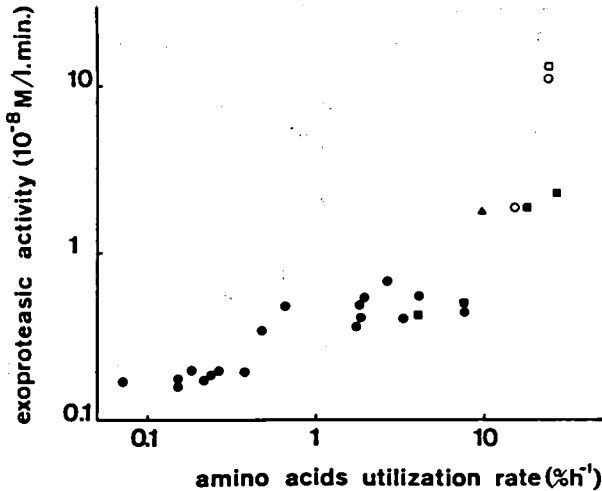
fig. 3.

Relation between exoproteasic activity and relative amino acids
utilization rate in various aquatic environments
(•) coastal seas
(▲) eutrophic pond — Bois de la Cambre, Brussels
(■) Oise river — France
(○) Scheldt estuary
(□) Rupel river — Belgium

With the method described, proteasic activity can be determined after gentle filtration of the sample through 0.8 or 0.2 µmembranes. The former have been shown by numerous studies in sea-water (Derenbach and Williams, 1974; Azam and Hodson, 1977) to retain most phytoplanktonic organisms while most free living bacteria pass them; the latter retain all microorganisms. By this procedure, it is therefore possible to assess to which fraction of the natural microbial community exoenzymes are associated. The data obtained with sea-water collected in June 1981 in the eutrophic Belgian coastal zone of the North Sea, show that most oligotrophic waters from the Eastern English Channel at the same period, most of them appear to be bound to particles 0.8 and 0.2 µ. In the last case, exoproteases are probably linked to

the external surface of bacterial cells, as demonstrated in some instance by Christison and Martin (1971).

This suggests the existence of two strategies in exoenzymatic hydrolysis of macromolecules : the first one consists for the bacteria to produce free dissolved exoenzymes and wait until the hydrolysis products diffuse again to the cell, the second one consists in keeping the exoenzymes linked to the cell membranes so that the hydrolysis products have more chance to be taken up.

It is probably significant that this last, more active strategy, in which the bacteria "chase" in a sense the macromolecules, prevails in oligotrophic situations, while the former dominates in the summer in the eutrophicated Belgian coastal zone.

2.2.2.– Uptake of direct substrates.

Following the work of Wright and Hobie (1966), numerous measurements of the uptake kinetics of various substrates by intact natural communities of aquatic microorganisms have been determined. In most situations, the uptake was found to obey a Michaelis-Menten-Monod relationship :

$$(1) \qquad V = \sum_i v_i \; \frac{V_{max} \, S}{S + K_t}$$

where V is the total rate of uptake, v_i is the rate of uptake by population i in the microbial community, V_{max} is the maximum total rate of uptake, S is the substrate concentration and K_t is the half-saturation constant of uptake.

The validity of this approach when dealing with heterogeneous microbial communities has been discussed in detail by Williams (1973).

The validity of relation (1) indicates either that one single microbial strain dominates all the others in the utilization of the substrate, or that all the strain utilizing the substrate have a very similar value of K_t. Therefore, the K_t value obtained from this kind of measurement with natural communities characterizes the affinity toward the substrate of the dominant microbial populations.

When comparing a whole range of aquatic environments, a relationship is found between the K_t value of the microbial community and its natural rate of substrate utilization : the lower the flux of the substrate, the lower the K_t value (figs. 4 and 5) [Billen, in preparation]. As the rate of utilization of a substrate rather than its concentration is the best index of the "richness" of an environment in this substrate, this trend can be interpreted as reflecting the competition between microorganisms for their substrates : the lower the availability of the substrate, the higher the selective pressure for developing sophisticated and expensive permease systems with great affinity for this substrate.
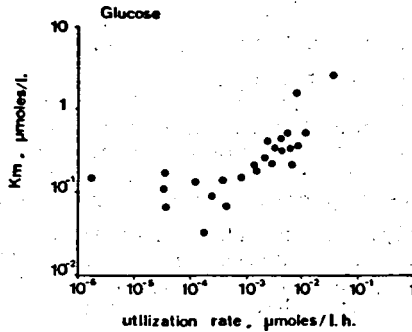
fig. 4.

Half-saturation constant for glucose uptake measured
in various aquatic environments, plotted against
the total flux of glucose utilization. (Data from
Japonese marine and brackish environments, and from
canadian lakes, Seki et al., 1975, 1980a,b).
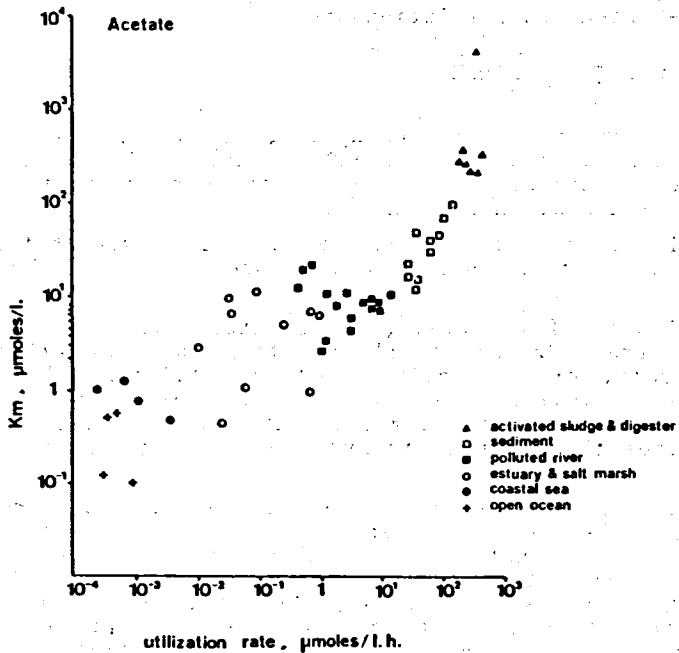
fig. 5.

Half-saturation constant for acetate uptake measured
in various aquatic environments, plotted against
the total flux of acetate utilization. (Data from
Seki et al., 1974, 1980; Strayer and Tiedje, 1977;
Stanley et Staley, 1977; Russel and Baldwin, 1979;
De Staercke, 1980; Billen et al., 1980; Billen,
unpublished).

Note that these high affinity permeases require substantial energy costs and result therefore in lower growth rates (fig. 6 and 7).
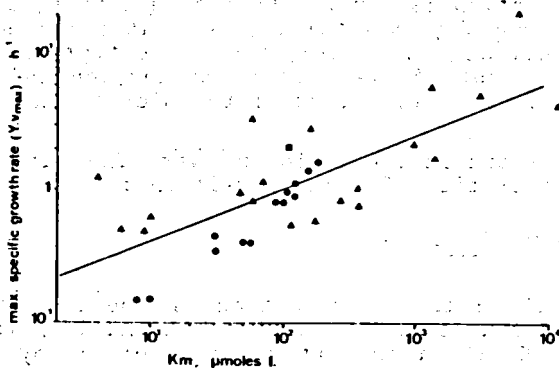
fig. 6.

Relation between the maximal specific rate of bacterial growth on a single substrate and their affinity for this substrate. (Data from Jannasch, 1968; Herbert et al., 1956; Russel and Baldwin, 1979).
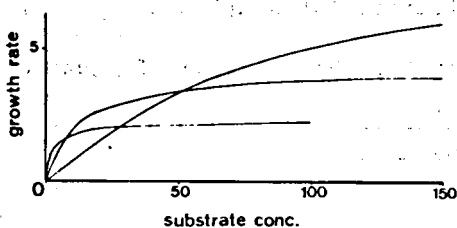
fig. 7.

Illustration of the various strategies during the competition of bacteria for a direct substrate

Again in the competition between microorganisms for direct substrates, r- and K-strategies are possible : the K-strategy consists in developing high affinity transport systems, at the expense of growth rate, while the r-strategy consists in maximizing the growth rate at the expense of affinity for the substrates. Figures 4 and 5 show that r-strategy prevails in rich environments.

The knowledge of the Km for direct substrates is important because, as we shall now see, its value controls the *in situ* concentration of the substrate.

The concentration of a particular substrate results from the balance between the production rate of this substrate (e.g. by phytoplanktonic excretion, exoenzymatic hydrolysis, etc.) and the rate of uptake by the dominant microorganisms populations.

If this population is limited by the substrate, and is able to grow fast enough for maintaining a stationary state between substrate production and uptake, the concentration of the substrate is independent of its rate of production and depends only on physiological characteristics of the microorganisms (Billen et al., 1980).

This can be shown in the following way. The rate of change of substrate concentration S can be written :

(2) $\dfrac{dS}{dt} = P - \dfrac{V_{max}\,S}{S + K_t}\,B$

where P is the rate of production of the substrate, B the mass of organisms utilizing S , V_{max} the maximum rate of uptake per organism, K_t the transport constant of S by the organism.

On the other hand, the rate of change of organisms biomass can be written :

(3) $\dfrac{dB}{dt} = \dfrac{Y\,V_{max}\,S}{S + K_t}\,B - k_d\,B$

where Y is the yield constant (i.e. the mass of organisms formed by unit substrate taken up) and k_d a first order mortality constant.

At stationary state, the solution of equations (2) and (3) is

(4) $S = \dfrac{K_t}{\dfrac{Y\,V_{max}}{k_d} - 1}$

(5) $B = \dfrac{Y}{k_d}\,P$

showing that at stationary state only the biomass of microorganisms is affected by the rate of production of the substrate. The concentration of the substrate depends only on the transport constant, and on the ratio between the maximum growth rate ($\mu_{max} = Y\,V_{max}$) and the death rate of organisms.

The question is of course to know how closely a stationary state is approached by natural aquatic systems. It has been shown by very simple simulations (Billen et al., 1980) that the time required for reaching a stationary state, or for restoring it after a sudden perturbation, is about $1/k_d$, i.e. is of the order of the generation time of the microorganisms at steady state. Thus bacteria, having smaller generation times, can be thought effectively to control the concentration of the direct substrates they use predominantly. On the contrary, algae, having longer generation times (and moreover, being limited by other factors

like light intensity) are not always able to maintain the concen-
tration of their direct substrates at a stationary state.

The validity of relation (4) for organic substrates predomi-
nantly used by bacteria can be tested with experimental concen-
tration and K_t data for glucose and acetate, reported in the
literature for various aquatic environments (fig. 8). Both sets
of data agree to relation (3), showing that a steady state is
not far from being reached, and that the control of substrate
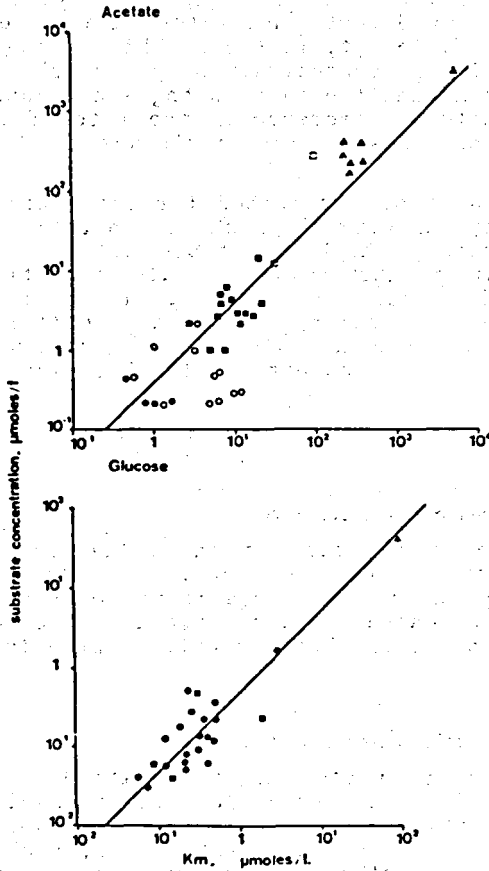
fig. 8.

Relation between the natural concentration of glucose and acetate
and their half-saturation constant of uptake in various aquatic
environments. (Data from Seki et al., 1974, 1980; Walher and
Monk, 1971; Russel and Baldwin, 1979; Stanley and Staley, 1977;
Kaspar and Wuhrman, 1978; De Staercke, 1980; Billen et al., 1980).

concentration by microorganisms uptake is effective. The relation
obtained is in both cases :

$$S \sim \frac{K_t}{3}$$

suggesting that in all the environments considered the ratio
V_{max}/k_d is about 4.

As can be seen in figures 4 and 5, which compare an extreme
range of environmental situations, Km values vary much less
from one environment to another than the rate of uptake of the
substrates. This explain why no significant variations exist in
the concentration of direct substrates between environments of
similar, although different, richness. This is the case, e.g. for
amino-acids and glucose concentration in the Scheldt estuary, the
Belgian coastal zone and the Eastern English Channel, in spite of
one order of magnitude difference in the rate of uptake of these
substrates between these three environments (Billen et al.,
1980).

This is the basis of our method for estimating heterotrophic
bacterial activity. Knowing the mean concentration of all classes
of direct substrates, which is stable in time and space, and
measuring accurately the relative rate of utilization which
varies a lot both in time and space, it is possible to calculate
by multiplication the total rate of organic matter utilization by
bacteria. This approach allowed us to estimate heterotrophic
bacterial activity independently of phytoplankton endogenous
respiration.

2.3.– *Degradation of organic matter in sediments.*

The importance of the benthos in carbon cycling is another
important characteristics of shallow coastal systems. The
factors affecting the flux of organic matter sedimenting and the
consequences of increased input of organic matter on the physico-
chemical conditions of the benthos will be briefly examined.

2.3.1.– *Flux of organic material to the sediment.*

From a compilation of sediment trap data from various sites,
Suess and Muller (1980) showed a clear relationship with depth of
the flux of sedimenting organic carbon expressed in percentage of
primary production. In shallow coastal seas, such as the Belgian
shelf, up to 50 % of net primary production is deposited on the
benthos. Faecal pelets and zooplankton corpses can only make up a
small fraction of this sedimentation flux. It is therefore likely
that phytoplanktonic cells and phytoplanktonic-derived detritus
constitute the bulk of the organic matter deposited on the
sediment. Direct confirmation of this has been obtained by
analysis of the vertical profile of chlorophyl pigments and
particulate nitrogen in sediment cores (Joiris et al., 1982).

The local distribution of the flux of sedimenting organic
material in the Belgian coastal zone, as revealed by the spatial
distribution of organic matter content of the uppermost layer
of the sediment, can be explained on basis of a hydrodynamical
model of the tidal circulation, by considering the role in the
sedimentation-erosion process of both the energy available for
the erosion of the sediment and the bottom stress indicating
whether or not suspended sediments are taken away by the flow
(Adam et al., 1981). Some places like the mud accumulation zone
in front of Zeebrugge (with low energy and low bottom stress)
appears to act as traps for organic material produced in the
whole coastal zone. The annual flux of organic carbon depo-
sition there has been estimated to 390 g C/m^2.year, while the
mean value for the Belgian coastal zone as a whole is only
160 g C/m^2.year and is 70 g C/m^2.year in the offshore zone.

2.3.2.– Redox state of the sediment as a result of organic matter degradation.

Microbiological organic matter degradation involves the
consumption of an equivalent amount of mineral oxidants, either
directly in the case of respiratory metabolisms, or indirectly in
the case of fermentative metabolisms, the reduced products of
which (organic acids, alcohols, H_2) have to be further oxidized
by respirative organisms. Oxidants susceptible to be used in
microbial metabolisms are oxygen, manganese oxides, nitrate
and nitrite, ferric oxides, sulfate and carbon dioxide. Orga-
nic matter degradation within the sedimentary column causes a
depletion of these oxidants X_i and an accumulation of the
corresponding reduced species Y_i.

The concept and measurement of redox potential in natural
environments have been discussed by several authors (Bass-Becking
et al., 1960; Stumm, 1966; Thorstenson, 1970; Billen, 1978b). It
has been stated that the concept of redox potential in natural
environments is meaningful owing to the fact that an internal
thermodynamic equilibrium is reasonably approached within the
subsystem formed by the main mineral redox species (X_i, Y_i)
involved in energy yielding metabolisms of microorganisms. The
redox potential Eh is defined with respect to this subsystem
only and does not take into account the presence of highly
reduced organic matter : it only characterizes the availability
of oxidants susceptible to use by microbial respirations. Direct
measurements of Eh in sediments with a platinum electrode must
be interpreted with caution, but can provide valuable relative
indications (Whitfield, 1969; Bågander and Niemislö, 1978).

Defined as above, the redox potential in sediments can be
viewed as the result of microbial metabolisms. Organotrophic
metabolisms generate a flux of electrons to the subsystem formed
by mineral redox couples, while chemolithotrophic metabolisms
tend to restore the internal thermodynamic equilibrium by oxi-
dizing reduced mineral species at the expense of oxidized ones,
when thermodynamically possible. Knowing the intensity and
distribution of organotrophic processes to which all oxidants and

their reduced forms are subject it is possible to calculate the
vertical profiles of all oxidants and of redox potential. This is
the principle of a general idealized, redox model of marine
sediments proposed by Billen and Verbeustel (1980). Their model,
however, was based on complete internal thermodynamic equili-
brium, including nitrogen species, which is rather unrealistic.
Moreover, only one mixing coefficient for both solid and dissol-
ved species was considered. An improved version of this redox
model, based on data collected in the sandy sediments of the
North Sea, has been presented by Billen (1982). It considers the
following equilibria :

$$O_2 + 4 e^- + 4 H^+ \rightleftharpoons 2 H_2O$$

$$MnO_2 + 2 e^- + 4 H^+ \rightleftharpoons Mn^{++} + 2 H_2O$$

$$Mn^{++} + HCO_3^- \rightleftharpoons MnCO_3 + H^+$$

$$Fe(OH)_3 + e^- + 3 H^+ \rightleftharpoons Fe^{++} + 3 H_2O$$

$$Fe^{++} + HCO_3^- \rightleftharpoons FeCO_3 + H^+$$

$$SO_4^{--} + 8 e^- + 9 H^+ \rightleftharpoons HS^{--} + 4 H_2O$$

$$HS^- + FeCO_3 \rightleftharpoons FeS + HCO_3^-$$

$$HCO_3^- + 8 e^- + 9 H^+ \rightleftharpoons CH_4 + 3 H_2O$$
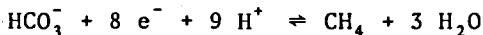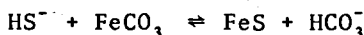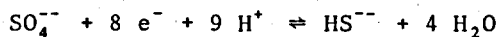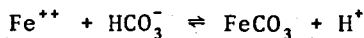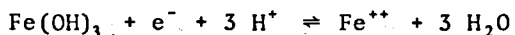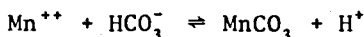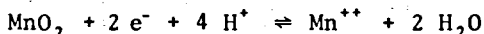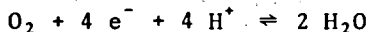
Nitrate is not considered to be at equilibrium with respect to
the other redox couples. It is assumed to be produced from
ammonium through nitrification above a critical value of redox
potential (Billen, 1975) and to be reduced into dinitrogen
through denitrification below this potential. Organic material is
assumed to be degraded according to first order (one G) kinetics
and the resulting organotrophic activity causes an electron flux
which is absorbed by the various oxidants according to the
reactions listed above. In this model, the flux of depositing
organic matter is taken as the independent variable, so that it
is possible to relate the input of organic material in the
sediment to its redox state.

The redox profiles, theoretically predicted by this model for
two different values of the input of fresh organic matter to
marine sediments, are shown in figure 9. Figure 9a corresponds to
the situation termed "suboxic diagenesis" by Froelich et al.
(1979), in which the organic matter input to the sediment is low
enough with respect to the diffusion flux of oxidants that only
oxygen consumption, manganese-reduction, denitrification and
ferrireduction are involved in organic matter degradation. This
is the case in most sandy sediments of the Southern Bight of
the North Sea, which does not receive important amounts of
organic matter. Figure 8b, on the other hand, shows a situation
of "anoxic diagenesis", where oxygen, manganese oxide, nitrate
and ferric oxides are rapidly exhausted and sulfate reduction
dominates organotrophic activity. This is the case of the mud
accumulation zone.
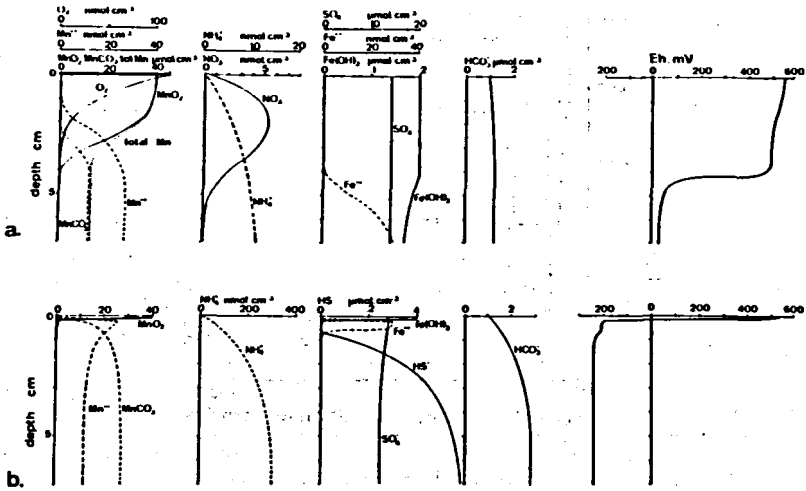
fig. 9.

Calculated vertical profiles of mineral redox species in sediments
according to thermodynamic equilibrium model (Billen, in press) for two
different values of the flux of organic matter depositing on the
sediment surface (Di = 10^{-4} cm^2s^{-1}; Ds = 10^{-7} cm^2s^{-1}; k = 3 10^{-8} s^{-1}).
a. "Sub-oxic diagenesis" : case of a marine sediment with a flux of
organic matter of 8 10^{-9} mmoles $cm^{-2}s^{-1}$ (30 g C/cm^2.year) [overlying
water containing 230 mM oxygen, 0 mM nitrate, 28 mM sulfate and 2 mM
bicarbonate; upper sediment containing 40 μmole/cm^3 manganese and
200 μmole/cm^3 reactive iron - porosity : 0.5].
b. "Anoxic diagenesis" : case of a marine sediment with a flux of
organic matter of 10^{-7} mmoles $cm^{-2}s^{-1}$ (378 g C/m^2.year) [some overlying
water and sediment composition].

    The vertical profiles calculated by this equilibrium model
are of course idealized. Moreover, they depend strongly on
the values chosen for the various parameters (D_i, D_s, k). Ne-
vertheless, they display the same general trends as numerous
experimental observations.

    Pearson and Stanley (1979) have recently used the measurement
of redox potential in the sediments of a sea loch as a means of
assessing the effect of organic pollution by a paper mill. They
experimentally related the Eh reached in depth in the sedi-
mentary column to the input of organic material to the sediments.
Such a relation can be theoretically deduced from the redox model
discussed above, as shown in figure 10 for a set of value of the
mixing coefficients D_i and D_s.

    These curves show the "buffering capacity" of the various
redox couples present in sediment towards "titration" by depo-
siting organic matter. For oxidants present in the solid phase
this buffering capacity is closely dependent on the value of D_s,
both because the mixing coefficients directly determines the
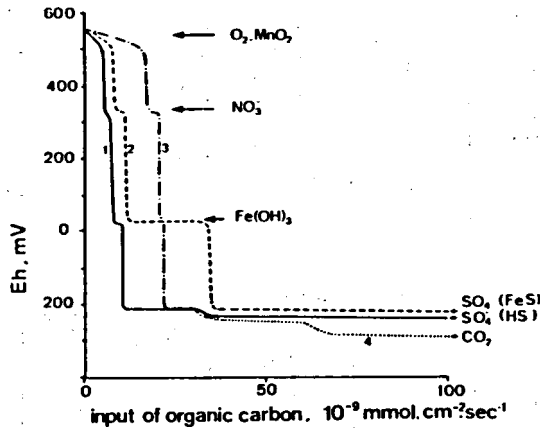
fig. 10.

Calculated relationship between the minimum redox
potential reached in depth in sediments and the
input of organic carbon depositing, according to a
thermodynamic equilibrium model, for different
values of the mixing coefficients.
1. $Di = 10^{-4}$ $cm^2 s^{-1}$, $Ds = 10^{-7}$ $cm^2 s^{-1}$, sea water
2. $Di = 10^{-4}$ $cm^2 s^{-1}$, $Ds = 5 \ 10^{-6}$ $cm^2 s^{-1}$, sea water
3. $Di = 10^{-4}$ $cm^2 s^{-1}$, $Ds = 10^{-8}$ $cm^2 s^{-1}$, sea water
4. $Di = 10^{-4}$ $cm^2 s^{-1}$, $Ds = 10^{-7}$ $cm^2 s^{-1}$, fresh water

"availability" of these oxidants within the sediment and because
it determines the penetration of organic material down into the
sediment and thus the depth distribution of organotrophic acti-
vity, for a given flux of depositing organic matter.

3.— Role of microorganisms in nitrogen cycling.

Following the introduction of the concept of the "Redfield
molecule", it has often been assumed that organic matter from
phytoplanktonic origin has a constant composition so that both
the uptake of nutrients by phytoplankton and the release of
nutrient upon organic matter mineralization obey a very simple
and constant stoechiometry. In the scope of this paradigm,
nitrogen cycling could be deduced from carbon measurements, if
the adequate C/N ratio is known.

The paper by Lancelot in this volume, has shown that the
composition of primary produced organic material is not constant
at all and widely varies according to nutrient concentrations in
the surrounding medium.

We will show that the relative amount of nitrogen released as a result of organic matter mineralization by planktonic and benthic bacteria also depends on environmental factors which can lead to important lack of parallelisms between carbon and nitrogen cycling, particularly in eutrophicated environments.

3.1.— *Ammonification*.

According to their biosynthetic or energetic fate in microbial metabolism, organic nitrogen compounds can be either incorporated into biomass or excreted as ammonia. In classical conceptions the latter process is thought to be the most important, and micro-organisms are viewed as direct mineralizers of organic matter. Some authors however (Rittenberg, 1963; Johannes, 1968) have stressed the possible importance of the former process, claiming that the bacteria account directly for only a minor fraction of nutrient regeneration or even compete with algae for mineral nitrogen. This question of organic nitrogen immobilization versus mineralization can now be reexamined in the light of recent physiological and ecological data.

The balance between N-mineralization and immobilization depends on the ratio carbon/nitrogen $(C/N)_S$ of the total organic matter utilized. The amount of ammonia released ΔNH_4 per unit carbon taken up ΔC is given by the following relation :

$$(6) \qquad \frac{\Delta NH_4}{\Delta C} = \frac{1}{(C/N)_S} - \frac{Y}{(C/N)_B}$$

where $(C/N)_B$ is the carbon/nitrogen ratio of bacterial biomass and Y the growth yield ratio.

The experiments of Hollibaugh (1978), Somville (1980) and Billen (unpublished), who supplemented natural sea water with mixtures of organic substrates and followed the consumption of the substrates and the release of ammonia, permit to test the validity of relation (6) [fig. 11]. Although the data come from two differnt environments and were obtained with different organic substrates, a very good fit is obtained with

$$\frac{Y}{(C/N)_B} = 0.1 \text{ g N/g C}.$$

If 4 g C/g N is taken as a reasonable estimate for $(C/N)_B$, Y can be evaluated as 0.4 in good agreement with the values cited in the literature.

Relation (6) and figure 11 also show the lack of parallelism between the role of bacteria in carbon nitrogen cycling. When the C/N ratio of the organic matter used by bacteria increases, ammonium release decreases and, for $(C/N)_S$ higher than 10 g C/g N, uptake instead of release can even occur during
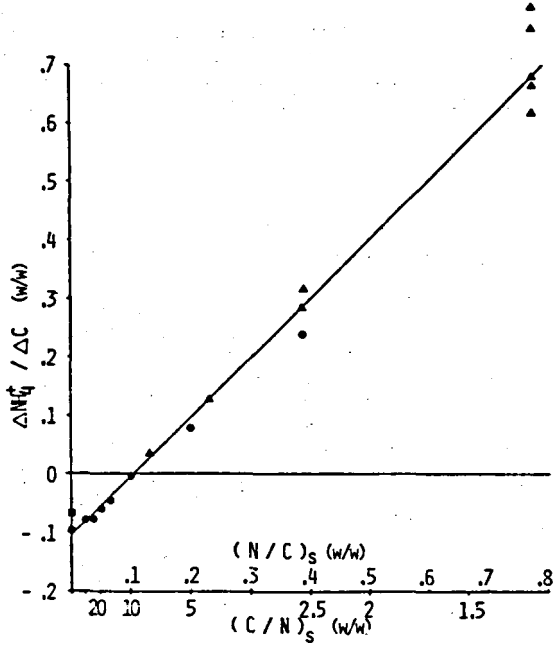
fig. 11.

Release or uptake of ammonia per unit carbon taken
up by microbiological communities of marine en-
vironments, supplemented with mixed substrates of
various C/N ratio. [Data from (▲) Hollibaugh,
1978; (■) Somville, 1980; (●) Billen, unpublished]

organic matter degradation. The role of bacteria as nitrogen mi-
neralizers thus not necessarily parallels their role as carbon
mineralizers. A striking example is provided by data obtained by
Joiris et al. (1982) and Billen (unpublished) in the Belgian
coastal zone, the English Channel and the Scheldt estuary, where
total heterotrophic activity has been estimated by measuring over
a whole year cycle the concentration and the relative rate of
bacterial utilization of the three main classes of direct organic
substrates (free amino acids, monosaccharides and glycollate)
[Table 2]. From these data, ammonium remineralization can be
calculated according to relation (6). As seen, the most important
ammonium release occurs in the Western Channel, where hetero-
trophic carbon utilization is the lowest. In the Belgian coastal
zone, the relative importance, as substrates for heterotrophic
activity, of carbohydrates, part of which coming from the muco-
polysaccharides excreted by *Phaeocystis poucheti* (Lancelot,
1982), severely limits ammonium regeneration by bacteria. In the

Table 2

Annual means of rates of organic substrates uptake
and calculated rates of ammonium release
in three marine environments

	Eastern Channel (off Boulogne)	Belgian Coastal zone (off Ostend)	Scheldt estuary (Hansweert)
Heterotrophic activity (mg C/ℓ.y)			
Amino acids uptake	3.1	2.4	6.5
Monosaccharides uptake	2.6	4.4	16.3
Glycollate uptake	0.3	1.2	15
Total	6	8	37.8
C/N of organic matter taken up	6	10	17
NH$_4$ release (mg C/ℓ.y)	0.4	0.08	− 0.115

heavily polluted Scheldt estuary, net ammonium uptake occurs, due
to the high ratio of the terrigenous organic material being
degraded.

3.2.— Nitrogen cycling in sediments.

Another cause of non parallelism between carbon and nitrogen
behaviour during organic matter mineralization results from the
occurrence of microbial transformations of nitrogen after the
stage of ammonification. This primarily concerns benthic mine-
ralization.

In the oxidized upper layer of the sediments, ammonium is
generally actively oxidized to nitrate by nitrifying bacteria.
Direct measurements in the sediments of the North Sea have shown
that nitrification rates are closely correlated with ammonifi-
cation rate and amount to 80 % of it (Billen, 1982). The depth
of the layer where nitrification is possible is therefore a major
factor in determining under which form (ammonium or nitrate)
nitrogen is released to the water column. Moreover, nitrates
formed in this oxidized layer can diffuse into the reduced layer
and be reduced into dinitrogen which is far less accessible for
primary production. The extend of this loss of nitrogen is also
determined, in a complex way, by the depth of the oxidized layer.

A general, although idealized, model of nitrogen recycling in
sediments based on data collected in the sandy sediments of the
North Sea has been presented by Billen (1982). It relates the
flux of organic material deposited on the sediments to the
release of ammonium- and nitrate-nitrogen to the overlying water.
The results of this model are shown in figure 12 for the same
values of the mixing coefficients as those used in the redox
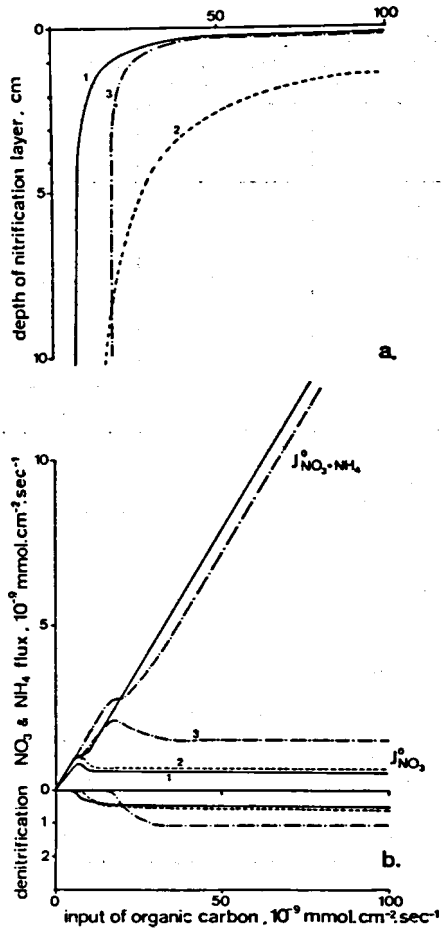
fig. 12.

Model of nitrogen recycling in marine sediments as a function of the input of organic material, for diffe-rent values of the mixing coefficients.

      1. $Di = 10^{-4}$ $cm^2 s^{-1}$, $Ds = 10^{-7}$ $cm^2 s^{-1}$
      2. $Di = 10^{-4}$ $cm^2 s^{-1}$, $Ds = 5 \cdot 10^{-6}$ $cm^2 s^{-1}$
      3. $Di = 10^{-4}$ $cm^2 s^{-1}$, $Ds = 10^{-8}$ $cm^2 s^{-1}$
              C/N ratio = β = 6

a. Depth of the nitrification layer.
b. Fluxes of nitrate and total mineral nitrogen across the water sediment interface and integrated rate of denitrification.

model of figure 9. It is seen that at low input of organic
material to the sediments (which can be considered as a "low"
flux of organic material depends on the mixing conditions pre-
vailing in the sediment) most of the nitrogen recycling occurs as
nitrate. With increasing input of organic matter nitrifying
activity is restricted to a shallower and shallower upper layer
(fig. 12a), vertically integrated nitrification reaches a maximum
and ammonium release becomes more important. The last process
prevails at high organic input (fig. 12b). Denitrification, being
dependent on nitrates formed in the nitrification layer, reaches
a plateau above a certain input of organic material. Paradoxi-
cally it implies that the relative value of denitrification in
the overall nitrogen cycle is maximum at an intermediate input of
depositing material and decrease at higher inputs.

   Because denitrification mostly results in producing of flux
of N_2 to the water column (although, some N_2O and NH_4^+ can
also be produced, it can be considered as causing a net loss of
nitrogen from the ecosystem. In the absence of nitrate in the
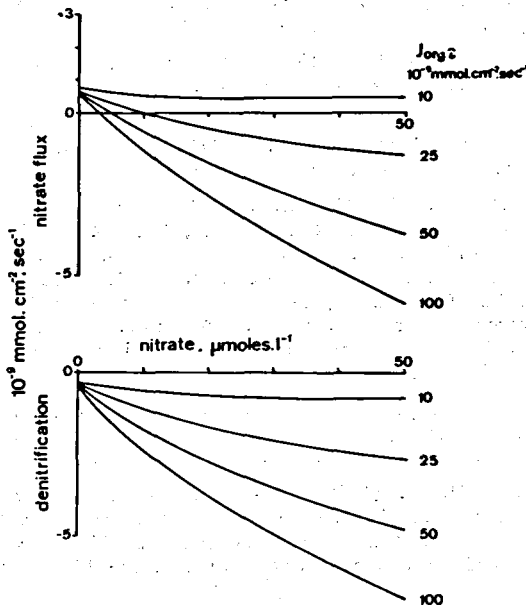
fig. 13.

Effect of nitrate concentration in the overlying water
on the flux of nitrate across the sediment-water inter-
face and on the rate of denitrification, calculated
with the model described for the same values of the
parameters as in fig. 6, and for various values of the
flux of depositing organic material (J_{org} C), indicated
in 10^{-9} mmoles $cm^{-2}s^{-1}$.

overlying water, this loss is not expected to concern more than
about 30 % of the flux of nitrogen remineralized. Only when
high nitrate concentrations exist in the overlying water, more
important denitrification rates can occur, with the sediment
acting as a sink for nitrates from the water column (fig. 13).
This effect of high nitrate concentration in the overlying
water on the rate of denitrification in the sediment is much
pronounced, however, at high organic content of the sediment
(i.e. at high organic flux to the sediment) than for organic poor
sediments (fig. 13).

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DETERMINATION OF MARINE PHYTOPLANKTONIC BIOMASS

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Abstract.

    Comparison between the classical spectrophotometric determinations of
chlorophyll a with a fluorometric analysis preceded by high-pressure liquid
chromatography, shows that interferences make the classical methods inaccu-
rate. In extreme cases the latter induce an overestimation of the concen-
tration up to ·30 % .

    As the pigments are to be extracted, the extraction efficiencies of
three solvents are compared. It appears that 95 % methanol is superior com-
paring to DMSO and to 90 % acetone. Moreover, the efficiency improves by
using a grinder.

1.— Introduction.

    The phytoplanktonic biomass is an improtant parameter in se-
veral studies concerning natural waters (e.g. the fertility of
the sea, primary production, nutrient uptake kinetics of phyto-
plankton, ...). As chlorophyll a is the most important pigment
in photosynthesis and as it is present in every non-bacterial,
photosynthetic organism, in oceanology and limnology the concen-
tration of this product was obviously chosen as a measure for
the phytoplanktonic biomass.

    For the determination of chlorophylls, several methods are
developed throughout the years. The most widely used are the
spectrophotometric methods of Parsons and Strickland (1963),
with the trichromatic equations for each kind of chlorophyll (a,
b, c); and the monochromatic equations of Moss (1967) and Loren-
zen (1967), for the total concentrations of chlorophyll a and
pheopigments. Both methods are based on the specific absorption
coefficients of the chloropigments in 90 % acetone, which is
used as the extraction solvent.

    Also fluorometry is used for determinations of chlorophyll a
in extracts (Yentsch and Menzel, 1963; Holm-Hansen et al., 1965),
as well as for continuous measurements in vivo (Lorenzen, 1966;
Strickland, 1968). With the last method, one measures the con-
centration of chlorophyll a that is not incorporated in the
photosynthetic activity, what has been proved by the addition
of DCMU (Slovacek and Hannan, 1977). This is very useful for
direct measurement in order to become a notion of the ecosystem

(Ballester and Plana, 1973). The method is not very sensitive
and suffers from the disadvantage that not every species has
the same amount of fluorescent chlorophyll a in its photosys-
tem II (Tolstoy, 1977a).

During the last fifteen years, many investigators studied,
criticized and tried to improve these methods.

1.1.— *Extraction of phytoplanktonic pigments.*

Most of the searchers on this subject agree that digestion,
in an ice-box, with 90 % acetone, as proposed by Strickland
and Parsons (1968) is not sufficient to ensure complete extrac-
tion. The extraction efficiency is inversely proportional to
the ratio dry weight/weight chlorophyll a, by which the popu-
lation of algae is characterized (Subba Rao and Platt, 1969).

In order to maintain the same spectroscopic determinations,
which are only valid for measurements with 90 % acetone, peo-
ple have tried other extraction methods as overnight shaking,
grinding and disruption in an ultrasonic bath (Daley *et al.*,
1973a). Some confusion exists, however, as to which method is
preferable. Garside and Riley (1969) found no denaturation pro-
ducts when using an ultrasonic disruptor, but Yentsch and Menzel
(1963) obtained the opposite results. To ensure complete extrac-
tion, some workers propose to combine digestion and grinding, or
even to extract at room temperature (Tolstoy, 1977b).

Because of these contradictory results, and the fact that
90 % acetone is not efficient enough as extraction solvent, a
lot of time has already been spent in finding other solvents.
Marker (1972) found that 95 % methanol gave better results.
For the measurements, however, it is preferable to transport the
pigments into 90 % acetone because the absorption spectrum of
pheofytine a is pH-dependent in methanol. Also pure methanol has
been tested for the extraction (Holm-Hansen and Rieman, 1978;
Rieman, 1980) but it has to be saturated with antioxidants, such
as H_2S, to prevent the formation of oxidation products as chlo-
rophyll a' (Pennington *et al.*, 1967; Katz *et al.*, 1968). But even me-
thanol cannot always ensure complete extraction; in this case,
pre-freezing is suggested (Marker, 1980). Recently, ethanol is
proposed as solvent for this work (Nush, 1980); it has the great
advantage of being safer to handle.

A second type of solvent that was tested to extract more
quantitatively is dimethylsulfoxide (DMSO). It was used with
grinding (Shoaf and Lium, 1976; 1977), and more recently with
an incubation technique (Hiscox and Isrealstam, 1978; Burnison,
1980). Both methods gave better results than extraction with
acetone but no comparison has been made yet. An important advan-
tage of the use of DMSO as extraction agent, is that when the
pigment solution is diluted afterwards with 90 % acetone in

a 1:1 (v/v) ratio, the absorption spectrum of the chloropig-
ments stays virtually the same as in pure 90 % acetone. So the
same, classical, determination formulas can be used.

1.2.— Determination of chloropigments after extraction.

The classical spectroscopic equations suffer from many er-
rors. The principal problem is the great overlapping between
the absorption bands of the different chloropigments and their
degradation products present in the extract. Evidently the pos-
tulations of Lambert-Beer's law are not fulfilled in this case.

Comparison of direct trichromatic spectrometry and spectro-
metry preceded by chromatographic separation on thin-layer plates
shows that chlorophyll b and c are overestimated with the clas-
sical method (Rott, 1980). Abaychi and Riley (1979) even calcu-
lated negative chlorophyll c concentrations. Also the chloro-
phyll a concentration will be systematically overestimated when
the pheofytine/chlorophyll ratio increases (Rai, 1980).

Because of all these difficulties with the trichromatic
equations, many workers prefer the use of the monochromatic me-
thod, certainly when other extraction solvents than 90 % ace-
tone are used (Marker, 1972; Hussainy, 1973; Rai, 1980; Nusch,
1980). But even then the major problem of interferences remains
the same. Moreover, one has to deal with carotenoid interferences
in the spectrophotometric determination of pheofytine because of
spectral changes after acidification especially with fucoxanthine
(Riemann, 1978). Therefore the Moss and Lorenzen equations do not
give reliable results for the determination of pheopigments (Tol-
stoy, 1977a).

Using these spectrophotometric methods, incorrect values are
also obtained when photosynthetic bacteria occur in the samples,
due to the fact that *Chlorobium* chlorophyll shows an absorption
maximum near 750 nm (Tolstoy and Toth, 1980).

Analogous the fluorescence of accessory pigments and degra-
dation products influences the fluorometric determination of
chlorophyll a (Rai, 1980). To overcome these problems, Boto and
Bunt (1978) and Bazzaz and Rebeiz (1979) proposed multiwavelength
analyses. Their propositions demand however a whole series of mea-
surements, and very complicated calculations.

As conclusion, we can say that any routine spectrophotometric
or fluorometric method is not capable to differentiate the various
pigments and degradation products present in an extract. There-
fore, it is appropriate to perform a chromatographic separation
followed by a more precise, and interference-free, determination
of the different pigments and their concentrations.

1.3.— *Chromatographic separations of plantpigments.*

Because of the extreme sensitivity of chlorophylls to heat, it is only possible to use liquid chromatography at ambient temperature. In case of the determination of the phytoplanktonic biomass, both thin-layer (TLC) and high-pressure liquid chromatography is less appropriate because of the minor quantities to be handled.

Although good separations have been achieved with TLC (Garside and Riley, 1969; Daley *et al.*, 1973b; Jeffrey, 1981), the latest technique of HPLC has several advantages : the ease of operation, the sensitivity, the accuracy and the possibility of automatisation. Most important is the fact that the estimation of concentration follows immediately after the separation, which limits the number of handlings. The use of HPLC for the separation of natural porphyrins started with the work of Evans *et al.* (1975). In this field, two types of columns can be used : normal phase (a polar packing) or reversed phase (a non-polar packing). Although results have been published with the first type of column (Evans *et al.*, 1975; Iriyama *et al.*, 1978; Stransky, 1978; Abaychi and Riley, 1979; Rebeiz *et al.*, 1980), recently reversed phase columns are preferred for this type of molecules. A great number of scientists worked out many different elution systems (Eskins *et al.*, 1977; Rebeiz *et al.*, 1978; Shoaf, 1978; Prenzel and Lichtenthaler, 1979; Braumann and Grimme, 1979; Eskins and Harris, 1980; Brown *et al.*, 1981; Scholz and Ballschmiter, 1981; Falkowski and Sucher, 1981; W.W.C. Gieskes, personal communication). With the aim to estimate chlorophyll a concentration, we developed an isocratic elution method in combination with fluorometric detection (Goeyens *et al.*, 1982).

The results of our system are compared with the classical spectroscopic methods for the estimation of chlorophyll a in marine phytoplankton extracts. At the same time, we compared two types of extraction methods, digestion and grinding, and three frequently used extraction solvents : 90 % acetone, 95 % methanol and DMSO.

2.— Methods and materials.

2.1.— *High-pressure liquid chromatography.*

2.1.1.— *Apparatus.*

Solvent was delivered by a DuPont three pistonpump, model 870, to a semipreparative Altex RP-18 column (25 cm × 1.0 I.D.) industrially packed with 5 μm C_{18} material. Because of its greater sensitivity, detection was done with a fluorometer Gilson-Spectra/GLO [exitation filter : 5-60 X (420-460 nm), emission filter : 2-60 M (630-680 nm) and a flow-cell of 15 μℓ], which was connected with

a reporting integrator Helwett-Packard 3390 A. Runs were made at
37°C with a solvent rate of 3 mℓ/min and a three solvent mix-
ture of methanol, acetone and water in the ratio 75/22/3 (v/v).
Pump-pressure was nearly constant at 96 bars. The sample was in-
troduced to the column by mean of an automatic injector, Microme-
ritics model 725, fitted with a 50 µℓ sampling loop.

2.1.2.– *Calibration of the detector.*

This was done by three successive injections of a chlorophyll
a standard solution. The solution was prepared by dissolving 1 mg
dry chrorophyll *a* , purchased from Sigma Chem., in .1 000 mℓ di-
ethylether, spectroscopic grade. The exact concentration was esti-
mated spectrophotometrically by using our extinction coefficients
(Goeyens *et al.*, 1982). Then the ether was evaporated by gently
blowing nitrogen over the surface, the residue was redissolved in
the same volume of mobile phase.

2.2.– *Spectrophotometry.*

Readings of the absorbances were done on a Pye-Unicam, model
SP8-100 UV , with cells of 1 cm pathlength (Hellma, type 115 0S).
The bandwidth was set on 0.5 nm .

In using the equations of Lorenzen, three drops of a 50 %
chloric acid (analytical grade) solution were added to the sample
in the cuvette. The absorption was measured after the sample had
stand in the dark for 15 min .

2.3.– *Solvents.*

All solvents used were chromatographically pure, except the
DMSO and diethylether which were spectroscopic grade, and pur-
chased from Merck AG. The chromatographic solvents were degassed
and filtered on glass-fiber filters (Whatmann GF/C). The water
we used was suprapure demineralized (Millipore-Milli Q).

2.4.– *Sea-water sample.*

It was taken at the Belgian seashore at the end of August,
and brought immediately to the lab. Five liters ot it were poored
into a stirred vessel. Nutrients (N , P and Si) were added in an

overdose and irradiation was kept on for 24 hours. After one
day the sample was used for analysis.

A microscopic study revealed that the algae population con-
sisted mostly of young diatomeae (*Asterionella japonica, Rhizosolenia
stolterfothii, Biddulphia mobiliensis* and *Skeletonema costatum*); also some
dead species were present (J.P. Mommaerts, personal communication).

2.5.— *Extraction of the phytoplankton.*

Extractions were carried out in the dark. Each time 50 mℓ
of the sample were filtered on glass-fiber filters (Whatmann
GF/C). The filters were cut into pieces and brought in a gradu-
ated centrifuge tube.

Further work was done as follows :

2.5.1.— *Extraction with 90% acetone.*

- (Strickland and Parsons, 1968) 10 mℓ of acetone were added
  and shaken vigorously. Digestion was done during 22 hours in
  an ice-box (4°C). After the sample had come to room temperature
  in the dark, it was centrifuged for 10 min at 3 000 g .
- (SCOR/UNESCO, 1966) 3 mℓ of acetone were added to the filter
  and during 1 min homogenized with a grinder (Ilado, model
  10/20). The instrument was washed with two aliquots of 3 mℓ
  and the total volume was brought up to 10 mℓ . Centrifugation
  was done after the sample had stand in the dark for 10 min .
- The acetone extracts were injected immediately into the HPLC,
  because this solvent gives no interferences with the mobile
  phase.

2.5.2.— *Extraction with 95% methanol* (Marker, 1972).

Both the same extraction methods as with 90 % acetone were
used. For the spectroscopic analyses the pigments were transfer-
red into 90 % acetone after drying with a rotavapor at a tempe-
rature of 20°C . For chromatographic analyses the methanol ex-
tract was used immediately.

2.5.3.— *Extraction with DMSO.*

- (Shoaf and Lium, 1976) 4 mℓ of DMSO were added to the filter
  and grinding was carried out for 3 min . The grinder was washed
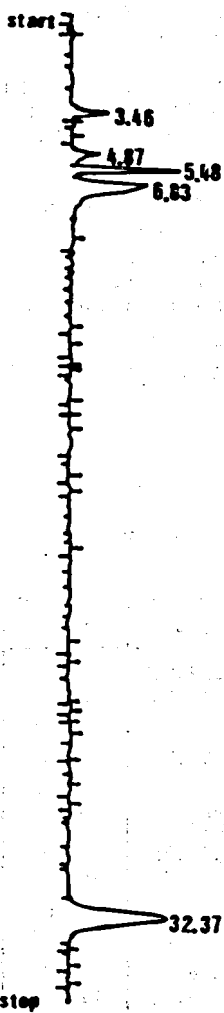
```
ZERO   = 10,-01
ATT 2↑ = 4
CHT SP = 0.7
PK WD  = 0.16
THRSH  = 4
AR REJ = 0
```

```
ESTD
RT              AREA        TYPE      CAL      AMOUNT
32.37         1564200       PV        1R       1.025


TOTAL AREA = 1564200
MUL FACTOR = 1.0000E+00
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AREA %
RT              AREA        TYPE      AR/HT     AREA %
3.46          165030        PB        0.171      5.111
4.87          195770        PV        0.260      6.063
5.48          425330        VV        0.144     13.171
6.03          878810        VV        0.439     27.215
32.37        1564200        PV        0.592     48.441


TOTAL AREA = 3229200
MUL FACTOR = 1.0000E+00
```

fig. 1.

Chromatographic profile of a methanol extract, with grinding
Sample size : 50 μℓ; flow-rate : 3 mℓ/min
Solventcomposition : methanol/acetone/water (75:22:3, v/v)

with three aliquots of 1 mℓ   90 %   acetone and the final volume
was brought up to   8 mℓ . The sample was shaken vigorously for
10 s   and centrifuged for   10 min .
- (Burnison, 1980)   4 mℓ   of DMSO were added to the filter and the
  closed tube was incubated in a waterbath at   55°C   for   10 min .
  The total volume was brought up to   8 mℓ   with   90 %   acetone,
  shaken vigorously and centrifuged for   10 min .
- For chromatography the volume was doubled with diethylether
  and shaken vigorously for   10 s , then   2 mℓ   of water were
  added dropwise. The sample was shaken again and then centri-
  fuged. Afterwards   1 mℓ   of the etherlayer, which contains
  the pigments, was pipetted off, evaporated untill dryness,
  by gently blowing nitrogen over the surface, and redissolved
  in an equal volume of mobile phase.

    For chromatogram of the phytoplankton extract and the results
of the experiments, see figure 1 and table 1.

Table 1

Comparison of the different extraction and determination methods
(concentration on mg/m^3)

Extraction method	Sample	S-P	S/U	M-L	HPLC
DMSO mechanical grinding	I	261.82	258.34	252.05	192.16
	II	249.60	248.06	216.04	183.19
DMSO incubation at 65 °C	I	210.33	210.02	196.51	141.92
	II	207.32	207.00	193.38	139.89
90 % acetone digestion	I	193.51	192.65	182.90	124.30
	II	196.32	195.47	201.59	126.10
90 % acetone mechanical grinding	I	233.34	232.56	226.95	189.00
	II	208.21	208.61	176.22	161.50
95 % methanol digestion	I	225.48	226.41	238.30	181.00
	II	222.24	223.15	216.20	178.40
95 % methanol mechanical grinding	I	271.34	272.24	256.32	205.00
	II	284.63	283.52	299.04	224.60

P-S : Parsons and Strickland (1963).
S/U : SCOR/UNESCO (1966).
M-L : Moss (1967) and Lorenzen (1967).
HPLC : High-pressure liquid chromatography.

3.— Results and discussion.

3.1.— Sample concentration.

The order of magnitude that was determined for the sample exceeds the main chlorophyll a concentration found in the North Sea.

This might be explained by the fact that the sea-water was sampled near the beach where at that time of the year high concentrations can occur (J.P. Mommaerts, personal communication). Moreover, it was grown in very favorable light and nutrient conditions and the microscopic study revealed the overall presence of young cells.

This however may be no point of doubt in view of the sensitivity of the method. We determined the detection limit of our detector to be 84 pg chlorophyll a (or ·16.8 $\mu g/m^3$, if 1 ℓ of seawater is filtered and extraction is done with 10 mℓ of extraction solvent) (Goeyens et al., 1982).

3.2.— Difference of extraction efficiency.

The results show clearly that simple digestion cannot compete with mechanical grinding. The last method gives up to 16 % more efficiency. Comparing the results at the three different solvents, it seems again that 90 % acetone is not capable to ensure complete extraction. The best figures are produced with 95 % methanol as extraction solvent (up to 18 % more compairing to 90 % acetone and 13 % more compairing to DMSO).

In using methanol as the extraction solvent, attention has to be paid to the exact concentration of water. This should not exceed 10 % otherwise extraction efficiency is reduced and the possibility of enzymatic oxidation or hydrolysis enhanced (Daley et al., 1973a). Of course, the extractions should be carried in the dark (Moreth and Yentsch, 1970).

3.3.— Comparison of detection methods.

A remarkable difference appears between chlorophyll a concentration determined by the direct spectrophotometric methods and the fluorometric determination preceded by chromatographic separation on HPLC. The latter always provides lower values, up to a maximum difference of 36 % . This can be explained by the earlier described interferences of all the pigments and their degradation products, the more the presence of dead algae was shown by the microscopic analysis.

4.— Conclusions.

Evidently interferences cannot be totally excluded by the classical spectrophotometric methods.

For more reliable results of the concentration of pigments in phytoplankton extracts chromatographic separations are necessary. The HPLC-technique is most suitable for interference free determination because of the great accuracy, the ease of operation and automatisation.

On the other hand, it is also possible to measure more pigments in a single run (chlorophyll b, c, their degradation products and carotenoids). Pheophytins, pheophorbides and chlorophyllides can offer information about the evolution of a phytoplankton crop.

The presence and quantity of accessory pigments, especially the different carotenoids, could be used as chemotaxonomic markers (Weber and Wettern, 1980) to get an idea of the different species present in the population.

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NUTRIENT UPTAKE BY MARINE PHYTOPLANKTON

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Abstract.

   The inorganic nitrogen assimilation by marine phytoplankton, in nitrate limiting medium, was determined by a dynamic method. The study consisted of laboratory experiments using natural phytoplankton communities from the North Sea.

   The chlorophyll *a* concentration was examined at the start and at the end of every experiment as an indicator of the phytoplanktonic biomass. During the experiment repeated sequential determinations of the nitrate concentration were performed in order to obtain the relation between nutrient uptake and the ambient nutrient concentration.

1.– Introduction.

   The assesment of nutrient uptake kinetics of phytoplankton populations in their natural environment is an important matter for the comprehension and the adequate modelisation of the ecosystem dynamics.

   Dugdale (1967) proposed a theoretical frame-work for the study of the relationship between nutrient uptake and the ambient concentration in the sea. Central to the theory is the Michaelis-Menten expression for enzyme kinetics, which seems valid in describing the nutrient controlled uptake for a great variety of species.

   Uptake experiments with unialgal cultures strongly support this Michaelis-Menten hyperbolic relation ship between uptake and phosphate concentration (Burmaster, 1979; Burmaster et al., 1979). Analogically, the silicic acid uptake by natural phyto-plankton populations has been described as a hyperbolic function of the concentration also (Goering et al., 1973).

   However for the Si uptake by diatoms, it was found that the plot of Si uptake against Si concentration does not start from zero Si concentration (Paasche, 1973).

   Also for the uptake of inorganic nitrogen there exists abundant evidence indicating that Michaelis-Menten kinetics are at least descriptively adequate in relating the uptake rate to the environmental concentrations. This appeared as well with studies with unialgal cultures (Eppley and Thomas, 1969; Eppley et al., 1969; Carpenter et al., 1971) as with natural populations (MacIsaac and Dugdale, 1969).

Perturbation studies and ^{15}N studies, carried out by Conway et al. (1976) indicated, however, a hyperbola that showed truncation. This was interpreted to be the result of internal cellular control. Moreover, Button (1978) did point out the importance of different mechanisms, which can produce different types of kinetic curves.

In addition it is easy to demonstrate that the saturation curve resulting from the combination of individual Michaelis-Menten curves differs significantly from the hyperbolic model at low limiting nutrient concentrations (Williams, 1973). The generalization of this type of formulation to the whole phytoplankton might therefore prove excessive.

This paper describes a continuous-flow technique which allows the study of nutrient uptake by natural phytoplankton populations. The transient uptake rates are measured in the useful range of substrate concentration by controlling the input mass rate of the limiting nutrient in such a way that only gradual and slow changes of the external substrate concentration are induced, either towards lower or higher concentrations.

The pronounced seasonal variations of the NO_3^- + NO_2^- concentrations in the Southern Bight of the North Sea, which in some periods fall to such values that one reasonably can estimate them as limiting (Mommaerts et al., 1979). led us to consider especially this particular nutrient.

2.— Methods and materials.

2.1.— Analytical methods.

Any nutrient concentration was determined according to earlier described methods (Mommaerts et al., 1979) by means of a *Technicon* Autoanalyzer.

In order to provide an index of the phytoplanktonic biomass in the particulate matter of the sea-water, the concentration of chlorophyll *a* is determined by the trichromatic method described by Parsons et al. (1963). As this method is largely criticized in the literature, it has recently been replaced by a fluorometric determination in combination with high-pressure liquid chromatography (Goeyens et al., 1982).

2.2.— The reactor.

The double-wall plexi-glass container (Goeyens et al., 1980) is equipped with an automatic sampling system (fig. 1). The rate of nutrient uptake is followed by repeated sequential determinations of the concentration. Therefore an inox needle, connected to the peristaltic pump and the autoanalyzer by polyethylene
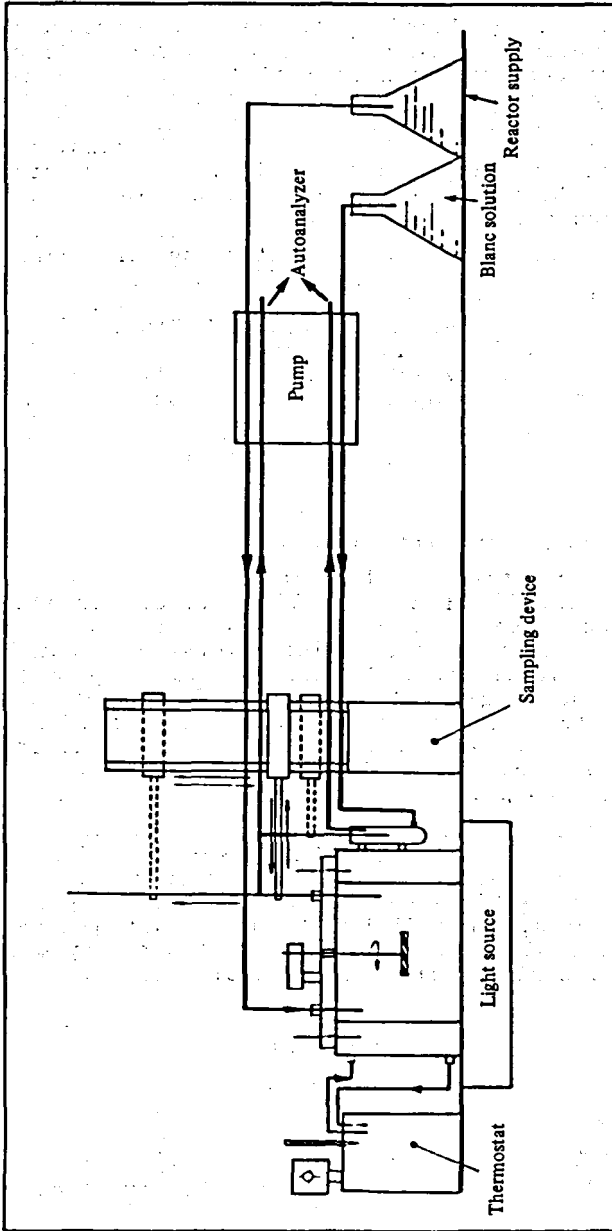
fig. 1.

Scheme of the reactor

tubing, alternatively samples reactor solution and a blank so-
lution (NaCl — 3.45 %). The transition from the NaCl-solution to
the reactor itself is realized by two timer-controlled pneumatic
pistons. This sampling system provides for determinations of the
external nutrient concentration in real time. Besides its advan-
tage of avoiding contamination between sampling and detection,
this method only needs very small sample volumes and the sampling
interval, which is variable between 1 and 10 minutes, is much
smaller than for manually taken samples. For our studies one
determination of the nitrate + nitrite concentration is carried
out every ten minutes.

Thermostatisation and mechanical stirring are performed as
described before (Goeyens et al., 1980). The lighting system on
the other hand is a modified version of the earlier described
one. The lightsources used here were *TL* tubes (*Philips 47*,
40 W), since their emitted light corresponds better with the
normal daylight. A light/dark cycle of twelve hours each is
determined by means of a simple *Velleman* microprocessor.

2.3.— *Sea-water sampling and preparation of the experiments.*

The samples were taken either at the Spuikom Bassin near
Ostend, or at point 33 of the sampling network in the Belgian
coastal area. Each time a volume of 50 ℓ or 75 ℓ was taken
with an immersible pump and stored in polyethylene containers for
transport to the laboratory. As the reactor experiments were
carried out at the original temperature of the water, this was
measured immediately after sampling. In the laboratory each
reactor was filled with 4.8 ℓ of sample. To eliminate zoo-
plankton and large particles in the sample, it was filtered
through a net of 100 μm.

Initial nutrial concentrations were determined for each
sample. For $NO_3^- + NO_2^-$ and NH_4^+ this was done in real time,
while for PO_4^{---} and SiO_2 a sample was stored for a few weeks
with chloroform at 4 °C. Also the initial chlorophyll *a* concen-
tration was measured. In addition to this last parameter, the
composition of the algal population was estimated by microscopic
analysis of samples fixed with acetic lugol's solution.

3.— Results and discussion.

3.1.— *Mathematical approach.*

Essential to derive the uptake rate is the law of mass conser-
vation :

(1) $$\frac{d(VC)}{dt} = Q_{in} C_{in} - Q_{out} C_{out} - \text{Uptake}$$

where V is the volume in ℓ in the reactor, C the concentration in the reactor (expressed in μg N/ℓ), Q the flow rate (expressed in ℓ/min), C_{in} the N-concentration in the alimentation of the reactor and C_{out} the N-concentration in the reactor at the moment of sampling.

As the volume is kept constant by egalizing the in- and out-flows, equation (1) reduces to

(2)   Uptake $= Q\,C_{in} - Q\,C_{out} - V\dfrac{dC}{dt}$,

where each term is expressed in μg N/min . Dividing by the biomass gives the uptake in μg N/min.(μg chlor. $a/\ell)^{-1}$.

The solution of this differential equation (2) depends highly on the way how the relationship between C and t is established. In our approach we made use of a polynomial regression of the fourth degree to determine C(t). From this relationship the corresponding values for C and dC/dt are derived and applicated in equation (2) for the derivation of the uptake rate. With these data the evolution of the uptake rate in function of the substrate concentration is determined.

3.2.— Description of two experiments.

3.2.1.— First experiment.

For the first experiment a sample was taken at the Spuikom bassin. Initially the algal population was very poor and the NO_3^- + NO_2^- concentration very high. Therefore the uptake study was undertaken, when the substrate concentration decreased at values below 200 μg N/ℓ. At that moment a rich population consisted especially of *Skeletonema* together with *Navicula*, the latter present to a less extent.

During the measurements the P- and Si-concentrations were kept at non-limiting levels (5 μmole/ℓ) and the NH_4-concentration was extremely low.

The chlorophyll *a* concentration increased from 304.4 μg/ℓ to 338.8 μg/ℓ during the experiment. The substrate concentration itself decreased from 193.5 μg N/ℓ to 56.2 μg N/ℓ : therefore an input solution with a NO_3^- + NO_2^- concentration of 1.3 mg N/ℓ was used.

The evolution of the uptake rate is represented in figure 2.

After one week a second series of data was obtained with a sample from the same origin. The algae population in this case was more diversified : *Skeletonema*, *Navicula* and *Nitzschia closterium* were very frequent, but also other species as *Melosira* chains and *Synedra* and even some flagellates as *Cryptomonas* and *Gymnodium* were present. The chlorophyll concentration increased from 328.7 μg/ℓ to 348.8 μg/ℓ during the experiment.
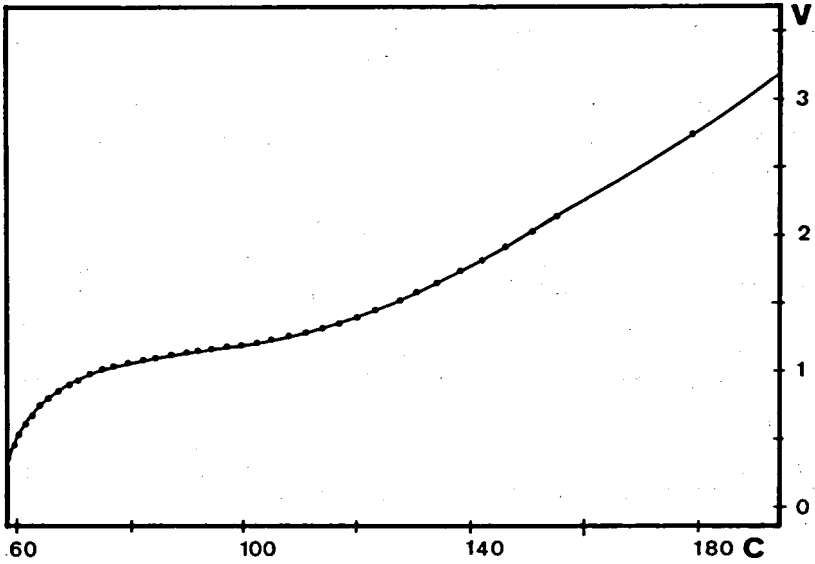
fig. 2.

Uptake rate versus concentration
(experiment 1, part 1)
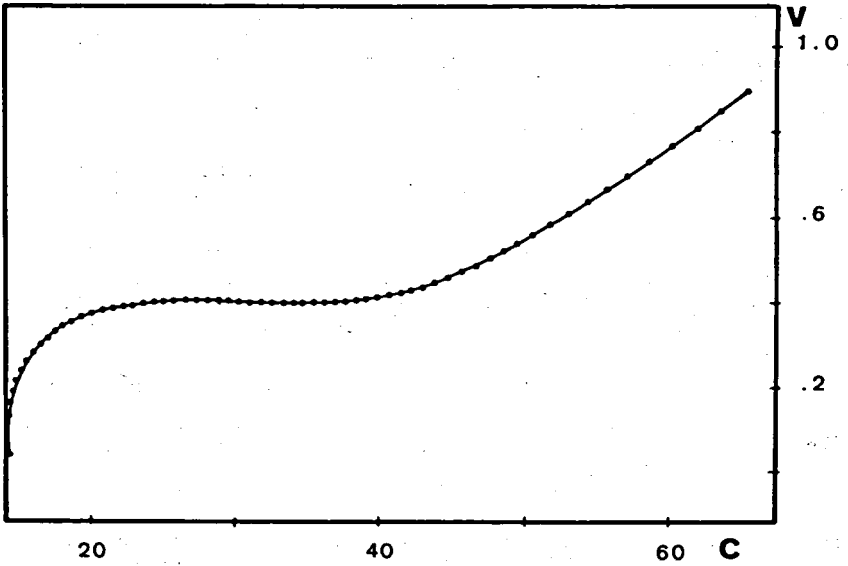

fig. 3.

Uptake rate versus concentration
(experiment 1, part 2)

For this experiment an evolution of the substrate concentra-
tion from   68.9   to   12.8 µg N/ℓ   was realized with an input
concentration of 1.4 mg N/ℓ. In figure 3, the evolution of the
uptake rate is shown as a function of the concentration.

3.2.2.– Second experiment.

A second experiment was performed with a sample from P 33
(51°24' - 2°48'), taken at the beginning of June 1981. The
experiment was divided into two parts. Primarily the input
concentration of NO$_3^-$ + NO$_2^-$ was low as to cause a decrease of
the ambient concentration in the reactor, due to uptake by the
phytoplankton. Secondly, after exhaustion the input concentration
in NO$_3^-$ + NO$_2^-$ was raised. In that case, the addition exceeded
the uptake and the ambient concentration increased in the
reactor.

This complete experiment was performed in one light-period,
during which a decrease in concentration from 51.8 to
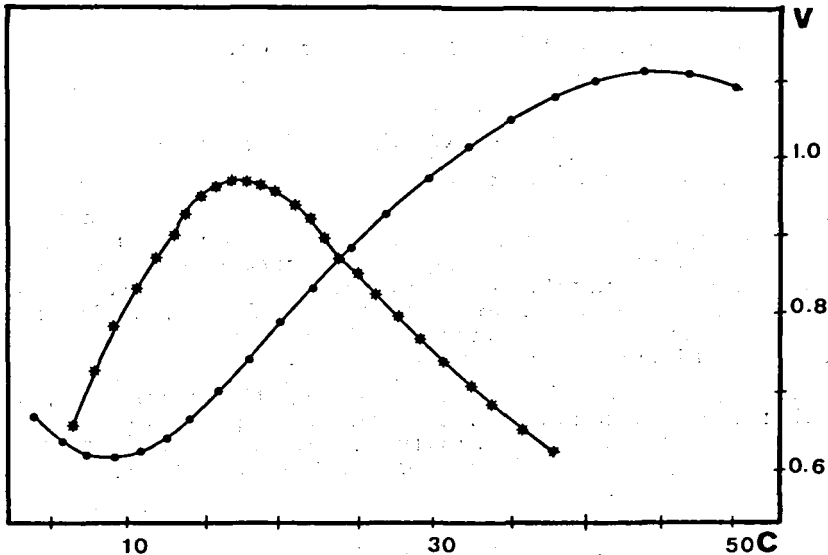2.7 µg N/ℓ as well as an increase to 37.9 µg N/ℓ were rea-
lized.

fig. 4.

Uptake rate versus concentration
for a decreasing (•) and an increasing (*) evolution of the concentration
(experiment 2)

The biomass increased from 67.6 to 75.3 µg chlor. a/ℓ; the dominant species were *Navicula* and *Asterionella japonica*, with some other diatoms present in very small quantities.

In figure 4, the uptake rate is given for both parts of the experiment.

3.3.— *Discussion.*

In the result of the first experiment three modes of uptake are distinguished. Conway et al. (1976) made already distinction between externally controlled uptake V_e, internally controlled uptake V_i and surge uptake V_s. V_e is represented as a function of the external substrate concentration, until the latter increases to a level sufficient to saturate the assimilatory enzyme system. At concentrations above this saturation concentration the uptake V_i is practically constant until the cellular nitrogen is increased to a non-deficient level.

For our experiment a grouping of the data into distinct modes of uptake seems also indicated. However, the V_e-segment appears at different concentrations in both cases. Evaluating the Michaelis-Menten constants from this externally controlled segment gives the following values :

$$V_{max} = 38.9 \times 10^{-3} \ \bar{\mu}g \ N/min.(\mu g \ chlor. \ a/\ell)^{-1}$$

for the first experiment, where the species *Skeletonema* was dominant, and

$$V_{max} = 11.8 \times 10^{-3} \ \mu g \ N/min.(\mu g \ chlor. \ a/\ell)^{-1}$$

for the more complex population.

The second experiment indicates a remarkable difference between the uptake rate for gradually increasing and decreasing external concentrations. During the decrease of the external concentration the uptake rate raises to a maximal value of 15.5×10^{-3} µg N/min.(µg chlor. $a/\ell)^{-1}$ at 45 µg N/ℓ. It is not very obvious from our experimental data wether the uptake rate decreases at higher concentrations or not.

On the other hand, during the phase of the experiment, where the substrate concentration increased, the uptake rate reached a maximum of 13.6×10^{-3} µg N/min.(µg chlor. $a/\ell)^{-1}$ at a concentration of 18 µg N/ℓ. At higher concentrations the uptake rate shows a net increase.

4.— Conclusion.

Obviously the uptake of nutrients by a natural population of phytoplankton depends on many parameters. The fact that the rectangular hyperbola of Michaelis-Menten is insufficient as

generalization for the uptake/concentration relationship is illustrated by both experiments. As a matter of fact the rate of assimilation of nutrient does not only depend on the external concentration. Diffusion, substrate tresholds, transport capacity and inhibition by competitive and/or non-competitive mechanisms may influence the uptake rate (Button, 1978).

A first remark concerns the study of the biomass itself. Most searchers agree that chlorophyll a concentrations are not sufficient in describing the biomass of a phytoplankton population. No information is available about age and physiological situation of the phytoplankton crop. Determinations of the overall composition on lugol's solution certainly was a very important datum, but pigment patterns of phytoplankton extracts on HPLC could give more information about the phytoplankton population and its evolution (Weber and Wettern, 1980).

On the other hand the experimental approach supplies a great number of data. Obviously, care has to be taken to treat these data on an objective and artefact-free way. In our calculations no model was imposed for the uptake; however, a control of the results by a different analytical method is recommendable. In view of this extension, the use of ^{15}N-labelled compounds is an important instrument (Stichelbaut, 1982).

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