Services du Premier Ministre
Programmation de la Politique scientifique
Rue de la Science 8
1040 BRUXELLES
BELGIQUE

Diensten van de Eerste Minister
Programmatie van het Wetenschapsbeleid
Wetenschapsstraat 8
1040 BRUSSEL
BELGIE

ACTIONS DE RECHERCHE CONCERTEES

GECONCERTEERDE ONDERZOEKSACTIES

ACTION INTERUNIVERSITAIRE

INTERUNIVERSITAIRE ACTIE

OCEANOLOGIE

OCEANOLOGIE

Rapport final

Eindverslag

Volume 3

Boekdeel 3

BIOLOGICAL PROCESSES AND TRANSLOCATIONS

edited by
C. HEIP and Ph. POLK

BIOLOGICAL PROCESSES AND TRANSLOCATIONS

edited by

C. HEIP and Ph. POLK

Services du Premier Ministre
Programmation de la Politique scientifique
Rue de la Science 8
1040 BRUXELLES
BELGIQUE

Diensten van de Eerste Minister
Programmatie van het Wetenschapsbeleid
Wetenschapsstraat 8
1040 BRUSSEL
BELGIE

ACTIONS DE RECHERCHE CONCERTEES

ACTION INTERUNIVERSITAIRE

OCEANOLOGIE

Rapport final

Volume 3

GECONCERTEERDE ONDERZOEKSACTIES

INTERUNIVERSITAIRE ACTIE

OCEANOLOGIE

Eindverslag

Boekdeel 3

BIOLOGICAL PROCESSES AND TRANSLOCATIONS

edited by
C. HEIP and Ph. POLK

TABLE OF CONTENTS

BENTHIC STUDIES OF THE SOUTHERN BIGHT OF THE NORTH SEA AND ITS ADJACENT CONTINENTAL ESTUARIES

CARBON CYCLING IN THE BELGIAN COASTAL ZONE AND ADJACENT AREAS

BENTHIC STUDIES

OF THE SOUTHERN BIGHT OF THE NORTH SEA

AND ITS ADJACENT CONTINENTAL ESTUARIES

SUMMARY

III

ENERGY FLOW THROUGH THE MEIOBENTHOS

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

IV

MEIOFAUNA OF THE BELGIAN COASTAL WATERS : SPATIAL AND TEMPORAL VARIABILITY AND PRODUCTIVITY

R. HERMAN, M. VINCX and C. HEIP

V

BENTHOS OF THE KWINTE BANK

(An exploited sandbank in the Southern Bight)

C. VANOSMAEL, K. WILLEMS, M. VINCX, D. CLAEYS and C. HEIP

VI

MACROBENTHOS IN THE WESTERN SCHELDE ESTUARY

Y.M. VERMEULEN and J.C.R. GOVAERE

VII

A SUMMARY OF BENTHIC STUDIES

IN THE SLUICE DOCK OF OSTEND DURING 1976-1981

L.K.H. THIELEMANS, C. HELP and D. VAN GANSBEKE

III

ENERGY FLOW THROUGH THE MEIOBENTHOS

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

Marine Biology Section, Zoology Institute, State University of Gent, Belgium

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, primary production and sedimentation in the Southern Bight of the North Sea are indeed higher than previously estimated. The research efforts in our laboratory were concentrated on the production 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 retained 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, nutrient 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 leaving it?

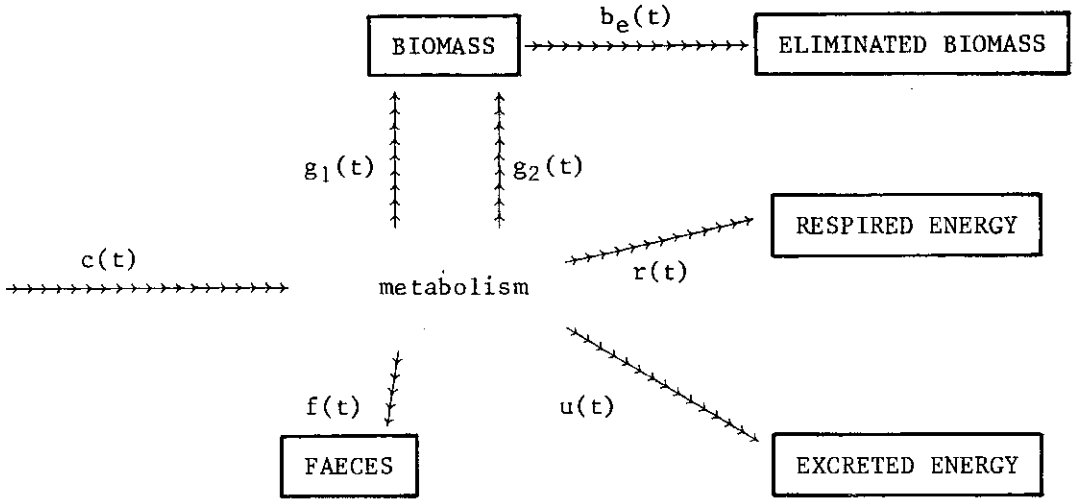


fig. 1.

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 individuals 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

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

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 :

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

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 cohort 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 *Microarthridion littorale*, another harpacticoid. Although nematodes are the numerically most important meiobenthic taxon, direct production 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 harpacticoid copepod species, *Tachidius discipes* and *Paronychocamptus nanus*, 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 measurable 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 accurately. Therefore we measured the respiration of the species mentioned. 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 turnover (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 temperature 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, mortality, growth).

Material and methods.

The Southern Bight area has been described in our first progress 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) brackish 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 solution, heated to 70°C. The animals were extracted according to

the method described by Heip *et al.* (1974), except that centrifugation 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 observed 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 salinity 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 maintain 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 (= medium used for *Monhystera microphthalma* and *Monhystrella parelegantula*). The constitution of this medium is as follows :

0.278 g $FeSO_4.7H_2O$
3 g $NaH_2PO_4.2H_2O$
30 g $NaNO_3$
0.47 g $MnCl_2.4H_2O$
50 g glycine per liter distilled water. For the other two species (*Paracanthonchus caecus* and *Chromadora nudicapitata*), 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 controlled 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 temperature a minimum of two replica's was examined and per replica a

known number (± 30) of gravid females deposited eggs during maximal 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 volume 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 cm^2 per 5 years.

Stage	Survival	Duration (days)	Recruitment	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 generations. 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 components (Bhattacharya, 1968) could be used. Fig. 3 shows the fit of the summed Gaussians on the density data of copepodite 3.

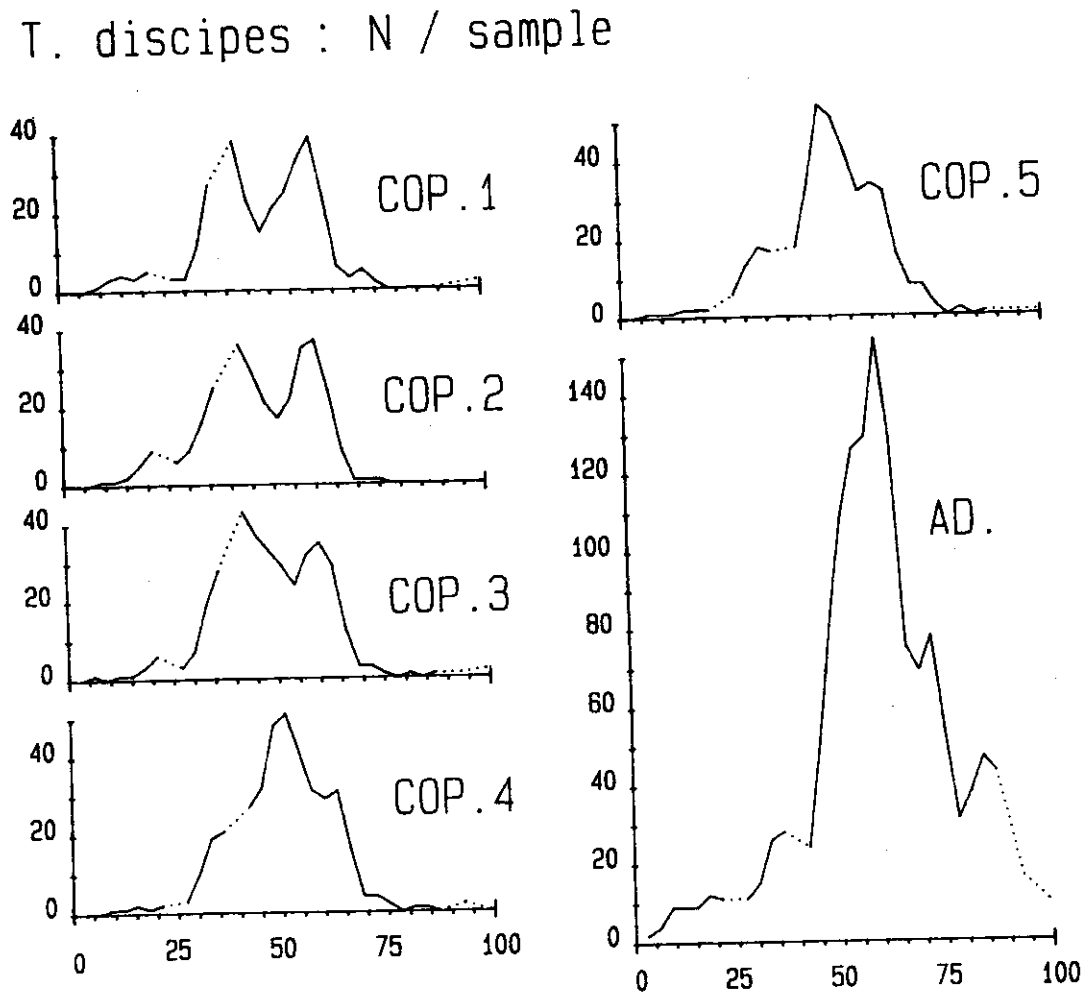


fig. 2.

Tachidius discipes : densities (N/sample of 6.06 cm^2)
of the different stages during spring 1979
(Day 0 : 27-03-1979)

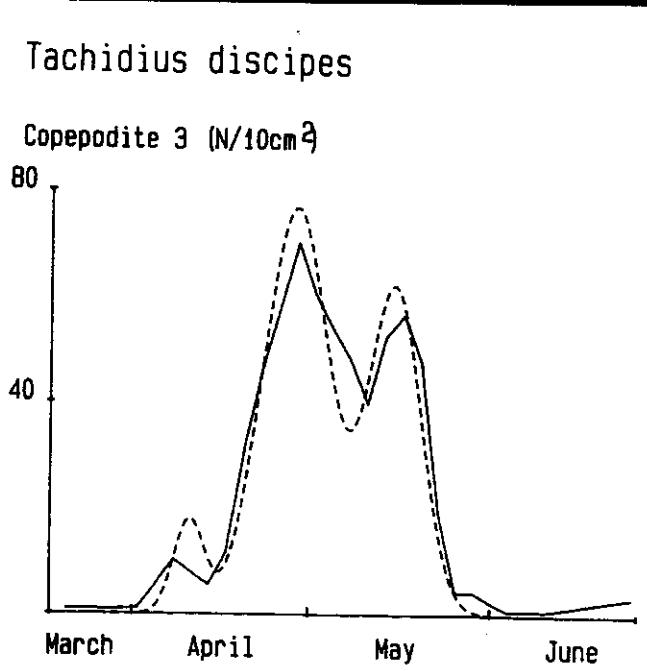


fig. 3.

Tachidius discipes : density (N/10 cm^2) 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 $O_2 ind^{-1} hr^{-1}$
W = µg dwt ind^{-1}

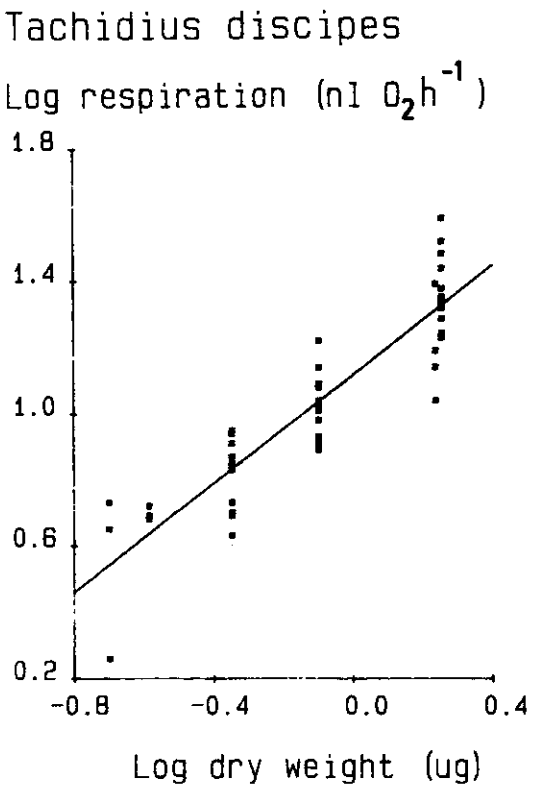


fig. 4.

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 copepodites 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 harpacticoid species was estimated with the size-frequency method. This requires the estimation of the generation time and of the proportion 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 physiological time scale. One unit on this time scale corresponds to one

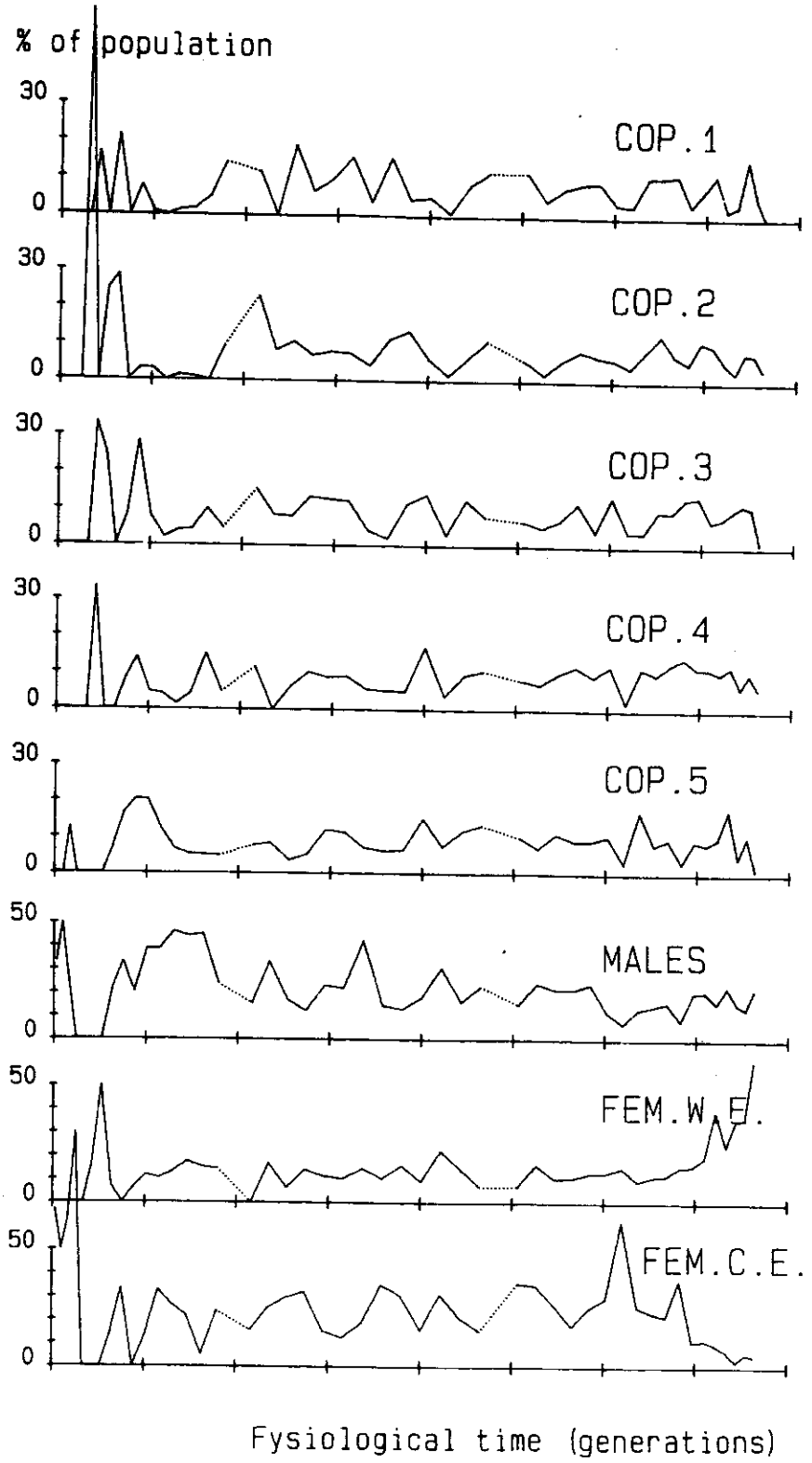


fig. 5.

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.

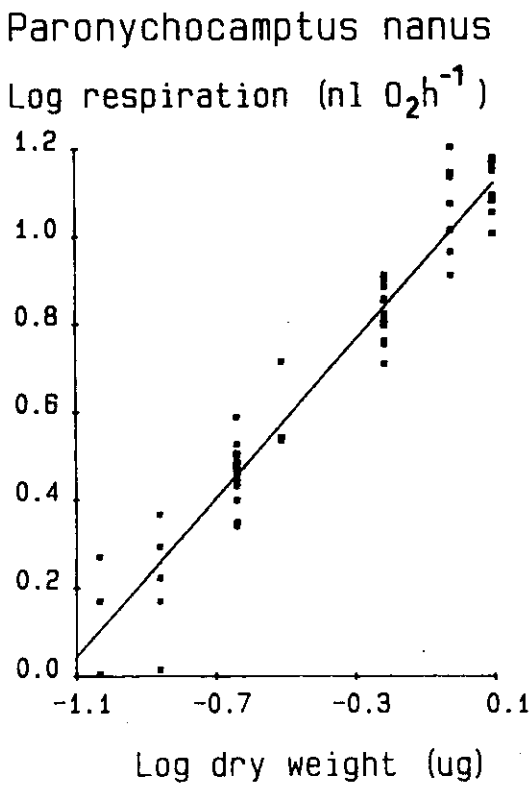


fig. 6.

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

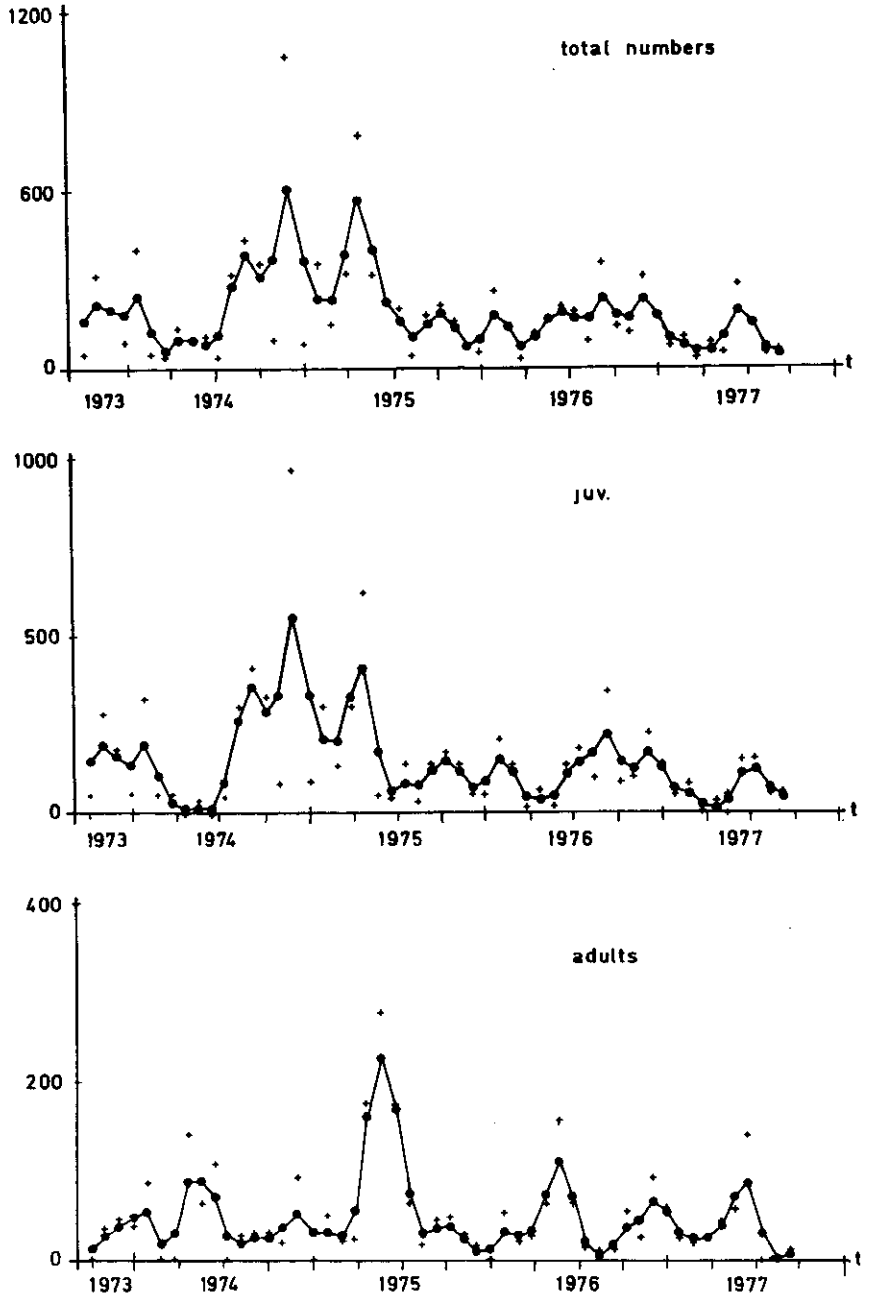


fig. 7.

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

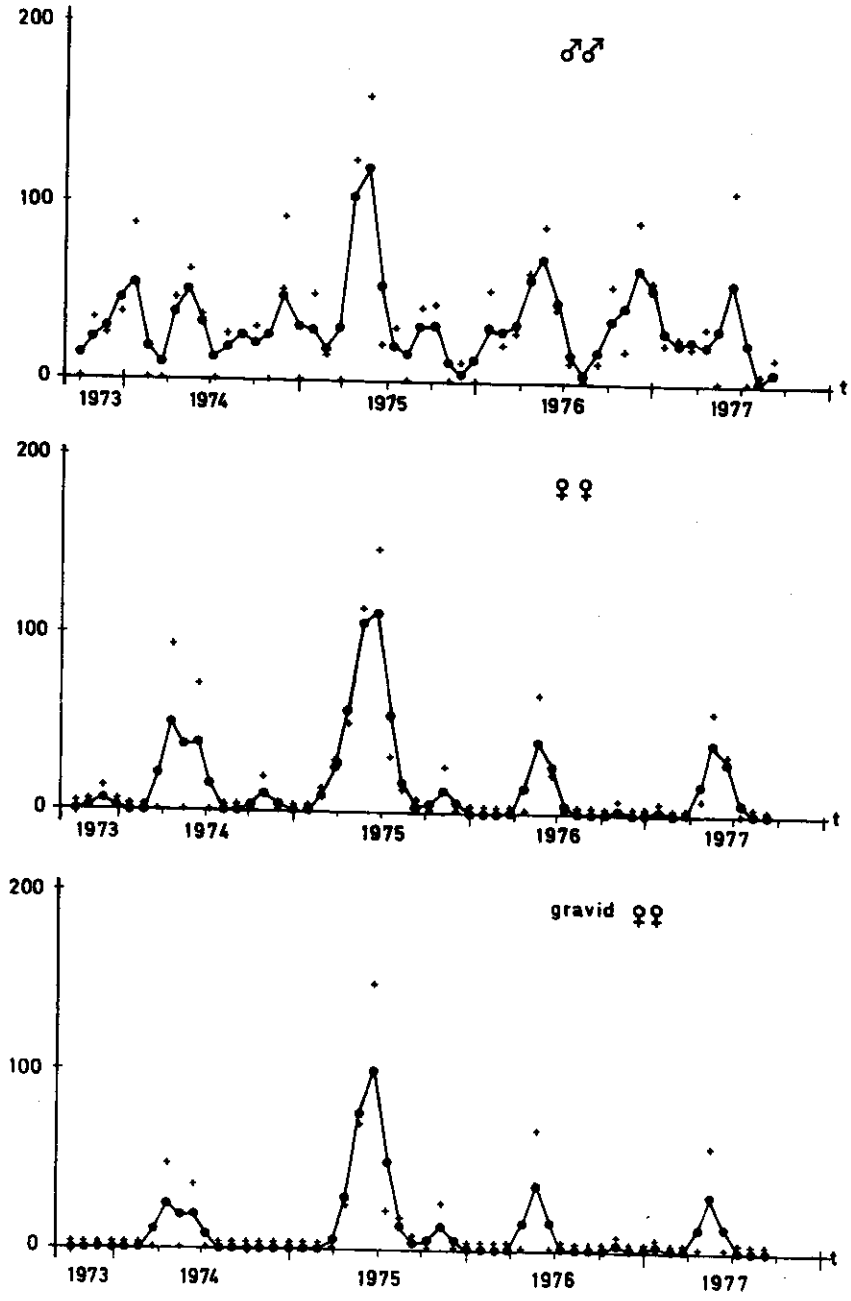


fig. 8.

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

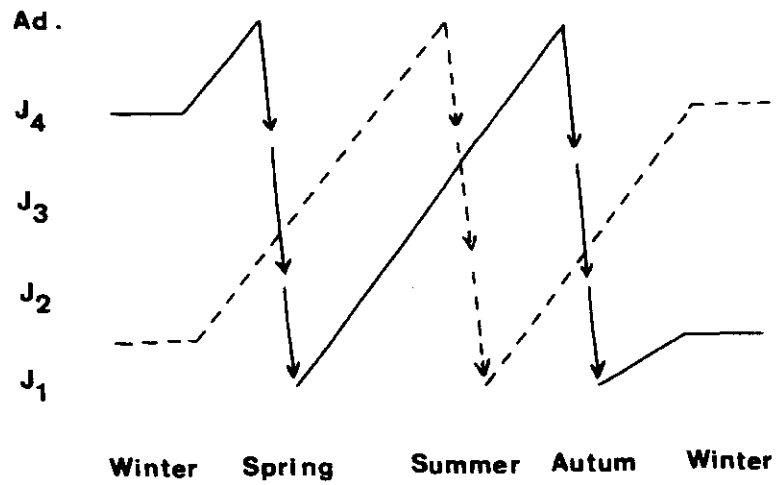


fig. 9.

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 complicated 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.

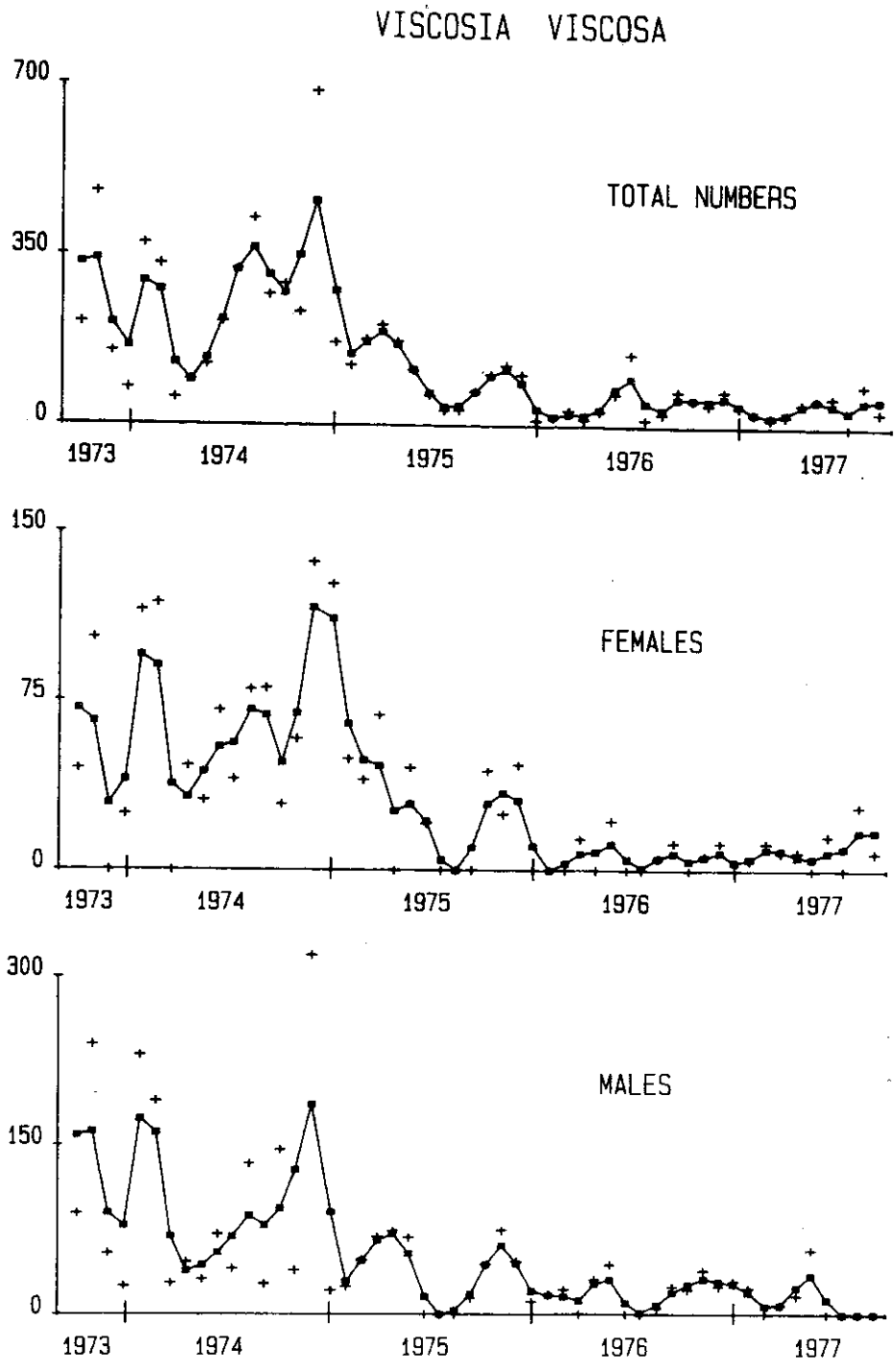


fig. 10.

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 $\ln D = \ln a + T \ln b + T^2 \ln c$ (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)

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

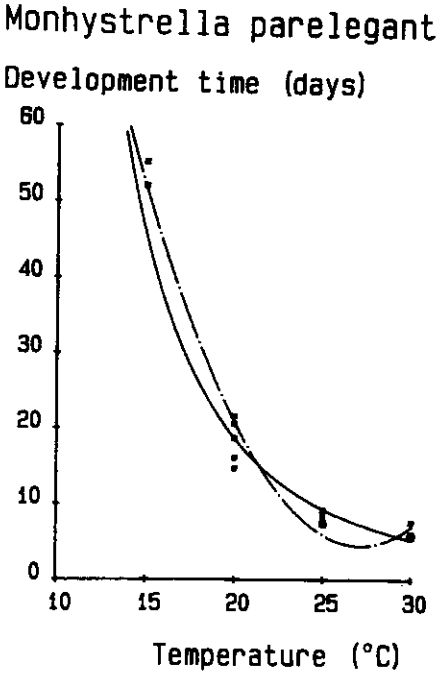


fig. 11.

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

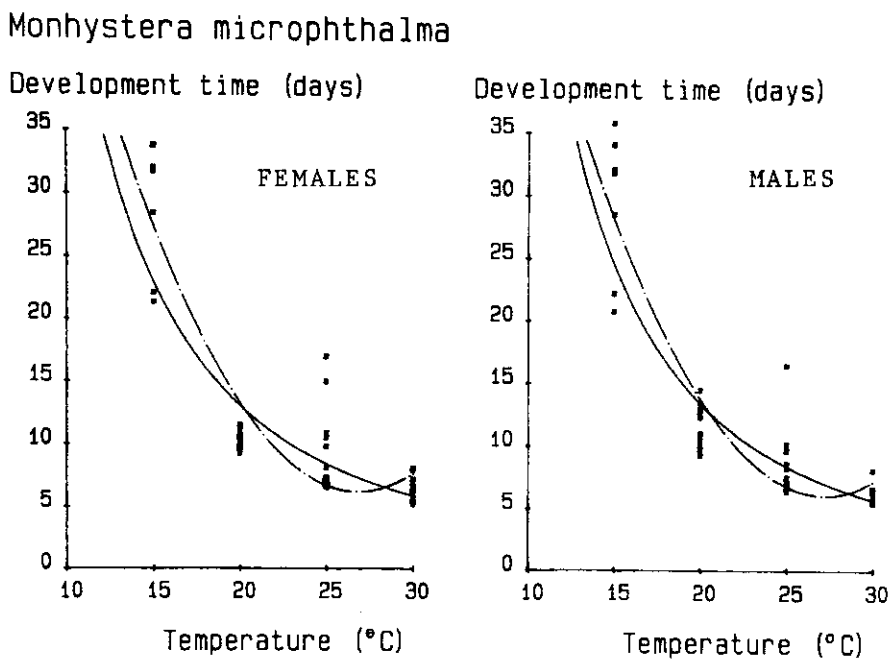


fig. 12.

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

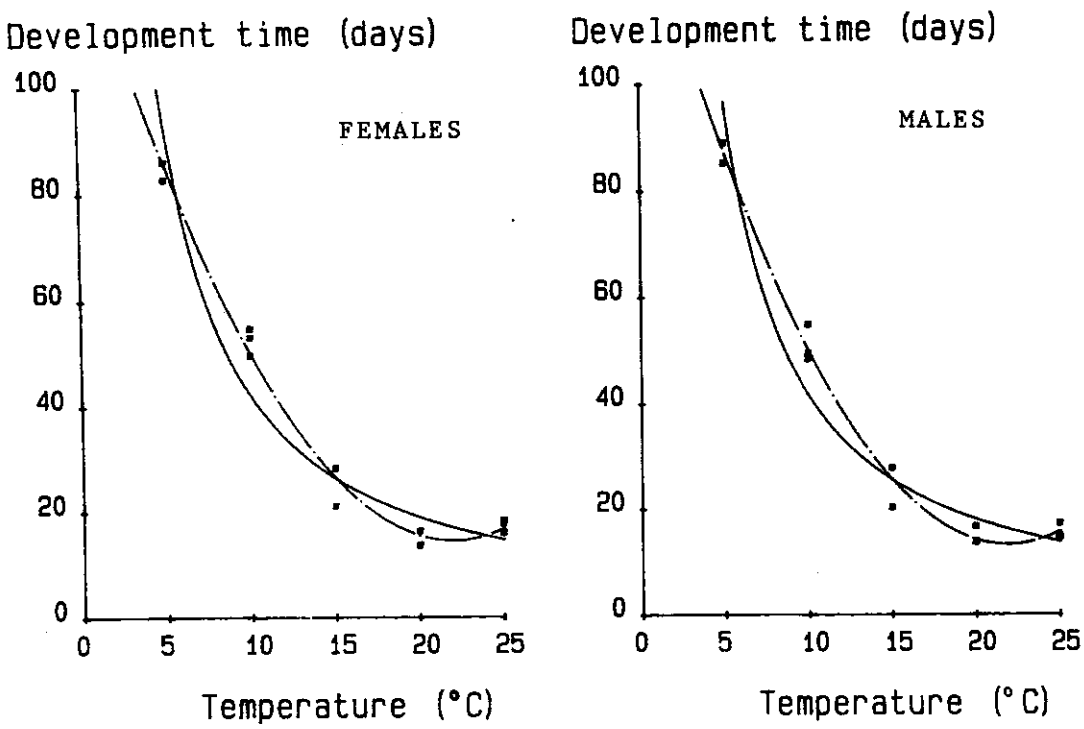


fig. 13.

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

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

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.

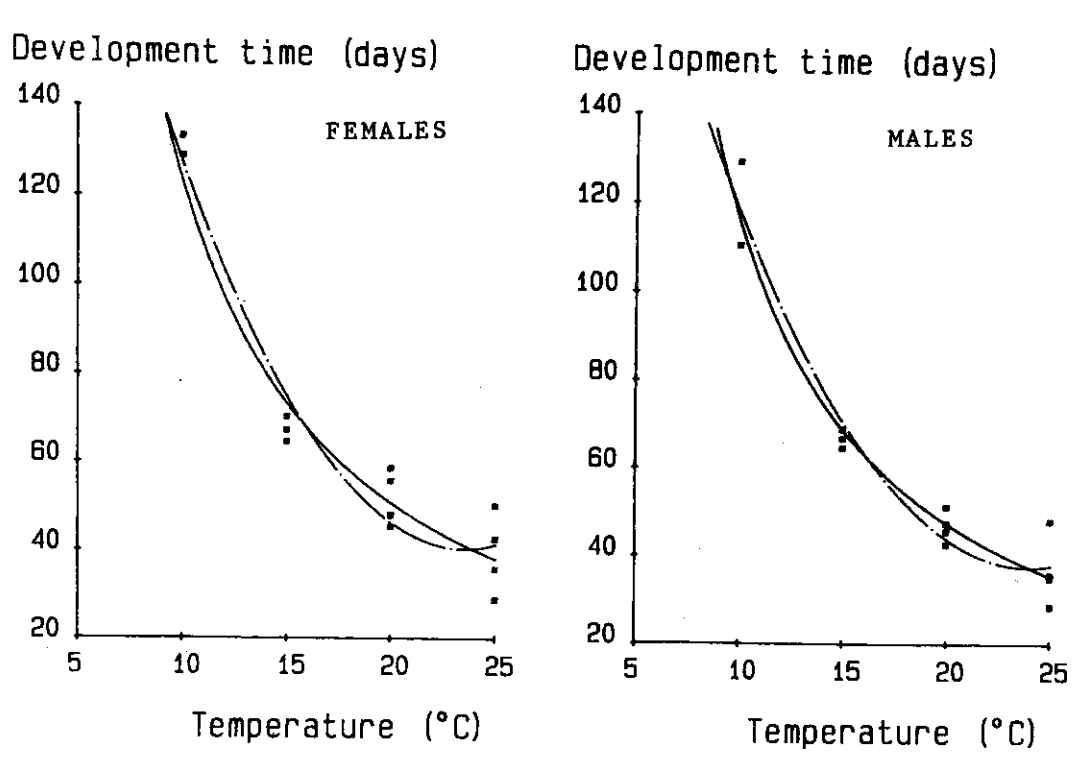


fig. 14.

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 biological zero's are accounted for the computations result in the numbers indicated with an asteriks (Table 5). The largest difference, 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 germanica* (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 meiobenthic populations (Table 6) already indicate that the use of a single annual P/B value for meiofauna (Gerlach, 1971) is inappropriate. In fact the annual P/B values show a considerable range, and the high diversity in life histories of meiobenthic populations is clearly reflected in the diversity of annual P/B values. In this respect, it is interesting to note that the P/B per generations 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 experiments 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 results 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 approximation 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. parelegantula* and *M. microphthalma* do not reproduce and occur only sporadically during approximately half a year, annual mean biomass 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 unrealistic. However, other shortcut methods may prove more useful. The data presented here indicate that the production efficiencies 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 nematodes are reviewed by Warwick & Price (1979). Table 8 summarizes 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 results 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 estuary (Teare & Price, 1979) makes clear that important differences 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 figure) 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 population 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 differences in feeding conditions. In their laboratory reared population, 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 productivity in the habitat may be an important factor in determining the respiratory activity of a population. Therefore, looking for a general value for the respiratory activity of meiofauna may be as spurious as looking for one overall P/B figure.

Acknowledgments.

C. Heip and P. Herman acknowledge a grant from the Belgian National Science Foundation N.F.W.O. G. Vranken and D. Van Brussel acknowledge a grant from the Institute for Scientific Research in Industry and Agriculture (I.W.O.N.L.). Part of this research was sponsored through grant 2.0010.78 from the Fund for Collective Fundamental Research F.K.F.O. We thank Wies Gijselinck for assistance in the laboratory.

References.

BANSE, K. & MOSHER, S., (1980). Adult body mass and annual production/biomass relationships of field populations. *Ecol.Monogr.*, 50, 355-357.

BARNETT, P.R.O., 1968). Distribution and ecology of harpacticoid copepods of an intertidal mudflat. *Int. Revue ges.Hydrobiol.Hydrogr.*, 53, 177-209.

BENKE, A.C., (1979). A modification of the Hynes method for estimating secondary production with particular significance for multivoltine populations. *Limn.Oceanogr.*, 24, 168-171.

BHATTACHARYA, D.G., (1968). A simple method of resolution of a distribution into Gaussian components. *Biometrics*, 23, 115-135.

BOTRELL, H.H., (1975). The relationship between temperature and duration of egg development in some epiphytic Cladocera and Copepoda from the River Thames, reading with a discussion of temperature functions. *Oecologia (Berl.)*, 18, 63-84.

COULL, B.C. & BELL, S.S., (1979). Perspectives in marine meiofauna ecology. In : R.J. Livingston (Ed.), *Ecological processes in coastal marine ecosystems*, Plenum, New York, pp. 189-216.

COULL, B.C. & VERNBERG, W.B., (1970). Harpacticoid respiration : *Enydrosoma propinquum* and *Longipedia helgolandica*. *Mar.Biol.*, 5, 341-344.

DE JONGE, V.N. & BOUWMAN, L.A., (1977). A simple density separation technique for quantitative isolation of meiobenthos using the colloidal silica Ludox-TM. *Mar.Biol.*, 42, 143-148.

FELLER, R.J., (1977). *Life history and production of meiobenthic harpacticoid copepods in Puget Sound.* Dissertation. Univ. of Washington, Seattle, Washington, 249 pp.

FLEEGER, J.W. & PALMER, M.A., (1982). Secondary production of the estuarine meiobenthic copepod *Microarthridion littorale*. *Mar.Ecol.Prog.Ser.*, 7, 157-162.

GERLACH, S.A., (1971). On the importance of marine meiofauna for benthos communities. *Oecologia (Berl.)*, 6, 176-190.

HAGERMAN, L., (1969). Respiration, anaerobic survival and diel locomotory periodicity in *Hirschmannia viridis* Müller (Ostracoda). *Oikos*, 20, 384-391.

HEIP, C., (1974). A comparison between models describing the influence of temperature on the development rate of copepods. *Biol.Jaarb.*, 42, 121-125.

HEIP, C., (1976). The life cycle of *Cyprideis torosa* (Crustacea, Ostracoda). *Oecologia (Berl.)*, 24, 229-245.

HEIP, C. & SMOL, N., (1976). Influence of temperature on the reproductive potential of two brackish-water Harpacticoids (Crustacea, Copepoda). *Marine Biology*, 35, 327-334.

HEIP, C., SMOL, N. & HAUTEKIET, W., (1974). A rapid method of extracting meiobenthic nematodes and copepods from mud and detritus. *Mar.Biol.*, 28, 79-81.

HEIP, C., SMOL, N & ABSILIS, V., (1978). Influence of temperature on the reproductive potential of *Oncholaimus oxyuris* (Nematoda : Oncholaimidae). *Mar.Biol.*, 45, 255-260.

HEIP, C., HERMAN, R., BISSCHOP, G., GOVAERE, J.C.R., HOLVOET, M., VAN DAMME, D., VANOSMAEL, C., WILLEMS, K.R. & DE CONINCK, L.A.P., (1979). Benthic studies of the Southern Bight of the North Sea and its adjacent continental estuaries. *Progress Report I, ICES CM/L*, 9, 133-163.

HEIP, C., VINCX, M., SMOL, N. & VRANKEN, G., (1982). The systematics and ecology of free-living marine nematodes. *Helminth.Abstr.*, Series B, 51, 1-31.

HERMAN, P.M.J. & HEIP, C., (in press). Growth and respiration of *Cyprideis torosa* Jones, 1850 (Crustacea, Ostracoda). *Oecologia (Berl.)*.

HUMPHREYS, W.F., (1979). Production and respiration in animal populations. *J.Anim.Ecol.*, 48, 427-453.

IVLEVA, I.V., (1980). The dependence of crustacean respiration rate on body mass and habitat temperature. *Int. Revue ges.Hydrobiol.*, 65, 1-47.

KINNE, O., (1977). Cultivation of animals : research cultivation. In : O. Kinne (Ed.), *Marine Ecology*, Vol. III, *Cultivation*, Part 2. Wiley, Chichester, pp. 579-1293.

KLEKOWSKI, R.Z., (1971). Cartesian diver microrespirometry for aquatic animals. *Pol.Arch.Hydrobiol.*, 18, 93-144.

LASERRE, P. & RENAUD-MORNANT, J., (1973). Resistance and respiratory physiology of intertidal meiofauna to oxygen deficiency. *Neth.J. Sea res.*, 7, 290-302.

LASERRE, P., RENAUD-MORNANT, J. & CASTEL, J., (1975). Metabolic activities of meiofaunal communities in a semi-enclosed lagoon. Possibilities of trofic competition between meiofauna and mugiliid fish. *10th European Symposium on Marine Biology, Ostend, Belgium*, (G. Persoone & E. Jaspers, Eds.), Vol. 2, 293-414.

LASKER, R., WELLS, J.B.J. & Mc INTYRE, A.D., (1970). Growth, reproduction, respiration and carbon utilization of the sand-dwelling harpacticoid copepod, *Asellopsis intermedia*. *J.mar.biol.Ass.U.K.*, 50, 147-160.

MACLAREN, I.A., (1963). Effects of temperature on growth of zooplankton and the adaptive value of vertical migration. *J.Fish.Res.Bd Can.*, 20, 685-727.

McNEILL, S. & LAWTON, J.H., (1970). Annual production and respiration in animal populations. *Nature*, 225, 472-474.

MENZIE, C.A., (1980). A note on the Hynes method of estimating secondary production. *Limnol.Oceanogr.*, 25, 770-773.

PALMER, M.A. & COULL, B.C., (1980). The prediction of development rate and the effect of temperature for the meiobenthic copepod, *Microarthridion littorale* (Poppe). *J.exp.mar.Biol.Ecol.*, 48, 73-83.

PODAMO, (1975). Ecometabolism of a shallow marine lagoon at Ostend (Belgium). *10th European Symposium on Marine Biology, Ostend, Belgium, Sept. 17-23*, Vol. 2, 549-562.

RIGLER, F.H. & COOLEY, J.M., (1974). The use of field data to derive population statistics of multivoltine copepods. *Limnol.Oceanogr.*, 19, 636-655.

SARVALA, J., (1979). Effect of temperature on the duration of egg, nauplius and copepodite development of some freshwater benthic Copepoda. *Freshwater Biology*, 91, 515-534.

SCHIEMER, F. & DUNCAN, A., (1974). The oxygen consumption of a freshwater benthic nematode, *Tobrilus gracilis* (Bastian). *Oecologia (Berl.)*, 15, 121-126.

SELLNER, B.W., (1976). Survival and metabolism of the harpacticoid copepod *Thompsonula hyaenae* (Thompson) fed on different diatoms. *Hydrobiologia*, 50, 232-238.

SMOL, N. & HEIP, C., (1974). The culturing of some harpacticoid copepods from brackish water. *Biol.Jb. Dodonaea*, 42, 159-169.

STEELE, J.H., (1974). *The structure of marine ecosystems*, Harvard University Press, Cambridge, Mass.

TEAL, J.M. & WIESER, W., (1966). The distribution and ecology of nematodes in a Georgia salt marsh. *Limnol.Oceanogr.*, 11, 217-222.

TEARE, M. & PRICE, R.,(1979). Respiration of the meiobenthic harpacticoid copepod, *Tachidius discipes* from an estuarine mudflat. *J.exp.Mar. biol.Ecol.*, 41, 1-8.

TIETJEN, J.H. & LEE, J.J., (1972). Life cycles of marine nematodes. Influence of temperature and salinity on the development of *Monhystera denticulata* Timm. *Oecologia (Berl.)*, 10, 167-176.

TIETJEN, J.H. & LEE, J.J., (1977). Life histories of marine nematodes. Influence of temperature and salinity on the reproductive potential of *Chromadorina germanica* Bütschli. *Mikrofauna Meeresboden*, 61, 263-

VERNBERG, W.B., COULL, B.C. & JORGENSEN, D.J., (1977). Reliability of laboratory measurements of meiofauna. *J.Fish.Res.Bd Can.*, 34, 164-167.

VAN DAMME, D. & HEIP, C., (1977). Het meiobenthos in de Zuidelijke Noordzee. In : *Inventaris van de fauna en flora, Nationaal Onderzoeks- en Ontwikkelingsprogramma - Leefmilieu - Projekt Zee*, Programmatie van het Wetenschapsbeleid, Brussel, pp. 1-113.

VRANKEN, G., THIELEMANS, L.K., HEIP, C. & VANDYCKE, M., (1981). Aspects of the life cycle of *Monhystera parelegantula* (Nematoda, Monhysteridae). *Mar.Ecol.Progr.* Ser. 6, 67-72.

WARWICK, R.M., (1982). The influence of temperature and salinity on energy partitioning in the marine nematode *Diplolaimelloides brucici*. *Oecologia (Berl.)*, 51, 318-325.

WARWICK, R.M. & PRICE, R., (1979). Ecological and metabolic studies on free-living nematodes from an estuarine mud flat. *Est.Coast.Mar. Science*, 9, 257-271.

WATERS, T.F., (1969). The turn-over ratio in production ecology of freshwater invertebrates. *Am.Nat.*, 103, 173-185.

IV

MEIOFAUNA OF THE BELGIAN COASTAL WATERS : SPATIAL AND TEMPORAL VARIABILITY AND PRODUCTIVITY

R. HERMAN, M. VINCX and C. HEIP

Marine Biology Section, Zoology Institute, State University of Gent, Belgium

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 m^2 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 cm^2). Each box core was subsampled by four plastic cores (surface : 10.2 cm^2). 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 decreasing tidal currents a clear gradient from coarse sands in the south to fine sands in the north of the area is a very significant feature of the Southern Bight.

Gullentops *et al.* (1977) gave some data on the origin, transport and on the behaviour of the fine sediments along the Belgian coast. The material along the Belgian coast is from two different origins ; the western part of the coast is influenced by the IJzer, French coast and by the Channel, the eastern coast is influenced by the Western Scheldt estuary. Based on sedimentological 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.

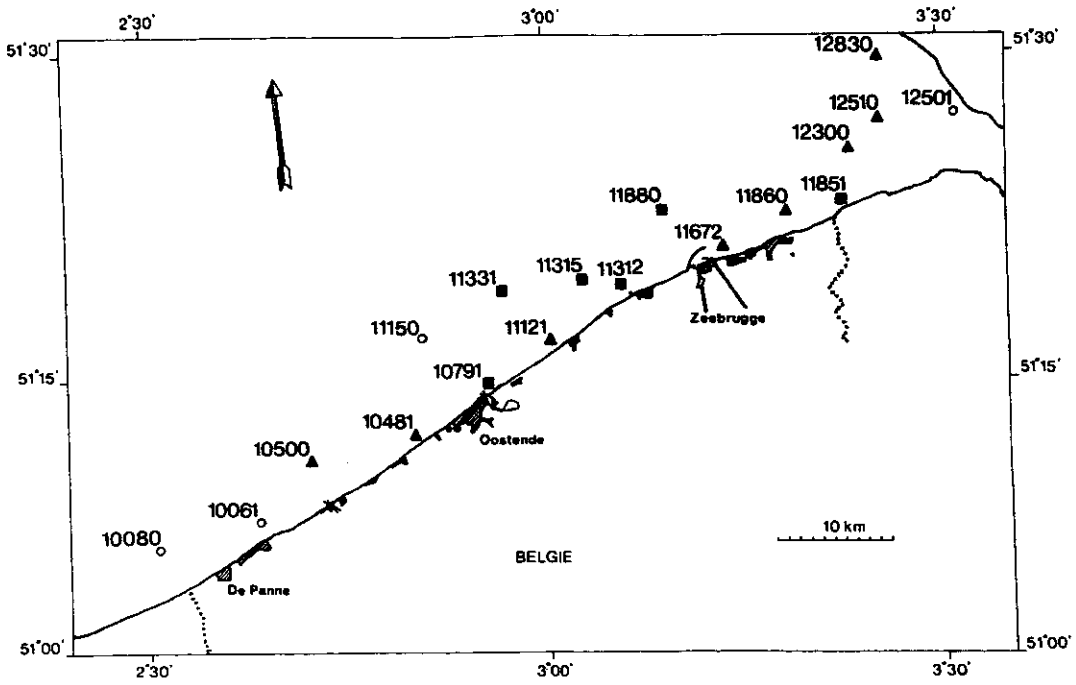


fig. 1.

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 stations can be classified into three groups (Fig. 1). Those stations 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 0.230 mm for the whole sampling 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).

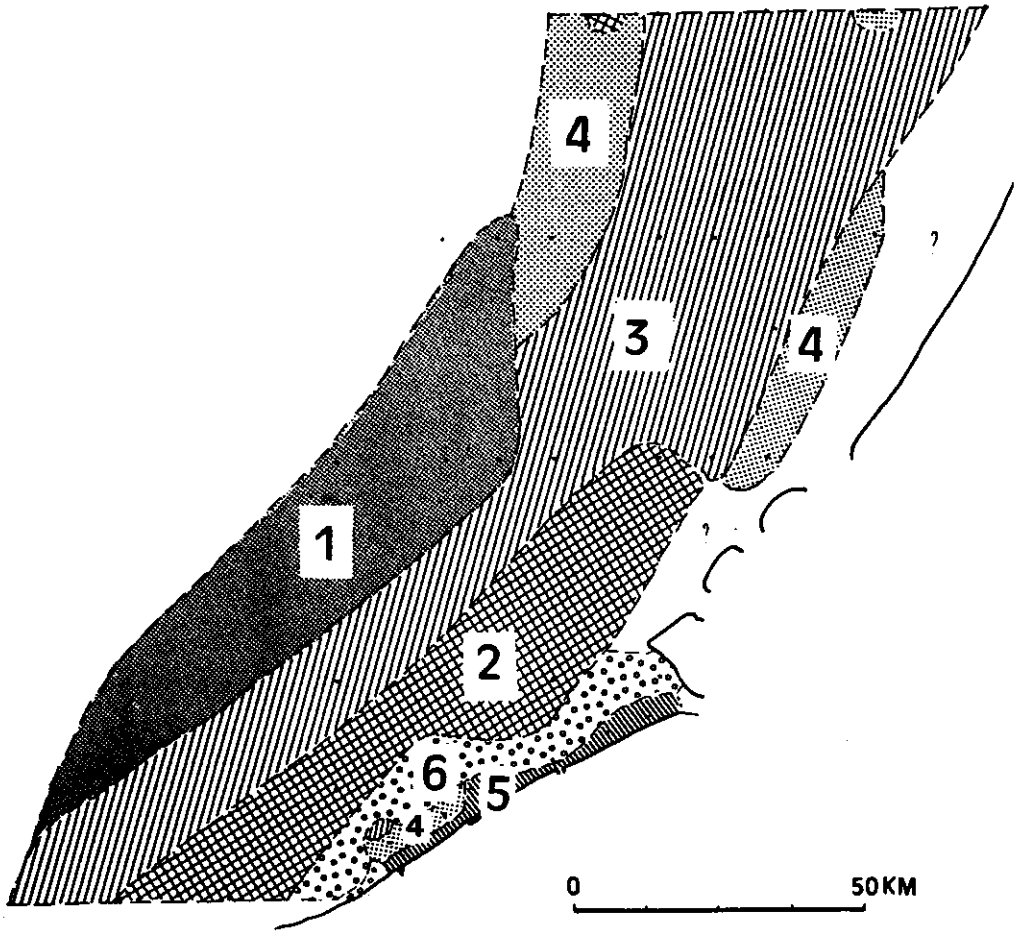


fig. 2.

Spatial distribution of nematode communities in the Southern Bight

Zone 1 :-the open sea region.
Characterized 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 order of importance) (abundance > 10%) are the Desmodoridae, 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 families.

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 recording 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 producers 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 cavity : 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 relative 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 bottoms), the four feeding types are more equally distributed, although epigrowth feeders are the most numerous group.

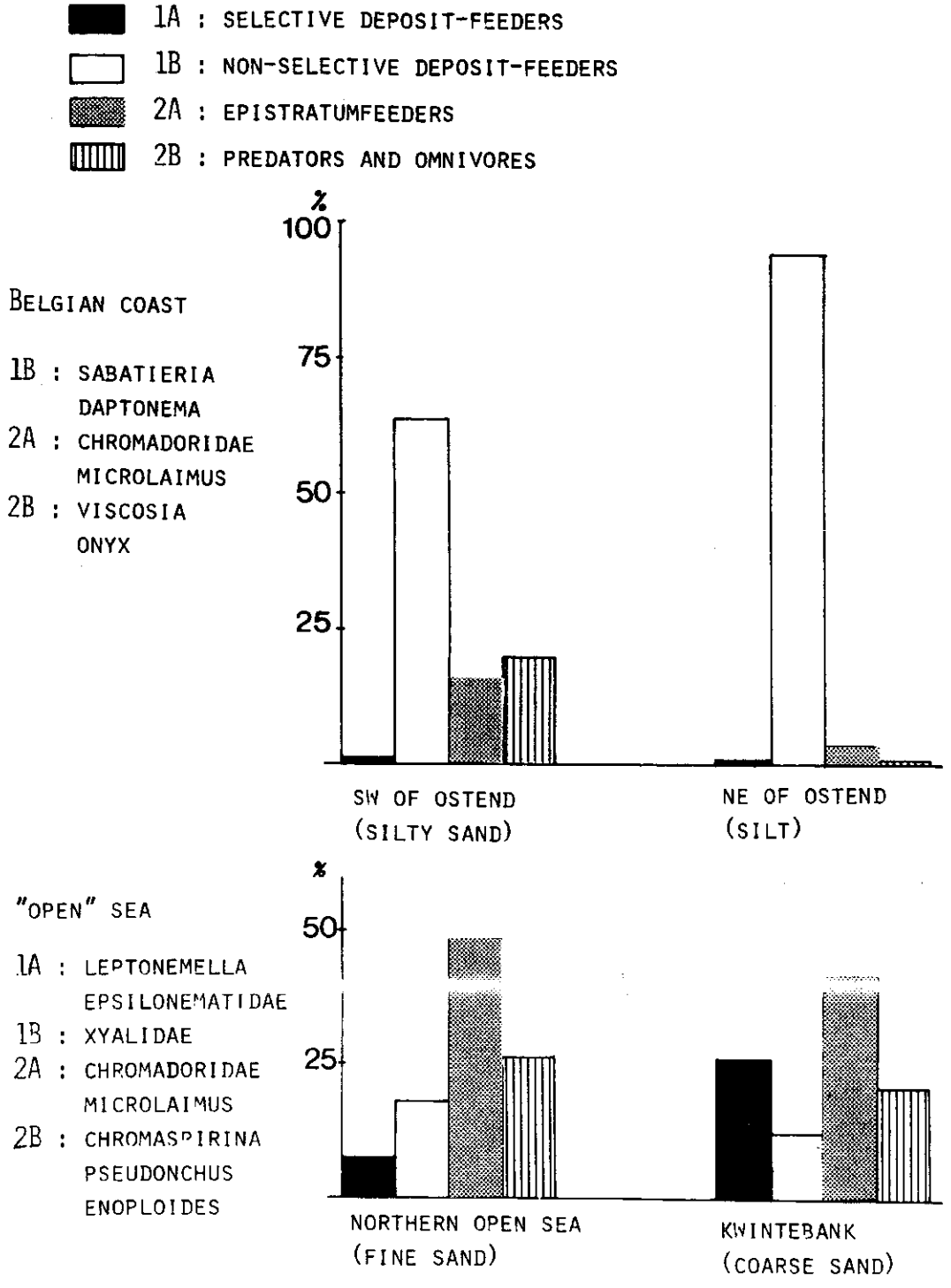


fig. 3.

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

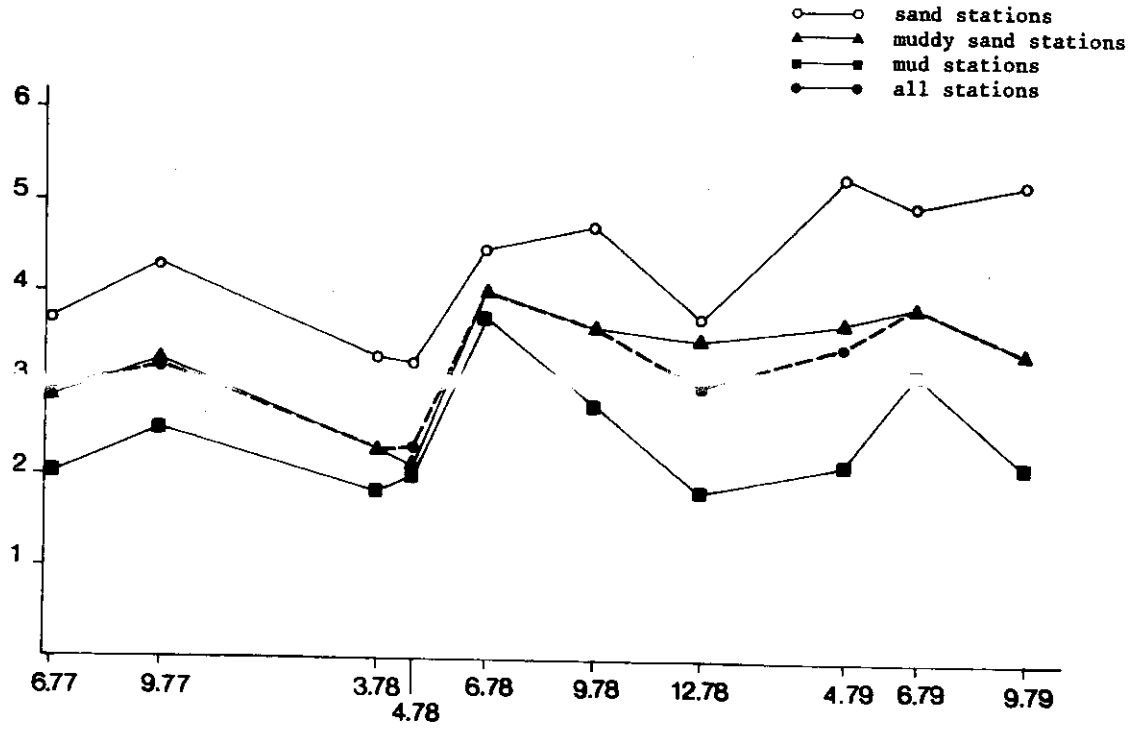


fig. 4.

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 study 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 stations (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 harpacticoids 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).

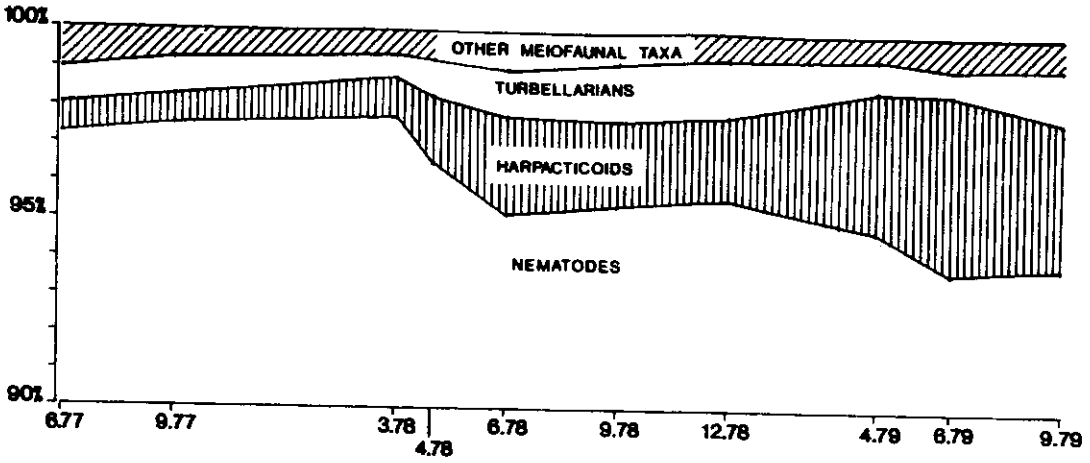


fig. 5.

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.

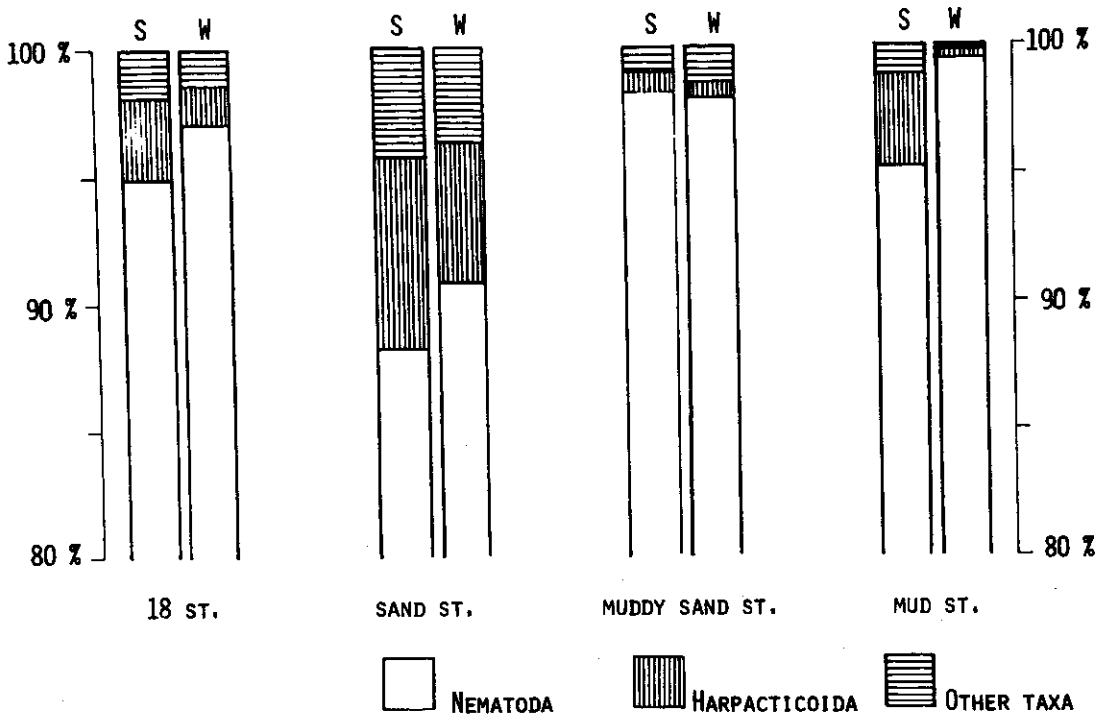


fig. 6.

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	$\bar{x}$/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
$\bar{x}$/cruise	1241 ±243	2592 ±551	926 ±358	1126 ±311	3678 ±997	2517 ±1108	1115 ±319	1061 ±229	1903 ±564	1120 ±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

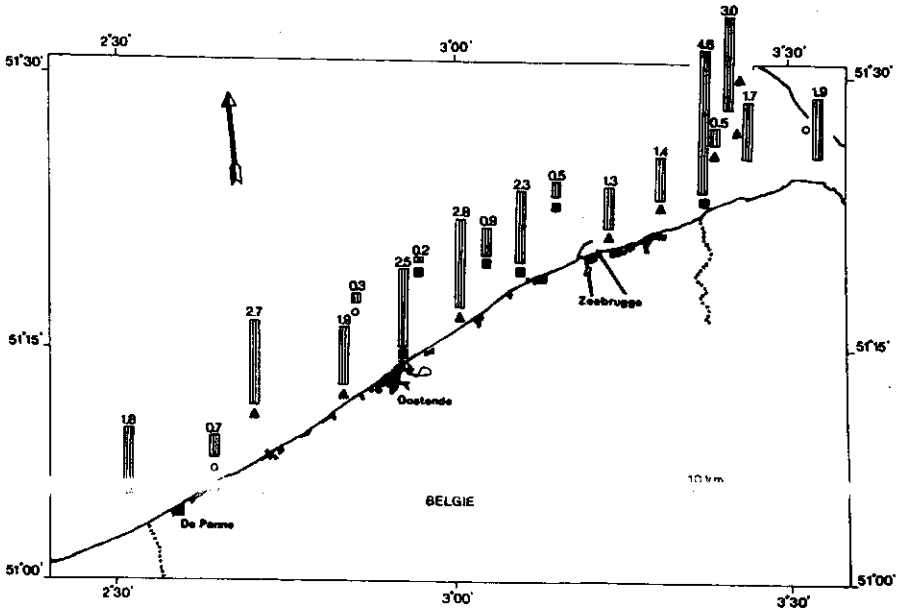


fig. 7.

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 treated 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 extreme poverty of the coastal area. The influence of the river Westerschelde 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.

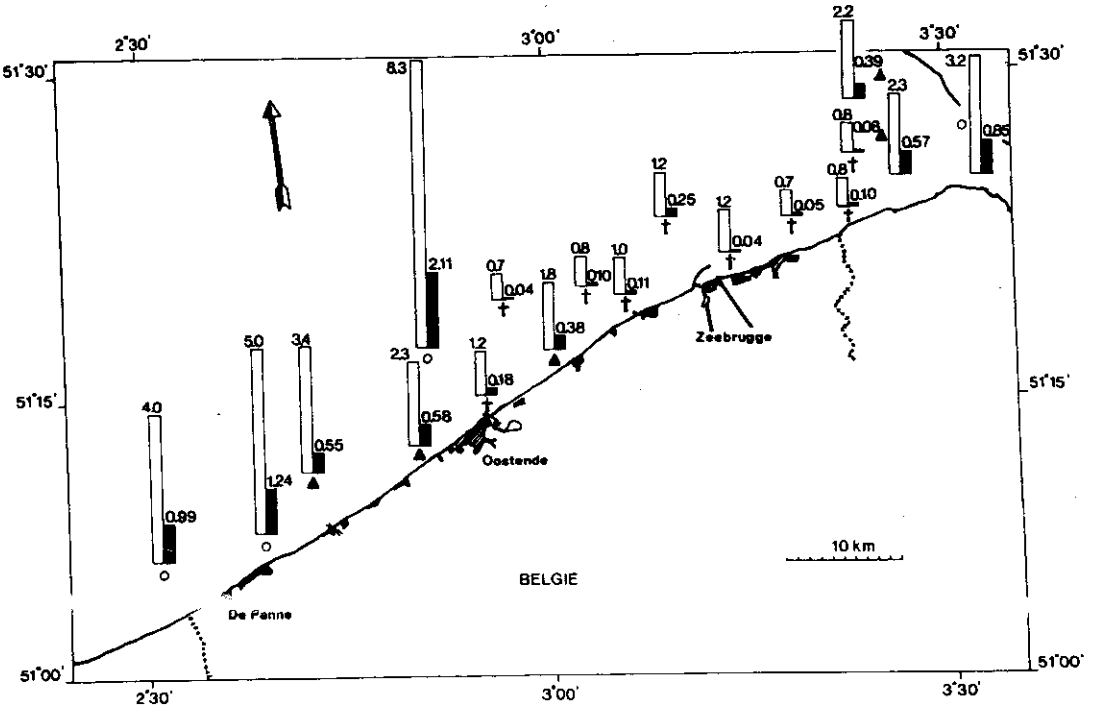


fig. 8.

Mean number of species $\bar{s}$ (open bars), mean diversity H (shaded bars) and mean density of harpacticoid copepods per station († < 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. Excluding 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 nematode.

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

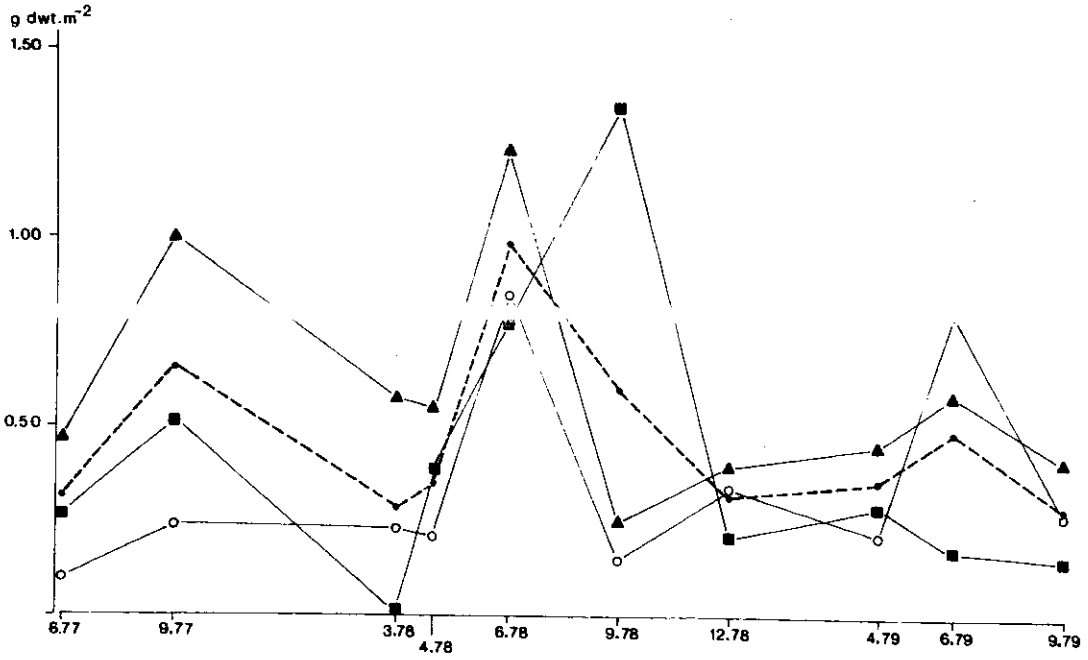


fig. 9.

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).

[illegible] tive 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

R lative abundance of nematodes in the Belgian coastal area

	M01		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	-	-	-	-
1B *Daptonema normandicum*	-	-	-	-	8	-	-	75	-	-	-	-	-
1B *Daptonema tenuispiculum*	-	-	-	-	-	75	-	-	-	1	-	15	-
1B *Theristus* sp. A	-	-	-	2	3	-	-	6	-	-	-	-	6
1B *Monhystera disjuncta*	-	-	1	-	-	-	-	-	10	-	-	35	-
1B *Metalinhomoeus* n.sp.	-	7	6	3	-	-	-	4	15	2	-	-	-
2B *Mononcholaimus separabilis*	2	-	-	-	-	-	-	-	-	-	-	-	5
2B *Mesacanthion diplechma*	-	-	-	-	-	-	-	-	-	-	-	-	2
2B *Viscosia viscosa*	-	-	-	4	1	-	1	-	-	-	-	-	-
1B *Richtersia inaequalis*	3	1	2	-	-	-	-	-	1	-	-	-	1
2A *Microlaimus marinus*	-	-	-	1	-	-	-	-	-	-	-	-	-
1B *Ascolaimus elongatus*	-	2	-	-	-	3	-	-	4	-	-	-	-
2B *Synonchiella* sp. A & B	-	-	-	1	-	-	-	-	-	-	-	-	-
2A *Hypodontolaimus* sp. A	-	2	-	-	5	-	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

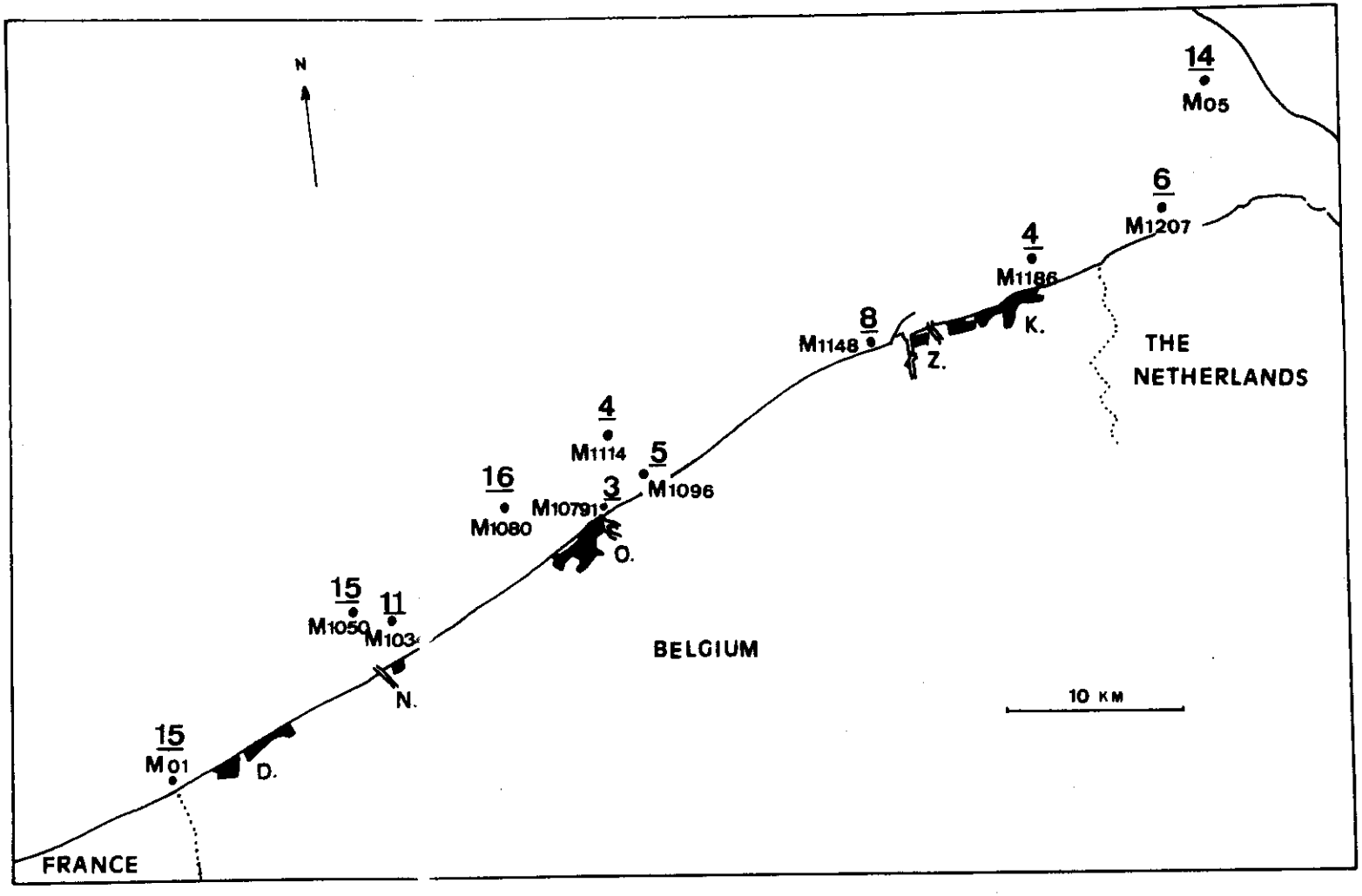


fig. 10.

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 macrobenthic *Abra alba*-community, and by a meiobenthic *Microarthridion littorale-Halectinosoma herdmani*-community. In their study nematodes were neglected.

Based on nematode species diversity (nematode family diversity) and on the presence absence of those species, the Belgian coastal waters can be clearly divided into two zones ; the division 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 harpacticoid species. According to Govaere *et al.* (1980) one can distinguish 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 community. In the muddy stations only five species, all large epibenthic or endobenthic forms, were found, while in muddy sand stations up to ten species were noted over the whole period. *Microarthridion littorale* is very dominant followed by *Halectinosoma herdmani*, *H. sarsi*, *H. gothiceps* and *Pseudobradya beduina*. The mean diversity is extremely low, as in 60% of the samples only one species (H = 0) or no species at alle were found. This results in a mean diversity of H = 0.13 bits/ind. for the muddy stations 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 $\bar{s}$ and Brillouin's diversity H per station group

	s	$\bar{s}$	H
sand	26	5.13	1.30
muddy sand	10	1.84	0.33
mud	5	0.95	0.13

Sandy stations are characterized by an impoverished harpacticoid 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 average 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 calculations are based on biomass and density measurements and the relationships between these parameters and respiration and production (Heip *et al.*, this volume).

Two contrasting situations are compared. In the Belgian coastal waters nematodes are extremely dominant and average densities are high. On the Kwintebank (Willems *et al.*, 1982),meiofauna is less abundant and nematodes are less dominant. The production 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 (l.half y^{-1}.m^{-2})	4.50	2.12
Respiration (l^{-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.µg $dwt^{-1}.h^{-1}$	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.

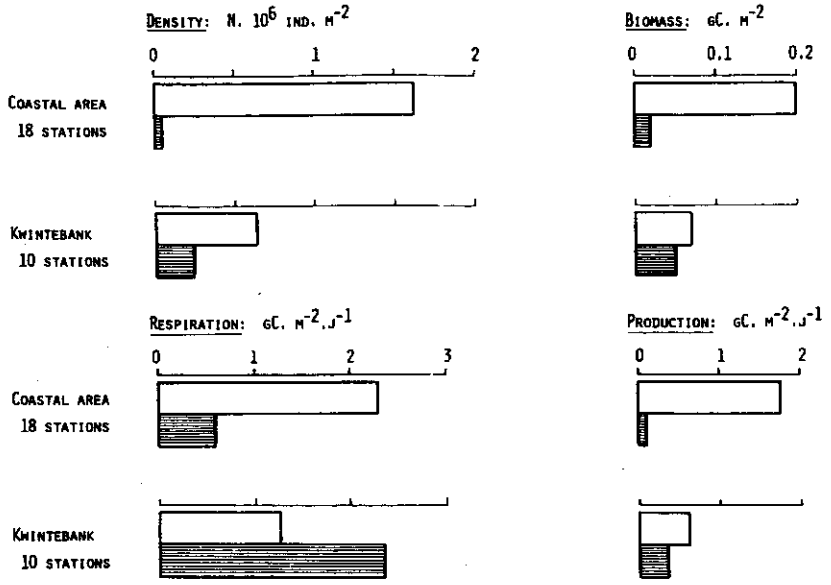


fig. 11.

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.

Acknowledgments.

We wish to thank the Management Team of the Mathematical Model of the North Sea (Ministry of Public Health) and the crew of the *R.V. Mechelen* and *R.V. Hasselt* for assistance on sea. Alex Braeckman, Mark De Keere and Dirk Van Gansbeke were responsable for competent technical assistance, for which we are immensely grateful. Carlo Heip and Magda Vincx acknowledge a grant from the Belgian National Science Foundation (N.F.W.O.).

This research was supported through the Concerted Actions Oceanography Project of the Ministry of Scientific Policy.

References.

GERLACH, S.A., (1971). On the importance of marine meiofauna for benthos communities. *Oecologia (Berl.)*, 6, 176-190.

GOVAERE, J.C.R., VAN DAMME, D., HEIP, C. & DE CONINCK, L.A.P., (1980). Benthic communities in the Southern Bight of the North Sea and their use in ecological monitoring. *Helgoländer wiss.Meeresunters.*, 33, 507-521.

GULLENTOPS, F., MOENS, M., RINGELE, A. & SENGIER, R., (1977). Geologische kenmerken van de suspensie en de sedimenten. In : C.F. Nihoul & F. Gullentops, *Nationaal Onderzoeks- en Ontwikkelingsprogramma - Projekt Zee*, Vol. 4, 1-121.

HEIP, C., HERMAN, R., BISSCHOP, G., GOVAERE, J., HOLVOET, M., VAN DAMME, D., VANOSMAEL, C., WILLEMS, K. & DE CONINCK, L.A.P., (1979). Benthic studies of the Southern Bight of the North Sea and its adjacent continental estuaries. *Progress Report I.ICES, CM* L/9, 133-163.

HEIP, C., HERMAN, P.M.J., SMOL, N., VAN BRUSSEL, D. & VRANKEN, G., Benthic studies in the Southern Bight of the North Sea and its adjacent continental estuaries. III. Energy flow through the meiobenthos. (This volume).

HERMAN, P.M.J. & HEIP, C., (in press). Growth and respiration of *Cyprideis torosa* Jones, 1850 (Crustacea, Ostracoda). *Oecologia (Berl.)*.

HUMPHREYS, W.F., (1979). Production and respiration in animal populations. *J.Animal.Ecol.*, 48, 427-453.

LANCE, G.N. & WILLIAMS, W.T., (1967). A general theory of classifactory strategies. I. Hierarchical systems. *Comput.J.*, 9, 373-380.

VAN DAMME, D. & HEIP, C., (1977). Het meiobenthos in de zuidelijke Noordzee. In : C.F. Nihoul & L.A.P. De Coninck (Eds), *Nationaal Onderzoeks- en Ontwikkelingsprogramma - Projekt Zee*, Vol. 7, 114 pp.

WARWICK, R.M. & PRICE, R., (1979). Ecological and metabolic studies on free-living nematodes from an estuarine mud flat. *Est.Coast.Mar.Sci.*, 9, 257-271.

WIESER, W., (1953). Die Beziehung zwischen Mundhohlengestalt, Ernahrungsweise und Vorkommen bei freilebenden marinen Nematoden. *Archives fur Zoologie*, 4(26), 439-484.

WILLEMS, K.A., VINCX, M., CLAEYS, D., VANOSMAEL, C. & HEIP, C., (1982). Meiobenthos of a sublittoral sandbank in the Southern Bight of the North Sea. *J.mar.Biol.Ass.U.K.*, 62, 535-548.

V

BENTHOS OF THE KWINTE BANK

(An exploited sandbank in the Southern Bight)

C. VANOSMAEL, K. WILLEMS, M. VINCX, D. CLAEYS and C. HEIP

Marine Biology Section, Zoology Institute, State University of Gent, Belgium

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 parallel 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, transported by the stream draining the waters from the present rivers Rhine, Meuse, Scheldt and Thames, before the Flandrian marine transgression. During and after the postglacial transgression of the North Sea these deposits got their present sandbank-shape (Houbolt, 1968).

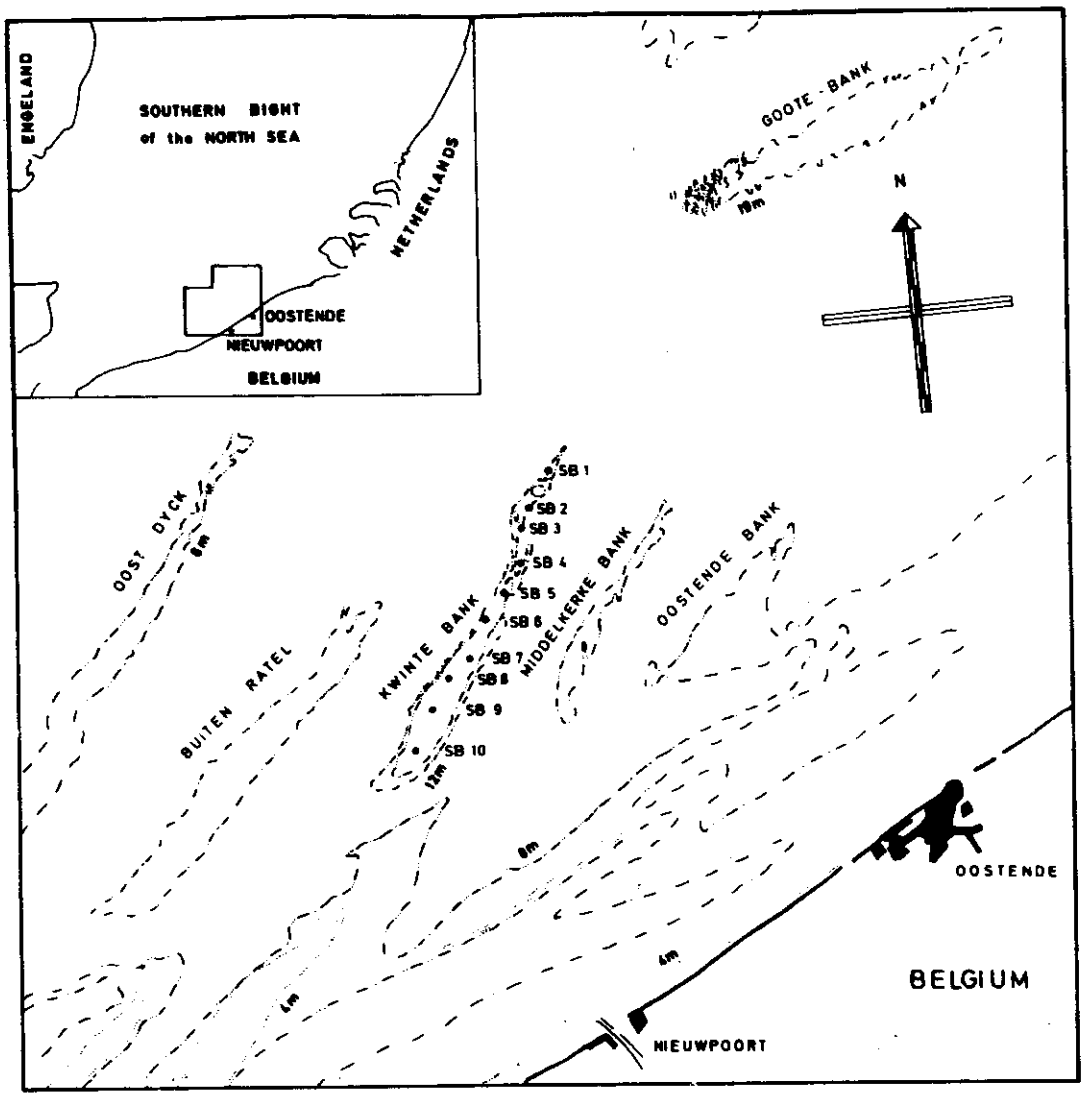


fig. 1.

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 environments 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.

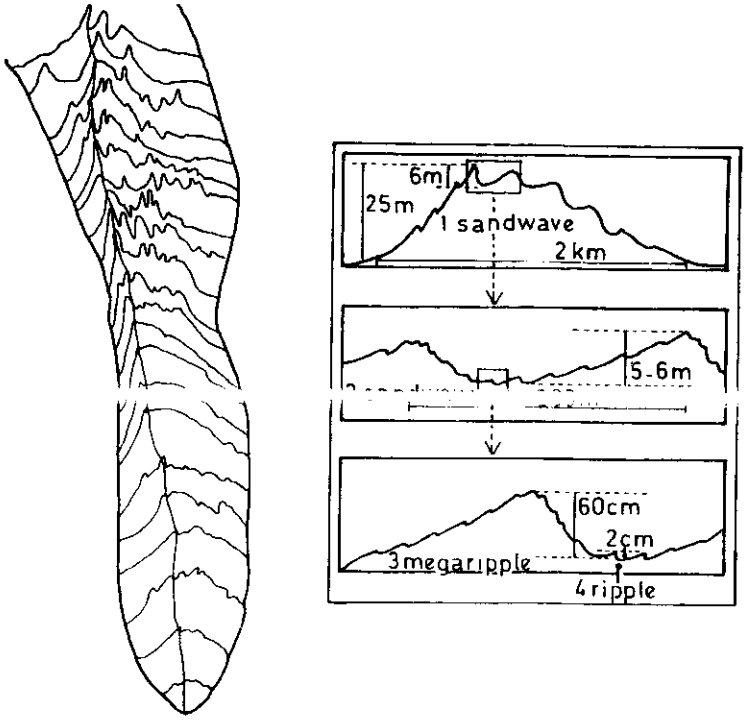


fig. 2.

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 ($\beta = -0.25$) [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 :

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

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 macrocrustaceans 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.		(Ø)	(µm)	QD Ø	Sk Ø			
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 ( ) 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	6 .3	542± 55	72.1	682±329	80.7	1047±151	68.3	602±64	82.8
Archiannelida	18± 14	.5	160± 58	21.3	104± 34	12.3	87± 28	5.7	68±14	9.4
Oligochaeta	26± 13	.7	16± 8	2.1	15± 3	1.8	80± 42	5.2	20± 6	2.8
Nemertini	21± 10	.3	20± 10	2.6	24± 6	2.9	62± 25	4.0	26± 4	3.6
Mollusca	34± 31	1 .3	13± 4	1.8	19± 1	2.2	165± 67	10.8	2± 1	0.3
Crustacea	3± 1	.8	0	0	*	0.1	5± 2	0.3	8± 2	1.2
Echinodermata	*	.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	6 .8	22±5	42.8	14± 3	27.7	67± 24	50.8	105± 49	50.6
Archiannelida	31± 11	1 .1	*	1.9	0	0	27± 19	20.4	61± 46	29.5
Oligochaeta	15± 3	.3	0	0	*	0.6	*	0.5	*	0.3
Nemertini	42± 19	1 .0	8±0.3	15.9	4± 2	7.4	23± 5	17.8	27± 15	12.9
Mollusca	6± 3	.2	*	1.9	0	0	2± 1	1.2	*	0.5
Crustacea	5± 1	.6	10±6	37.4	32± 7	64.3	12± 4	9.4	13± 6	6.1
Echinodermata	*	.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	7 .1
Archiannelida	56± 12	1 .4
Oligochaeta	17± 6	.5
Nemertini	26± 4	.3
Mollusca	24± 11	.9
Crustacea	10± 2	.0
Echinodermata	9± 7	.8
Total	491± 91	

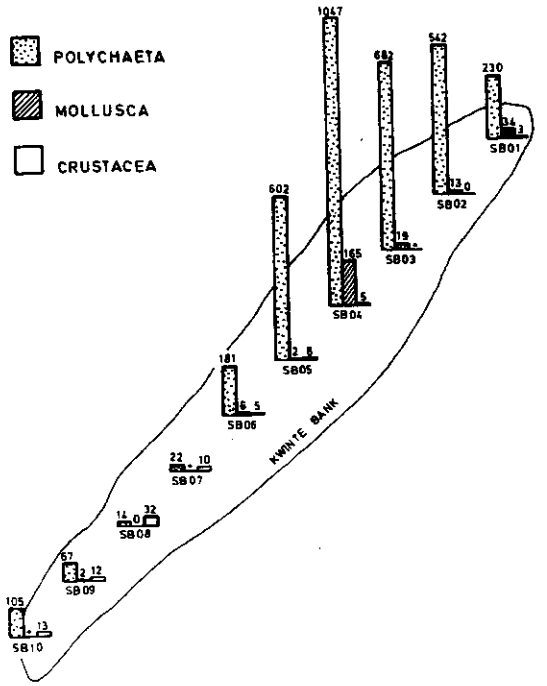


fig. 3.

Density of macrobenthos (N/0.1 m^2)

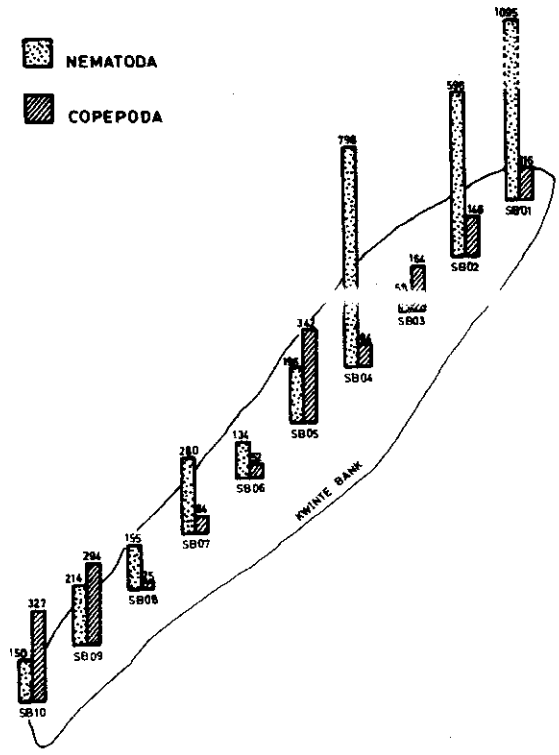


fig. 4.

Density of meiobenthos (N/10 cm^2)

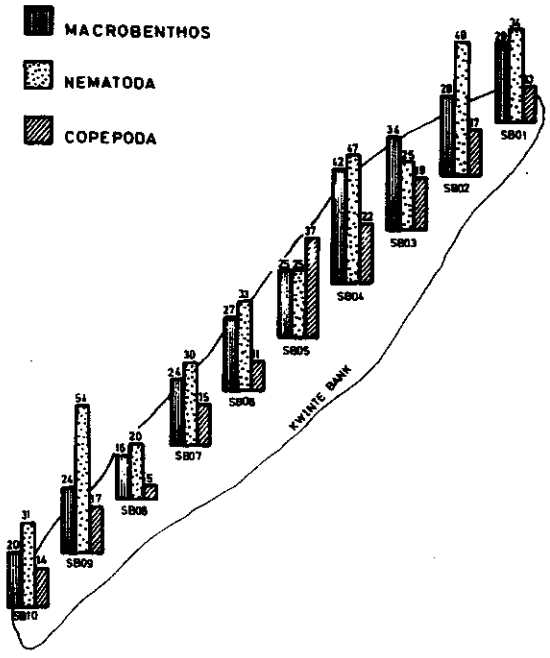


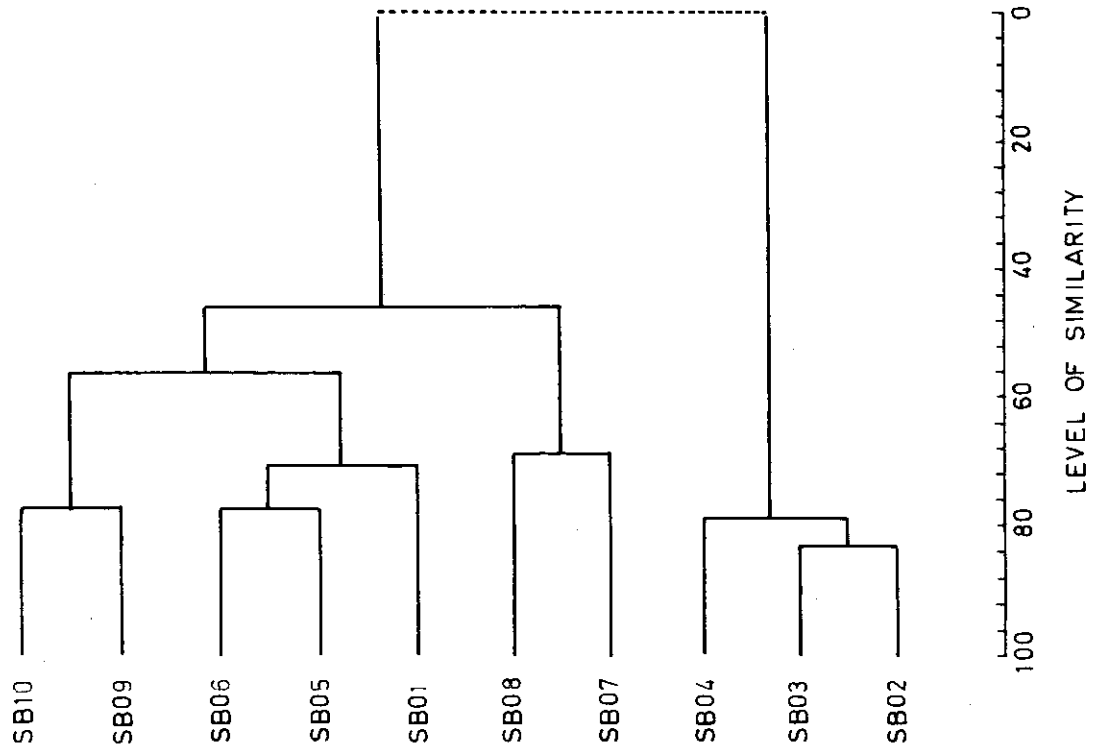
fig. 5.
Number of species per station

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	[illegible]	[illegible]
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 cm^2) 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		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
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	

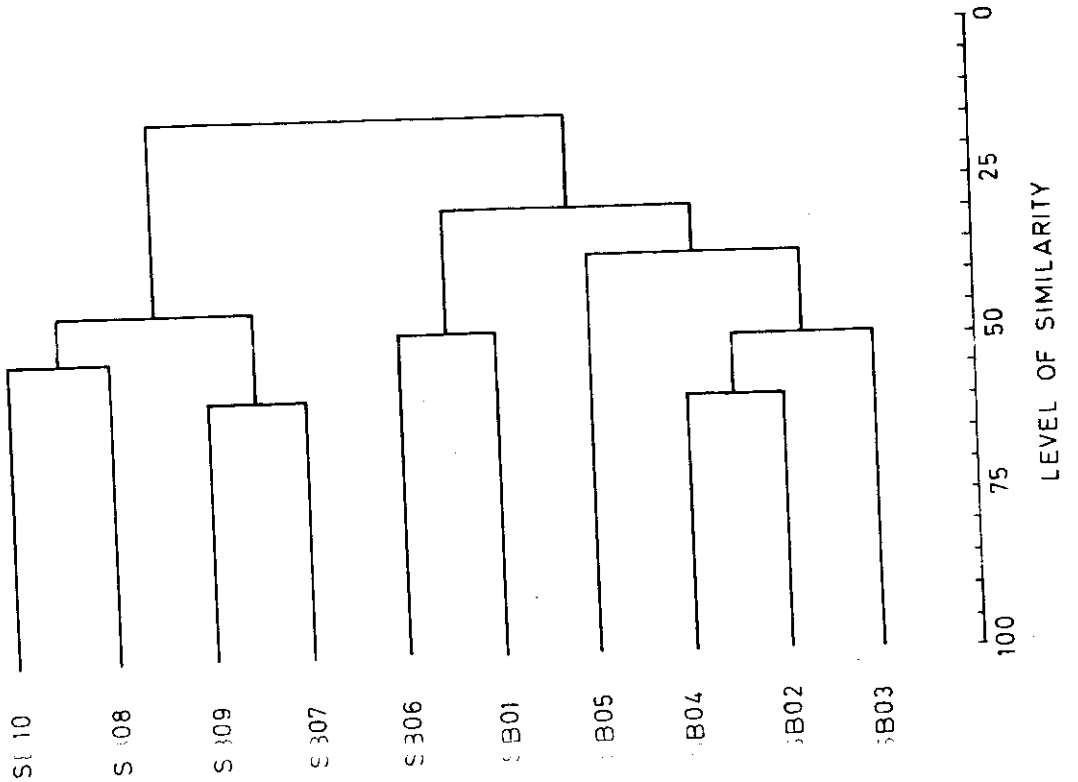
Meiobenthos.

(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).

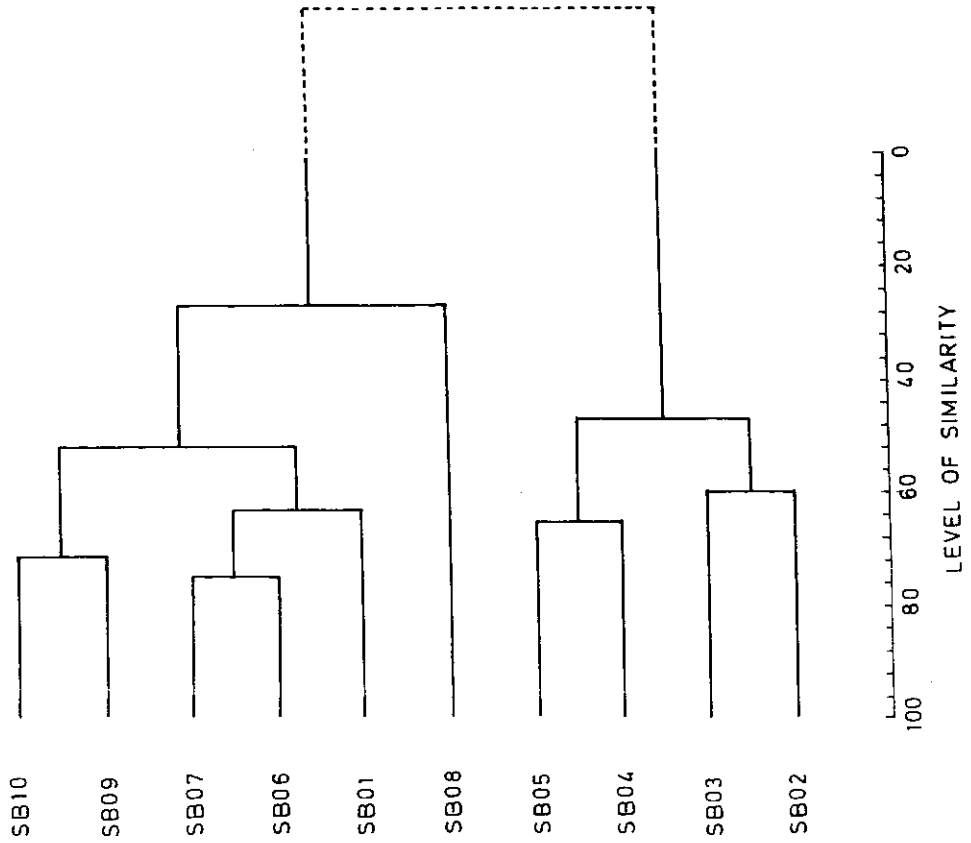
Group I (SB2, SB3, SB4, SB5) : clusters the medium to coarse sand stations and is characterized by high densities. The mean number of species per station is higher than in group II.

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



	Group II	Group I
Median particle diameter (μm)	188-281	375-654
Mud < 63 μm	0-1.6	0-0.3
[illegible]	[illegible]	0.2-10.6
Density (N/10 cm²; 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.

Acknowledgments.

This research was sponsored and actively supported by the Management Team of the Mathematical Model of the North Sea (Ministry of Public Health). We especially thank J.P. Mommaerts and H. Picard.

For help in the field we are grateful to the crew of the *R.V. Mechelen* and especially Alex Braeckman, Mark De Keere, R. Herman and Dirk Van Gansbeke.

Carlo Heip and Magda Vincx acknowledge a grant from the Belgian National Science Foundation (N.F.W.O.).

D. Claeys and C. Vanosmael acknowledge a grant from the Institute for the Encouragement of Scientific Research in Industry and Agriculture (IWONL).

References.

BASTIN, A. (1974). *Regionale sedimentologie en morfologie van de Zuidelijke Noordzee en van het Schelde-estuarium.* Ph. D. Thesis, Catholic University of Leuven, 91 pp.

BLOOM, S.A., SANTOS, S.L. and FIELD, J.G. (1977). A package of computer programs for benticc community analysis. *Bull. Mar. Sci.*, 27, 577-580.

BUCHANAN, J.B. and KAIN, J.H. (1971). *Measurement of the physical and chemical environment.* In : *Methods for the Study of Marine Benthos*, IBP Handbook N° 16, Eds. N.A. Holme and A.D. McIntyre, Blackwell Scient. Publ., Oxford.

CLIFFORD, H.T. and STEPHENSON, W. (1975). *An Introduction to numerical classification.* Academic Press, San Francisco, 129 pp.

GOVAERE, J.C.R. (1978). *Numerieke analyse van het makrobenthos in de Southern Bight (Noordzee).* Ph. D. Thesis, State University of Gent, 220 pp.

GOVAERE, J.C.R., VAN DAMME, D., HEIP, C. and DE CONINCK, L.A.P. (1980). Benthic communities in the Southern Bight of the North Sea and their use in ecological monitoring. *Helgoländer wiss. Meeresunters*, 33, 507-521.

HOUBOLT, J.J.H.C. (1968). Recent sediments in the Southern Bight of the North Sea. *Geologie en Mijnbouw*, 47, 245-273.

SØRENSEN, T. (1948). A method of establishing groups of equal amplitude in plant sociology based on similarity of species content and its application to analysis of vegetation on Danish commons. *Biologiske Skrifter*, 5, 1-34.

VANOSMAEL, C., WILLEMS, K.A., CLAEYS, D., VINCX, M. and HEIP, C. (1982). Macrobenthos of a sublittoral sandbank in the Southern Bight of the North Sea. *J. mar. biol. Ass. U.K.*, 62, 521-534.

WILLEMS, K.A., VINCX, M., CLAEYS, D., VANOSMAEL, C. and HEIP, C. (1982). Macrobenthos of a sublittoral sandbank in the Southern Bight of the North Sea. *J. mar. biol. Ass. U.K.*, 62, 535-548.

WILLEMS, K.A., VANOSMAEL, C., CLAEYS, D., VINCX, M. and HEIP, C. (1982). Macrobenthos of a sublittoral sandbank in the Southern Bight of the North Sea. General Considerations. *J. mar. biol. Ass. U.K.*, 62, 549-557.

VI

MACROBENTHOS IN THE WESTERN SCHELDE ESTUARY

Y.M. VERMEULEN and J.C.R. GOVAERE*

Marine Biology Section, Zoology Institute, State University of Gent, Belgium

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

* Present address : Oceanography Section, K.B.I.N., Vautierstraat, 29, 1040 Brussels (Belgium).

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 m^2 van Veen grab, littoral stations were hand-collected with a plastic core (5 cores of 77.8 cm^2 per sample), three samples on each station. The contents of the grabs or cores were fixed in buffered formaldehyde 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.

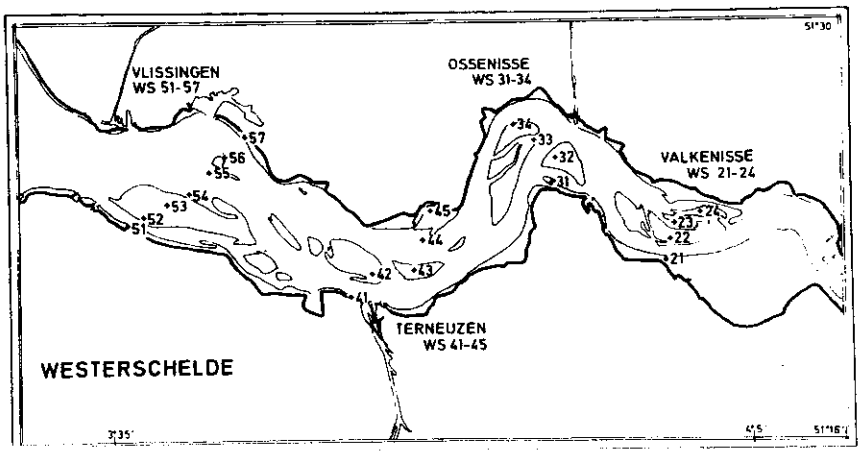


fig. 1.

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 i^{th} 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

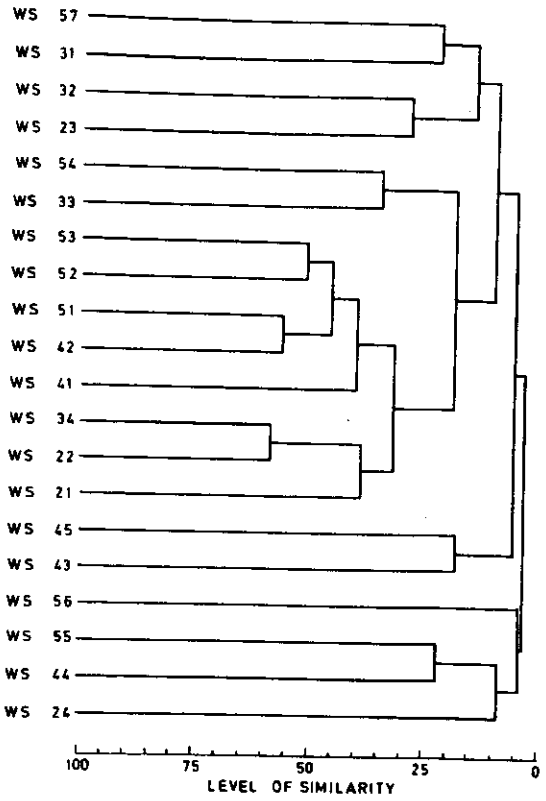


fig. 2.

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.

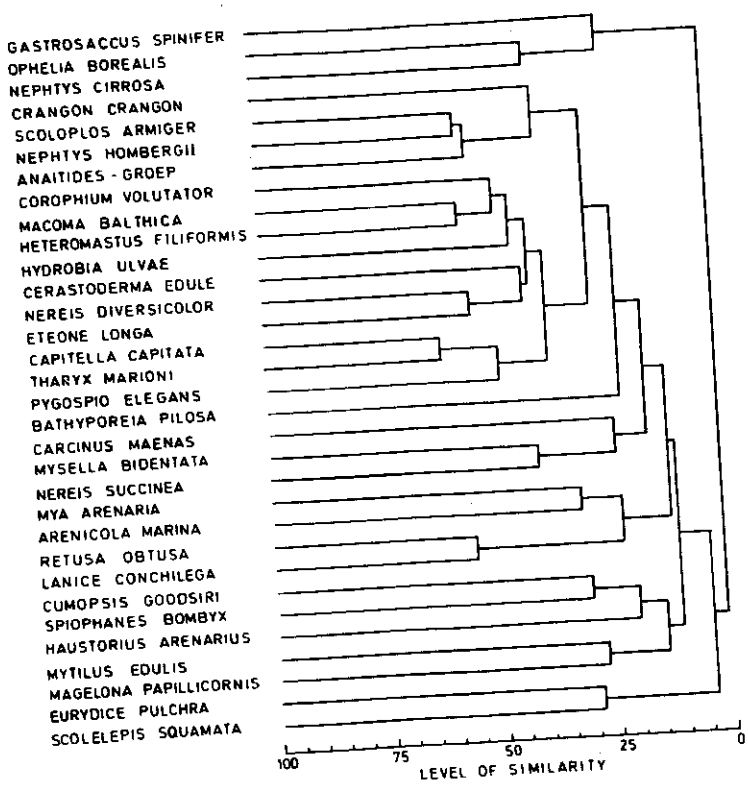


fig. 3.

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 correlation 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.

Acknowledgments.

We wish to thank Rudy Herman and the crews of the *R.V. Welsingen* and *Wijtvliet* (Rijkswaterstaat, The Netherlands) for their competent assistance in the field.

Y.M. Vermeulen acknowledges a grant from the Institute for Scientific Research in Industry and Agriculture (I.W.O.N.L.).

References.

BRILLOUIN, L.,(1956). *Science and information theory*, Academic Press, New York, 320 pp.

GOVAERE, J.C.R., VAN DAMME, D., HEIP, C. & DE CONINCK, L.A.P., (1980). Benthic communities in the Southern Bight of the North Sea and their use in ecological monitoring. *Helgoländer Meeresuntersuchungen*, 33, 507-521.

HEIP, C., HERMAN, R., BISSCHOP, G., GOVAERE, J.C.R., HOLVOET, M., VAN DAMME, D., VANOSMAEL, C., WILLEMS, K.R. & DE CONINCK, L.A.P., (1979). Benthic studies of the Southern Bight of the North Sea and its adjacent continental estuaries. Progress Report I. *International Council for the Exploration of the Sea*, C.M., 1979/L, 9, 1-30.

JONES, N.S., (1950). Marine bottom communities. *Biological Review*, 25, 283-313.

LANCE, G.N. & WILLIAMS, W.T., (1967). A general theory of classificatory sorting strategies. I. Hierarchical Systems. *Computer Journal*, 9, 373-380.

PETERSEN, C.J.G., (1914). Valuation of the sea. II. The animal communities of the sea bottom and their importance for marine zoogeography. *Report of the Danish Biological Station to the Board of Agriculture*, 21, 1-44.

SOKAL, R.R. & SNEATH, P.H.A., (1963). *Principles of Numerical Taxonomy*, Freeman, San Francisco, 359 pp.

VAN DAMME, D., HERMAN, R., SHARMA, Y., HOLVOET, M. & MARTENS, P., (1980). Benthic studies of the Southern Bight of the North Sea and its adjacent continental estuaries. Progress Report II. Fluctuations of the meio-benthic communities in the Westerschelde estuary. *International Council for the Exploration of the Sea*, C.M., 1980/L, 23, 131-170.

WAKEEL EL, S.K. & RILEY, J.P., (1957). The determination of organic carbon in marine muds. *Journal du Conseil*, 22, 180-183.

WOLFF, W.J., (1973). The estuary as a habitat, an analysis of data on the soft-bottom macrofauna of the estuarine area of the rivers Rhine, Meuse, and Scheldt. *Zoologische Verhandelingen*, 126, 1-242.

VII

A SUMMARY OF BENTHIC STUDIES

IN THE SLUICE DOCK OF OSTEND DURING 1976-1981

L.K.H. THIELEMANS, C. HEIP and D. VAN GANSBEKE

Marine Biology Section, Zoology Institute, State University of Gent, Belgium

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/m^2 and the harpacticoids decreased to 371,000 ind/m^2. 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/m^2. Its production was measured with a cohort-analysis and production amounted to 3.43 g/m^2 in 1976 and 1.73 g/m^2 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/m^2). In summer a maximum density of 29,000 ind/m^2 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/m^2. 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/m^2, with a maximum of 96,000 ind/m^2 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.

Acknowledgments.

This research was supported by grant 2.0010.78 of the Foundation for Collective Fundamental Research (F.K.F.O).

References.

BROCK, V. (1979). Habital selection of two congeneric bivalves *Cardium edule* and *C. glaucum* in sympatric and allopatric populations. *Marine Biology*, 54, 149-156.

BULTHEEL, M. (1981). *Recolonisatie door macrobenthos van een verstoord zandsediment in de Spuikom van Oostende*, R.U.G., Ms. Sc. Thesis, 57 pp.

FREDERICQ, S. (1980). *Fauna-associatie van* Ulva lactuca *in de Spuikom te Oostende*, R.U.G., Ms. Sc. Thesis, 59 pp.

HUYSSEUNE, A. (1978). *Levenscyclus en produktie van* Canuella perplexa *in de Spuikom te Oostende*, R.U.G., Ms. Sc. Thesis, 49 pp.

LELOUP-MILLER (1940). La flore et la faune du Bassin de Chasse d'Ostende (1937-1938). *K.B.I.N.*, 94, 122 pp.

LELOUP and VAN MEEL (1965). La flore et la faune du Bassin de Chasse d'Ostende. *K.B.I.N.*, 154.

LELOUP and POLK (1967). La flore et la faune du Bassin de Chasse d'Ostende. *K.B.I.N.*, 157, 114 pp.

PEARSON and ROSENBERG (1978). Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanogr. Mar. Biol. Ann. Rev.*, 16, 229-311.

PODAMO, Jo. (1976). Ecometabolism of a shallow marine lagoon at Ostend (Belgium), I-V. *10th E.M.B.S.*, 485-562.

POLK, Ph. (1978). The Sluice Dock at Ostend. *Rapp. P. v. Réun. Cons. int. Explor. Mer*, 173, 43-48.

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

J.M. BOUQUEGNEAU[1], G. BILLEN[2], C. JOIRIS[3], M.H. DARO[3], C. LANCELOT[2], J.H. HECQ[4], A. BERTELS[3], M. BOSSICART[3], M. SOMVILLE[2], J.P. MOMMAERTS[5], J. WIJNANT[3] and J.C. WEHREN[1]

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).

1. Laboratoire d'Océanologie, Université de Liège.
2. Laboratoire d'Océanographie, Université libre de Bruxelles.
3. Laboratorium voor Ekologie en Systematiek, Vrije Universiteit Brussel.
4. Laboratoire de Biologie marine, Université de Liège.
5. Unité de Gestion du Modèle mathématique de la mer du Nord et de l'estuaire de l'Escaut, Ministère de la Santé publique.

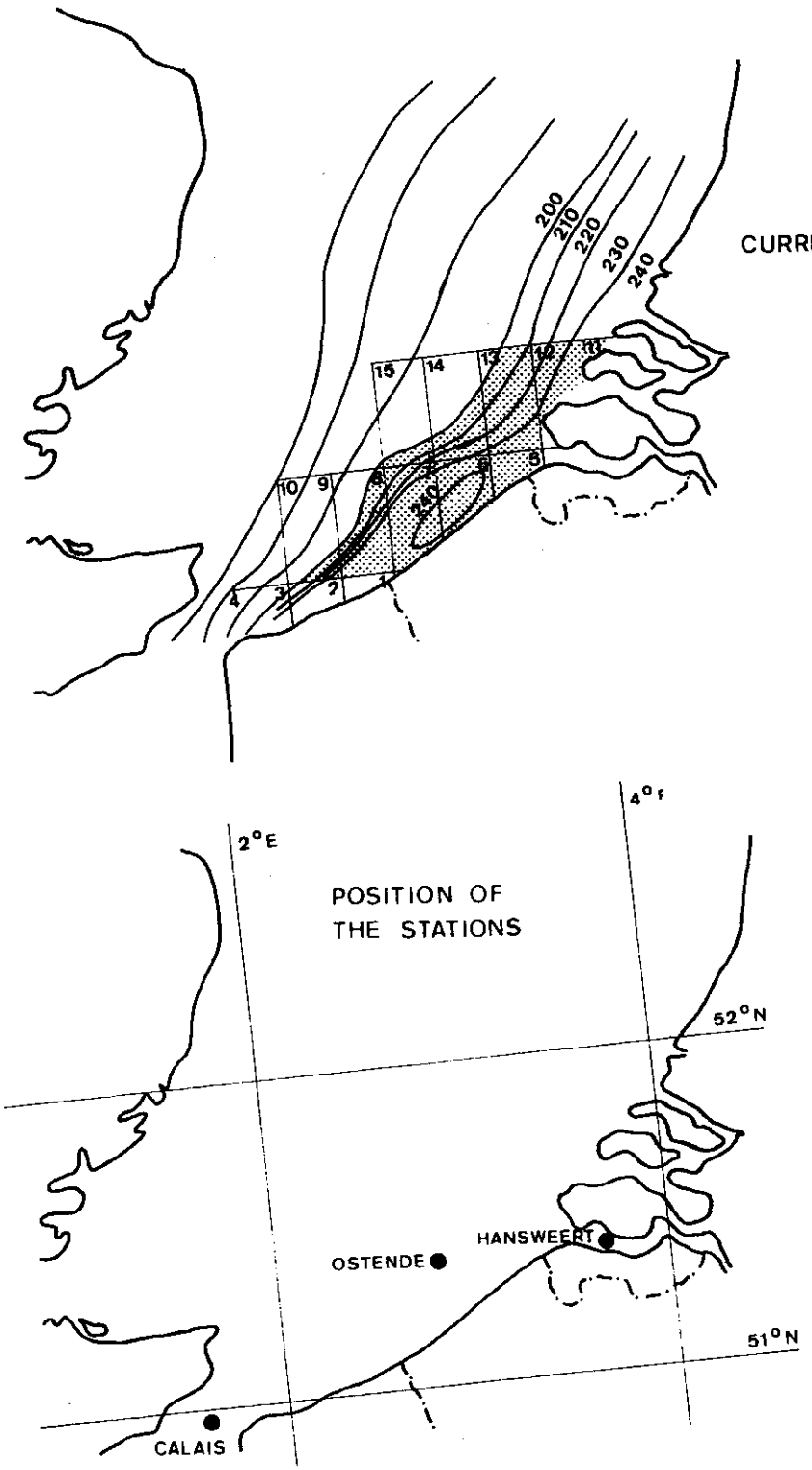


fig. 1.

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) g C/m^2.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).

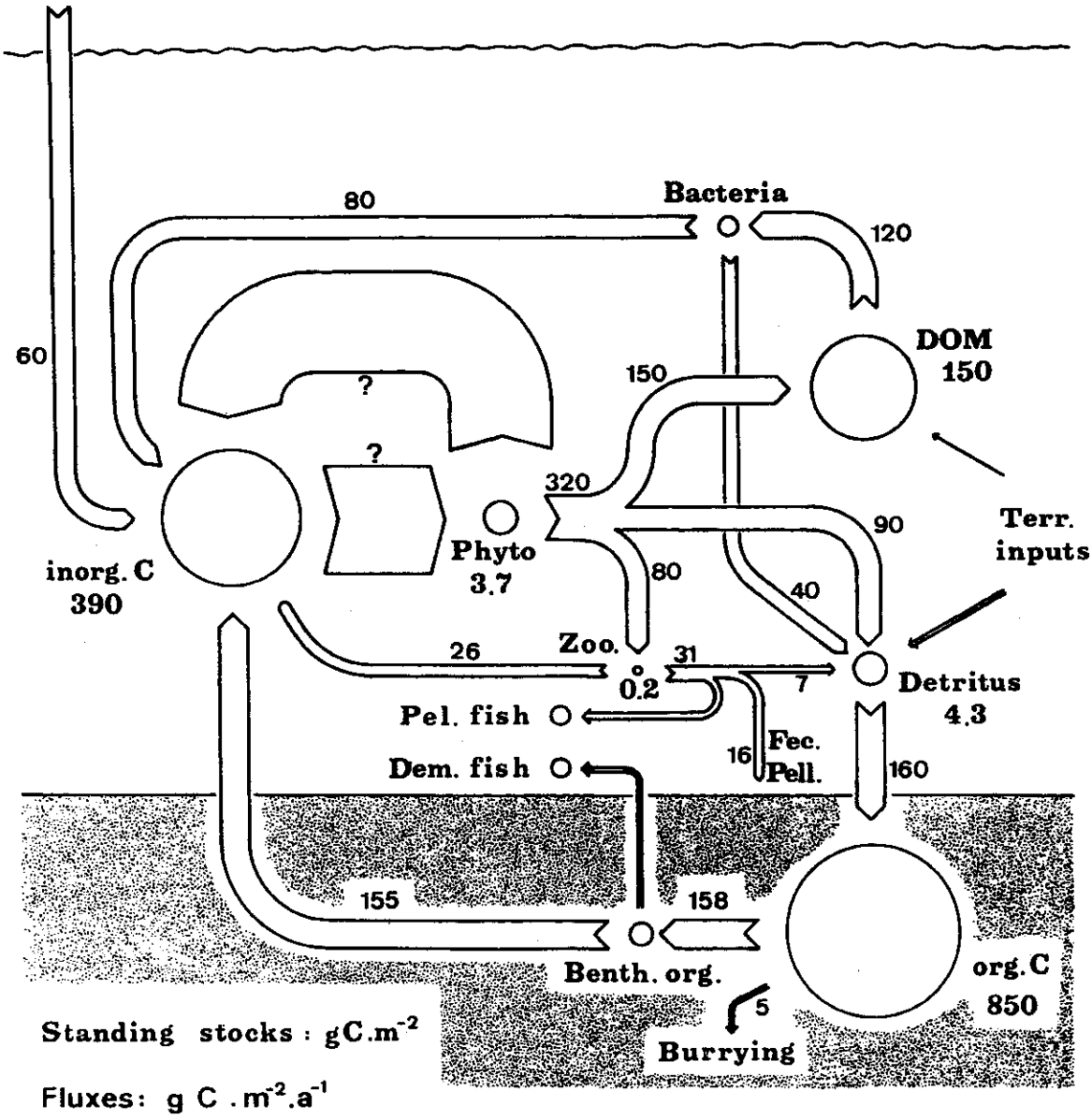


fig. 2.

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

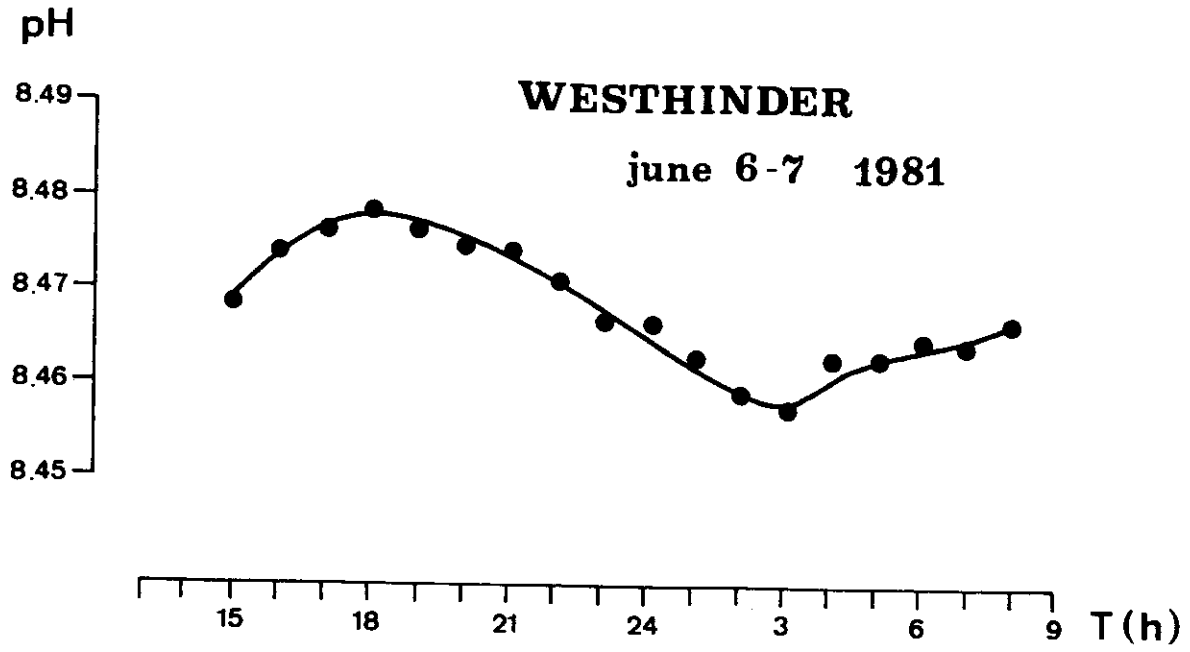


fig. 3.
Nycthemeral variation of pH

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

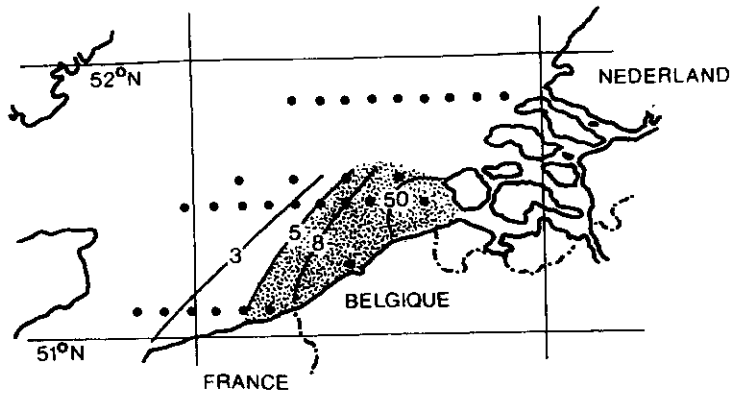


fig. 4.

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 phytoplankton biomass and of detritus concentrations (fig.5b), obtained during 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 production 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 detected in an adjacent area, cannot be excluded. In situ and semi-situ measurements of dissolved primary production show a relationship between extracellular release, expressed as a percentage of the total primary production, and the concentration of mineral nitrogen : excretion decreases with increasing nitrogen concentration (fig.6). This empirical relation was used for calculating the curve of seasonal evolution of dissolved primary production (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

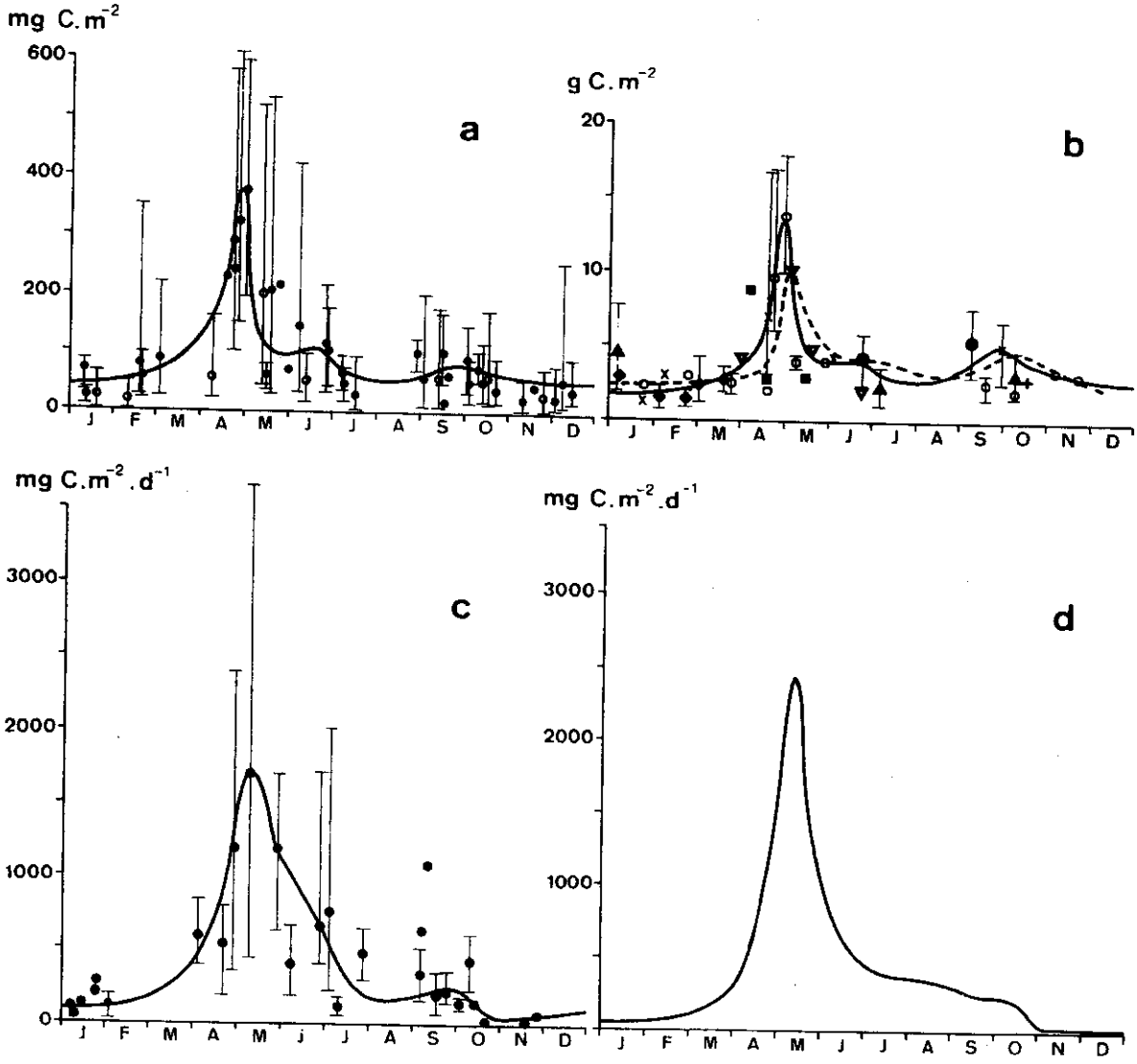


fig. 5.

(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/m^2 and the net primary production 320 g C/m^2.year.

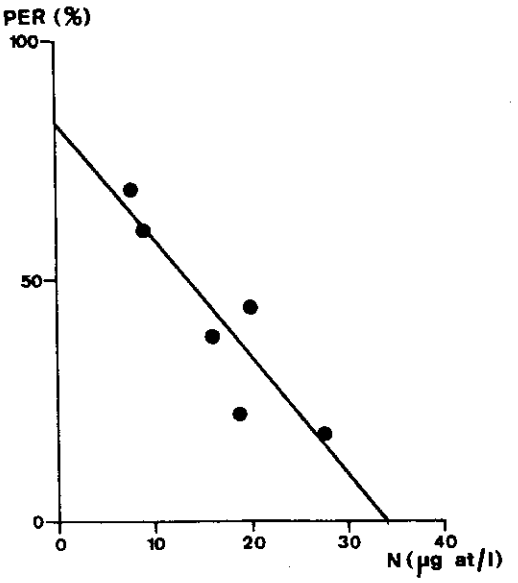


fig. 6.

Relationship between extracellular release and concentration of mineral nitrogen

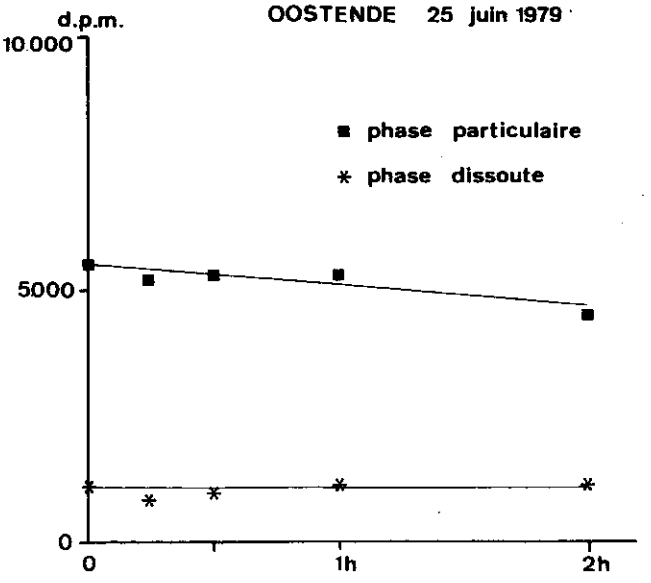


fig. 7.

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 heterotrophic 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 dissolved 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-heterotrophs (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 characterizing the Belgian coastal zone compared with the more "Atlantic" water masses. The station "Ostend", for which more data on heterotrophic activities are available, seems reasonably representative of the whole area. Planktonic respiration rates, on the other hand, do not display any clear pattern of spatial distribution.

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 utilization rate (reciprocal of the turnover time) obtained from the incorporation kinetic of the same radioactive substrate. The natural concentration of small substrates was shown to vary little around its equilibrium value set by the affinity of the microorganisms utilizing them (Billen et al, 1980). Most surveys of the concentration of amino acids or monosaccharides do indeed not reflect any significant seasonal evolution (e.g. Andrews & Williams,

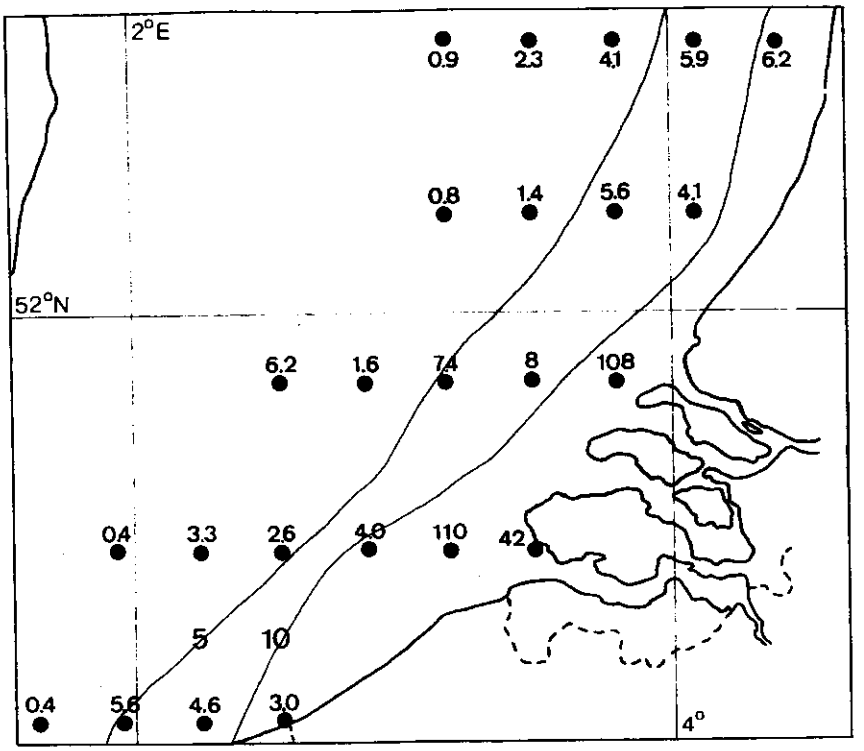


fig. 8.

Distribution of plate counts of bacteria in springtime

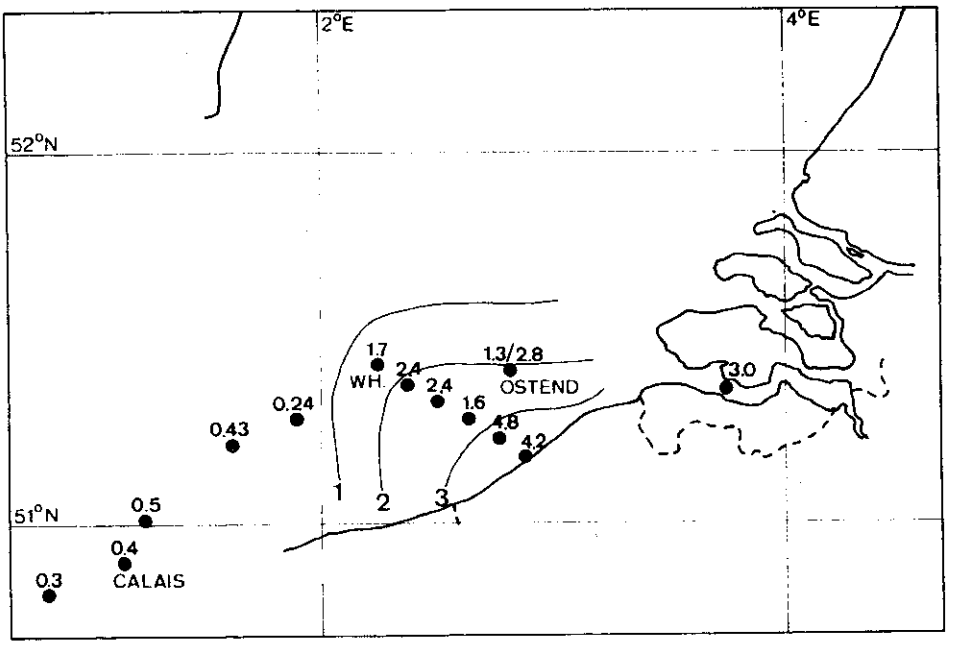


fig. 9.

Distribution of glucose utilization rates (% h^{-1})

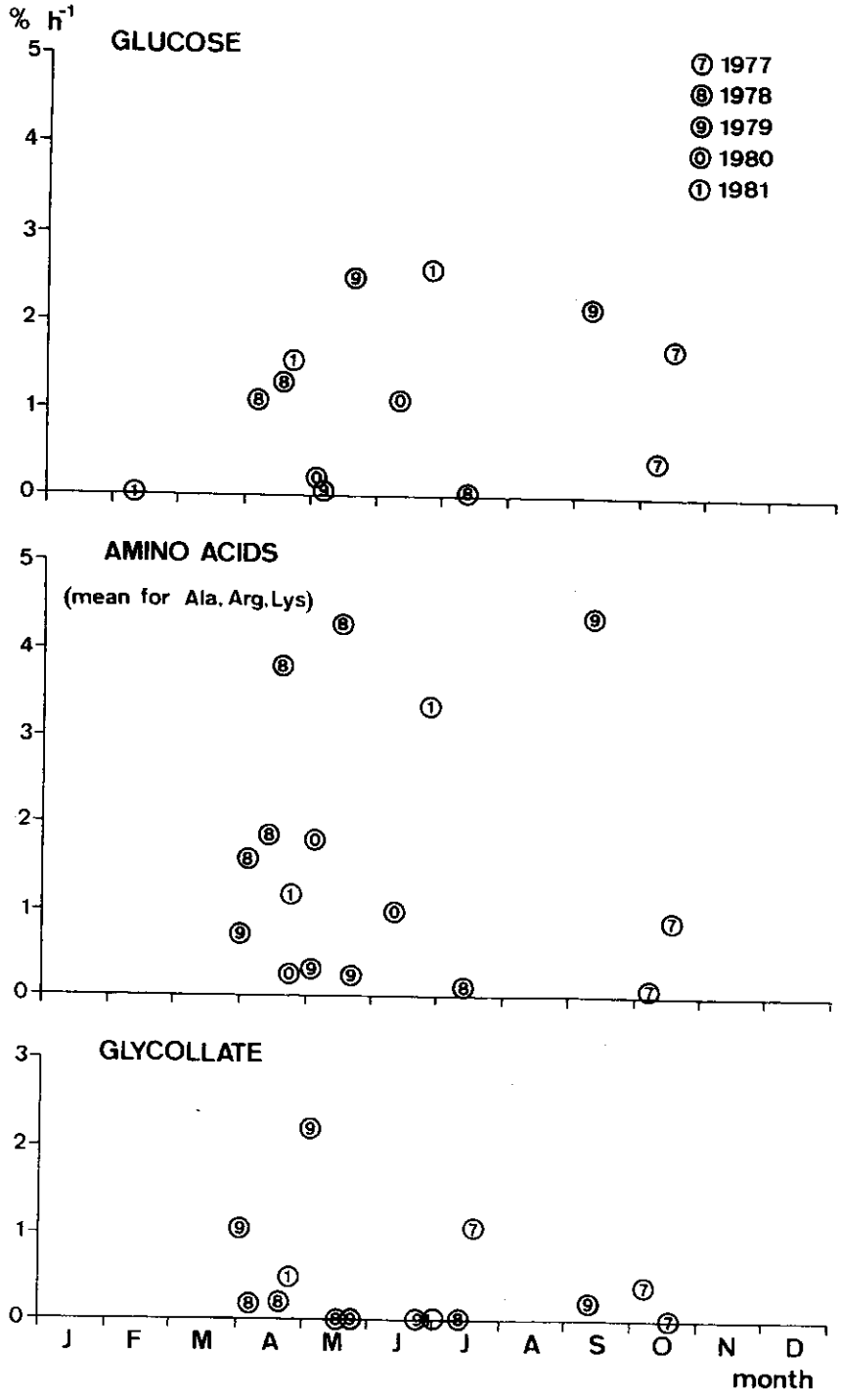


fig. 10.

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

1971 ; Crawford et al, 1974) : the variations of the absolute utilization rates are thus reflected by the variations of the relative 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 resulting 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 control by microheterotrophs.

Table 1

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

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 monosaccharides (0.8 µmol.ℓ^{-1}, 60 µg C.ℓ^{-1}); free amino acids (0.5 µmol.ℓ^{-1}, 25 µg C.ℓ^{-1}); glycollate (2 µmol.ℓ^{-1}, 50 µg C.ℓ^{-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.

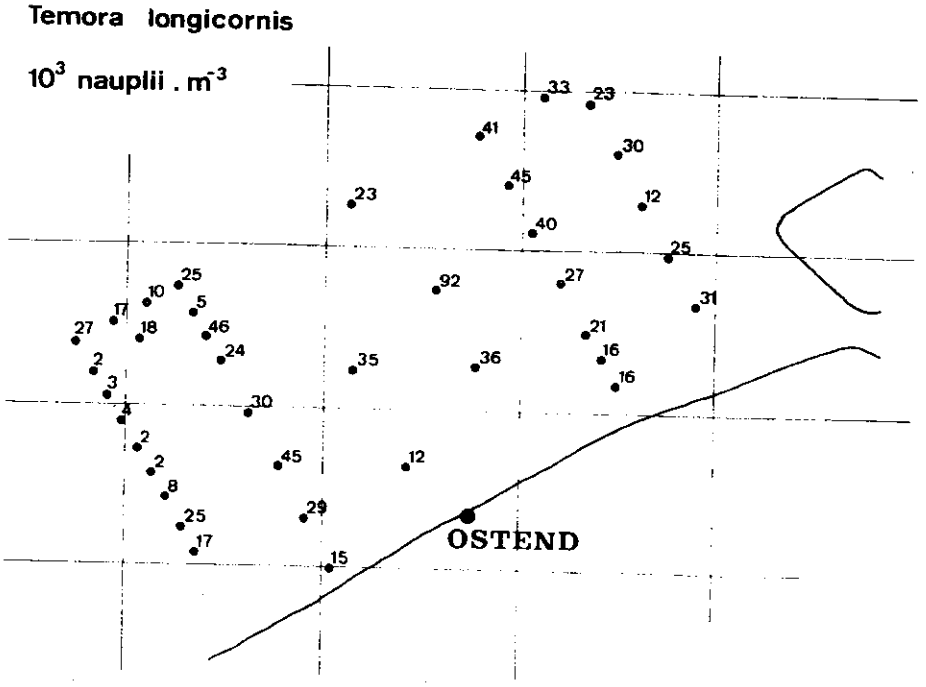


fig. 11.
Distribution of *Temora longicornis*

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).

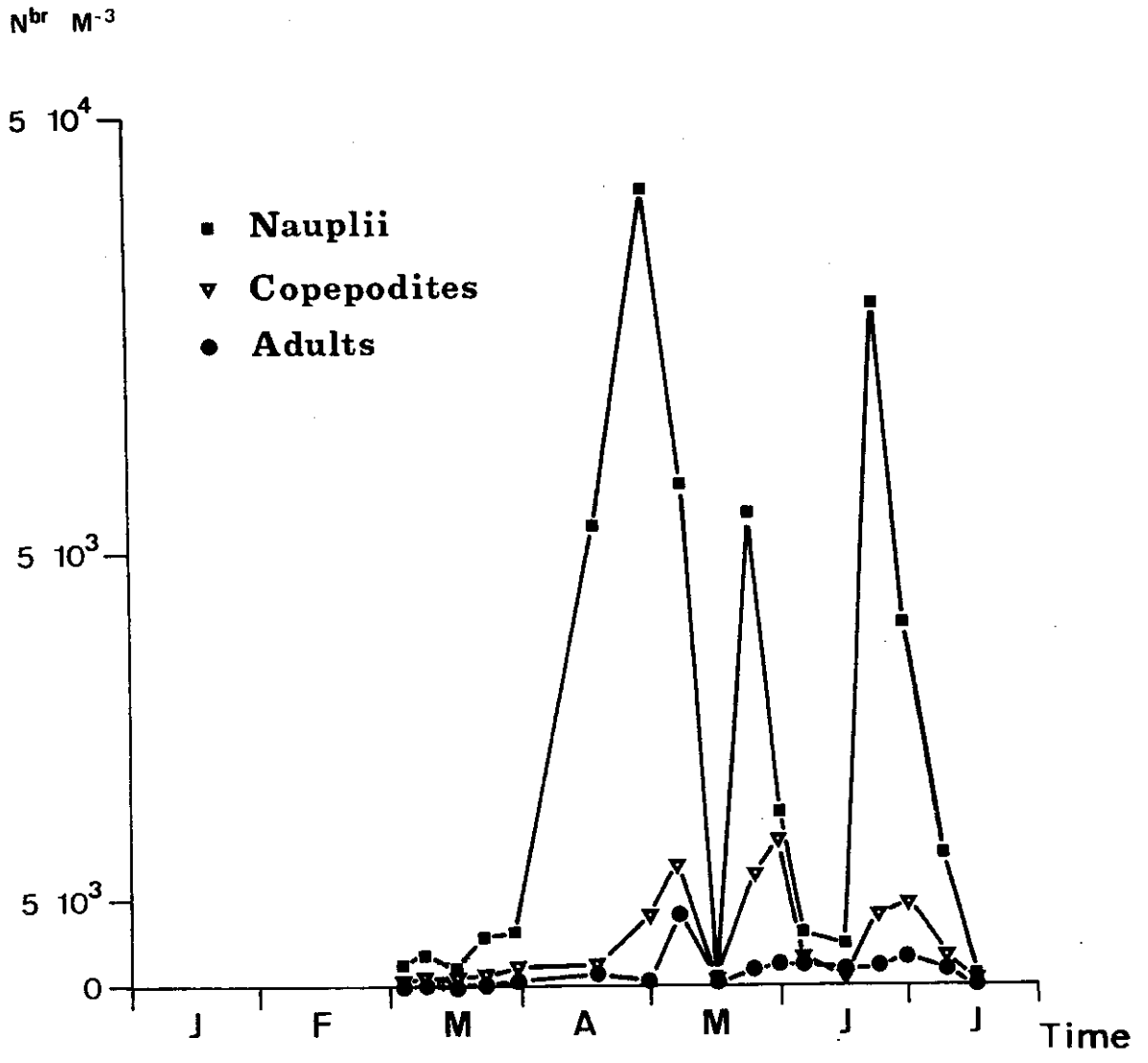


fig. 12.

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).

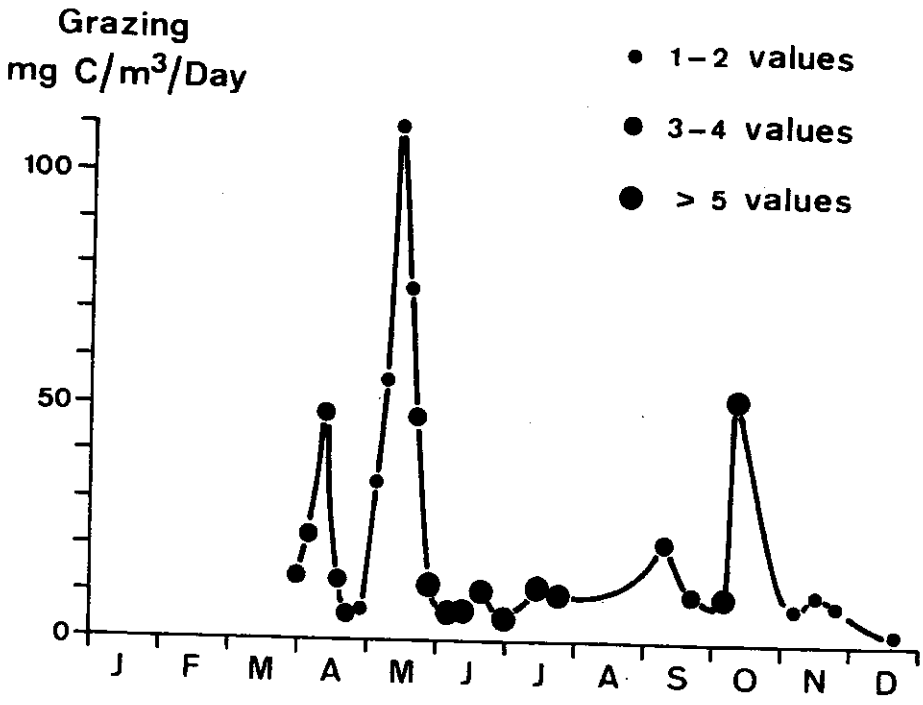


fig. 13.
Annual variation of grazing activity of zooplankton

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 matter content of the upper 1 cm of the sediment was used as an index 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 accumulation just in front of the Belgian coast.

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

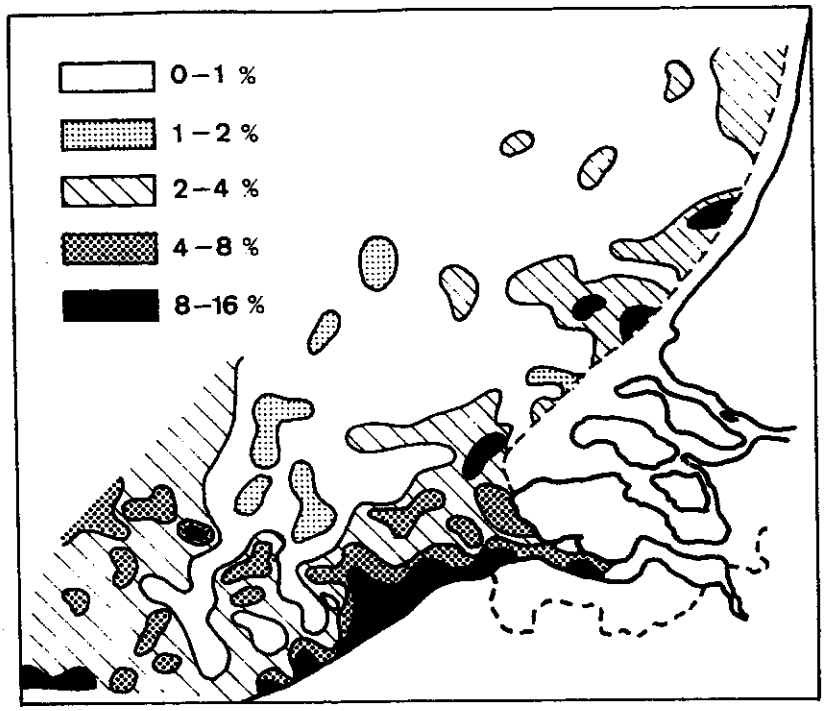


fig. 14.

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

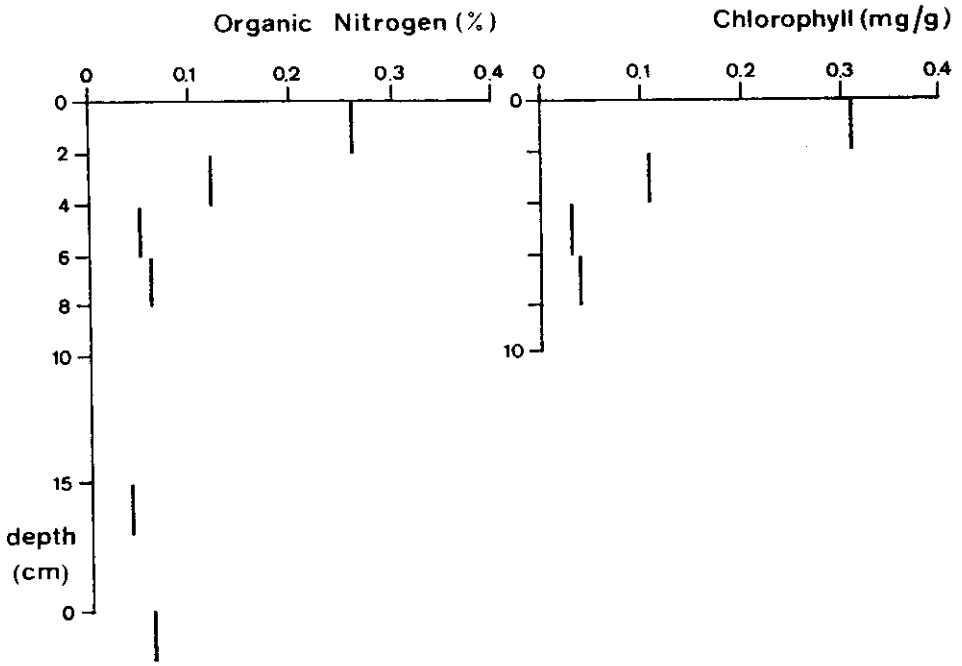


fig. 15.

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/m^2	Vernal budget (15 March - 15 July) g C/m^2
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/m^2.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 ecosystems 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 ecosystems, 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 % particulate primary production	Bacterioplankton activity % particulate primary	Bacterioplankton activity % dissolved 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 $C.m^{-3}d^{-1}$	

References.

ANDREWS, P. & WILLIAMS, P.J., (1971). Heterotrophic utilization of dissolved organic compounds in the sea. *J. Mar. Biol. Ass. U.K.*, **51**, 111-125.

AL HASAN, R.H., COUGLAN, S.J., PANT, A. & FOGG, G.E., (1975). Seasonal variations in phytoplankton and glycollate concentration in the Menai Strait. *J. Mar. Biol. Ass. U.K.*, **55**, 557-565.

BILLEN, G., (1982). *Modelling the processes of organic matter degradation and nutrient recycling in sedimentary systems* in *Sediment Microbiology*, D.N. Nedwell (Ed.), Academic Press, 15-52.

BILLEN, G., JOIRIS, C., WIJNANT, J. & GILLAIN, G., (1980). Concentrations and metabolisation of small organic molecules in estuarine, coastal and open sea environments in the Southern North Sea. *Est. Coast. Mar. Sci.*, **11**, 279-294.

BOSSICART, M., (1980). Population dynamics of copepods in the Southern Bight of the North Sea (1977). *I.C.E.S.*, C.M. 1980/L:24.

BURNEY, C.M., JOHNSON, K.M., LAVOIE, D.M. & SIEBURTH, Mc N., (1979). Dissolved carbohydrates and microbial ATP in the North Atlantic : concentrations and interactions. *Deep Sea Res.*, **26A**, 1267-1290.

CRAWFORD, C.C., HOBBIE, J.E. & WEBB, K.L., (1974). The utilization of dissolved free amino acids by estuarine micro-organisms. *Ecology*, **55**, 551-563.

CRISP, D. (Ed.), (1964). *Grazing in terrestrial and marine environments*. Blackwell Sci. Publ. Oxford, 322 pp.

CUSHING, (1958). The effect of grazing in reducing the primary production : a review. *Rapp. P.V. Réun. Cons. Perm. Int. Explor. Mer*, **144**, 149-155.

DARO, M.H., (1980). Field study of the deel feeding of a population of *Calanus finmarchicus* at the end of a phytoplankton bloom. *Meteor. Forsch. Ergb. R.A.*, **22**, 123-132.

DAWSON, R. & GOCKE, K., (1978). Heterotrophic activity in comparison to the free amino acid concentrations in Baltic sea water samples. *Oceanol. Acta*, **1**, 45-54.

EPPLEY, R.W., RENGER, E.H., VENRICK, E.L. & MULLIN, M.N., (1973). A study of plankton dynamics and nutrient cycling in the central gyre of the North Pacific Ocean. *Limnol. Oceanogr.*, **18**, 534-551.

FUHRMAN, J.A. & AZAM, F., (1979). Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica and California. *Appl. Environ. Microbiol.*, 1085-1095.

GOCKE, K., DAWSON, R. & LIEBEZEIT, G., (1981). Availability of dissolved free glucose to heterotrophic microorganisms. *Mar. Biol.*, **62**, 209-216.

GULLENTOPS, F., (1974). *Detrital sedimentology in the Southern Bight of the North Sea*, in *Math. Modelsea, I.C.E.S., Hydrography Committee*, CM 1974 : C-1 : 55-80.

GREFE, V.N., (1970). *The biomass and production of different trophic levels in the pelagic communities of South Seas in Marine Food chains*. J.H. Steele, Oliven & Boyd, Edinburgh.

HARRISSON, W.G., (1978). Experimental measurements of nitrogen remineralization in coastal waters. *Limnol. Oceanogr.*, **23**, 684-694.

HECQ, J.H., GASPAR, A. & PICARD, H., (1980). Spatial pattern and biochemical content of North Sea zooplankton (Belgian coast)(1979-1980). *I.C.E.S.*, 1980.

HOGETSU, K., (1979). *Biological productivity of some coastal region of Japan in marine production mechanisms.* IBP (Ed.), Dunbar. Cambridge University Press.

JAWED, N., (1973). Ammonia excretion by zooplankton and its significance to primary productivity during summer. *Mar. Biol.*, **23**, 115-120.

JOHNSON, K.M. & SIEBURTH, J.Mc N., (1977). Dissolved carbohydrates in seawater. I. A precise spectrophotometric analysis for monosaccharides. *Mar. Chem.*, **5**, 1-13.

JOIRIS, C., (1974). Variations spatiales et temporelles de la concentration bactérienne dans les eaux du Sud-Est de la Mer du Nord. *Ann. Soc. Roy. Zool. Belg.*, **104**, 39-50.

JOIRIS, C., (1977). On the role of heterotrophic bacteria in marine ecosystems: some problems. *Helgoländer Wiss. Meeresunters*, **30**, 611-621.

JOSEFFSON, B.R., (1970). Determination of soluble carbohydrates in sea water by partition chromatography after desalting by ion-exchange membrane electro-dialysis. *Anal. Chim. Acta*, **52**, 65-73.

KRAUSE, M. & RADACH, G., (1980). On the succession of developmental stages of herbivorous zooplankton in the northerh North Sea during FLEX '76. 1. First statements about the main groups of the zooplankton community. *"Meteor" Forsch. Ergebnisse*, **22**, 133-149.

LANCELOT, C., (1980). A statistical method to estimate the biochemical composition of phytoplankton in the Southern Bight of the North Sea. *Est. Coast. Mar. Sc.*, **10**, 467-478.

LANCELOT, C., DARO, M.H., BOUQUEGNEAU, J.M., BILLEN, G., JOIRIS, C., BOSSICART, M., BERTELS, A., VERBEUSTEL, S., NYS, J., NIHOUL, C., PUTMAN, J., WIJNANT, J., HEISE, A. & GOEYENS, L., (1979). Ecometabolism of the coastal area of the Southern Bight of the North Sea. Report of the workgroup "Organic Matter". *I.C.E.S.* C.M. 1980/L:22. Biological Oceanography Committee.

LARSSON, U. & HAGSTROM, A., (1979). Phytoplankton exudate release as energy source for the growth of pelagic bacteria. *Mar. Biol.*, **52**, 199-206.

LIEBEZEIT, G., BOLTER, M., BROWN, I.F. & DAWSON, R., (1980). Dissolved free amino acids and carbohydrates at pycnocline boundaries in the Sargasso Sea and related microbial activity. *Oceanol. Acta*, **3**, 357-362.

MENZEL, D.W. & RYTHER, J.H., (1961). Zooplankton in the Sargasso Sea of Bermuda and its relation to organic production.

MOENS, M., (1974). Suspended matter. *Math. Modelsea, I.C.E.S.*, Hydrography Committee, CM 1974 C/1 , 81-91.

MOMMAERTS, J.P., (1973a). The relative importance of nannoplankton in the North Sea primary production. *Br. Phycol. J.*, **8**, 13-20.

MOMMAERTS, J.P., (1973b). On primary production in the South Bight of the North Sea. *Br. Phycol. J.*, **8**, 217-231.

NIHOUL, J.C.J. & RONDAY, F.C., (1975). The influence of the tidal stress on the residual circulation. *Tellus*, **27**, 484-489.

OASIN, S.F., (1970). *Some problems related to the food chain in a tropical estuary*, in *Marine Food Chains*. J.H. Steele (Ed.), Oliver & Boyd, Edinburgh.

ODUM, E.P., (1971). *Fundamentals of Ecology*. Saunders, Philadelphia, London, Toronto, 574 pp.

POCKLINGTON, R., (1971). Free amino acids dissolved in North Atlantic Ocean waters. *Nature*, **230**, 374-375.

RILEY, G.A., (1956). Oceanography of Long Island Sound. 1952-1954. IX. Production and utilization of organic matter. *Bull. Bingham Oceanogr. Collect. Yale Univ.*, **15**, 324-344.

RILEY, J.P. & SEGAR, D.A., (1970). The seasonal variation of the free and combined dissolved amino acids at a station in the Irish Sea. *J. mar. biol. Ass. U.K.*, **50**, 713-720.

SUGIURA, Y., IBERT, E.R. & HOOD, D.W., (1963). *J. mar. Res.*, **21**, 11.

SHAH, N.M. & WRIGHT, R.T., (1974). The occurence of glycollic acid in coastal seawater. *Mar. Biol.*, **24**, 121-124.

SHUSKINA, E.A. & VINOGRADOV, N.E., (1979). *Trophic relationship in communities and the functioning of marine ecosystems. II. Some results of investigations on the pelagic ecosystems in tropical regions of the Ocean, Marine production mechanisms*. IBP (Ed.), Dunbar. Cambridge University Press.

SIEBURTH, J.Mc N., (1976). Bacterial substrates and productivity in marine ecosystems. *Ann. Rev. Ecol. Syst.*, **7**, 259-285.

SOROKIN, Y.I., (1973). Data on biological productivity of the western tropical Pacific Ocean. *Mar. Biol.*, **20**, 177-196.

SOROKIN, Y.I., (1978). Description of primary production and of the heterotrophic microplankton in the Peruvian upwelling region. *Oceanology*, **18**,(1), 62-71.

STEELE, J.H., (1974). *The structure of marine ecosystems*. Harvard University Press, Cambridge, Massachusetts, 128 pp.

STEEMANN NIELSEN, E., (1952). The use of radioactive carbon (^{14}C) for measuring organic production in the sea. *J. Cons. Perm. int. Explor. Mer.*, **18**, 117-140.

STEEMANN NIELSEN, E., (1972). The rate of primary production and the size of standing stock of zooplankton in the Ocean. *Int. Rev. Ges. Hydrobiol.*, **57**, (4), 513-516.

SUESS, E. & MULLER, P., (1980). *Productivity, sedimentation rate and sedimentary organic matter in the Oceans. II. Elemental fractionation*, in *Colloques internationaux du C.N.R.S.*, n° 293. Biogéochimie de la matière organique à l'interface eau-sédiment marin.

WALSH, J.J., (1981). A carbon budget for overfishing off Peru. *Nature*, **290**, 300-304.

WALSH, J.J., ROWE, G.T., IVERSON, R.L. & ROY, P.Mc, (1981). Biological export of shelf carbon in a sink of the global CO_2 cycle. *Nature*, **291**, 196-201.

WOLLAST, R., (1976). *Transport et accumulation de polluants dans l'estuaire de l'Escaut*, in *L'Estuaire de l'Escaut*. Programme national de recherche et de développement. Environnement Eau. Projet Mer, vol. 10, J.C.J. Nihoul & R. Wollast (Eds), Bruxelles.

WRIGHT, R.T. & SHAH, N.M., (1975). The trophic role of glycollic acid in coastal seawater. I. Heterotrophic metabolism in seawater and bacterial cultures. *Mar. Biol.*, **33**, 175-183.

Part 2

NATURE OF THE PRIMARY PRODUCTS

Ch. LANCELOT[1]

1.– Introduction.

As pointed out by the general pattern of organic carbon circulation 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 nitrogen, 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.

---

1. Laboratoire d'Océanographie, Université libre de Bruxelles.

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 mechanisms 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 relationship 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 statistically 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

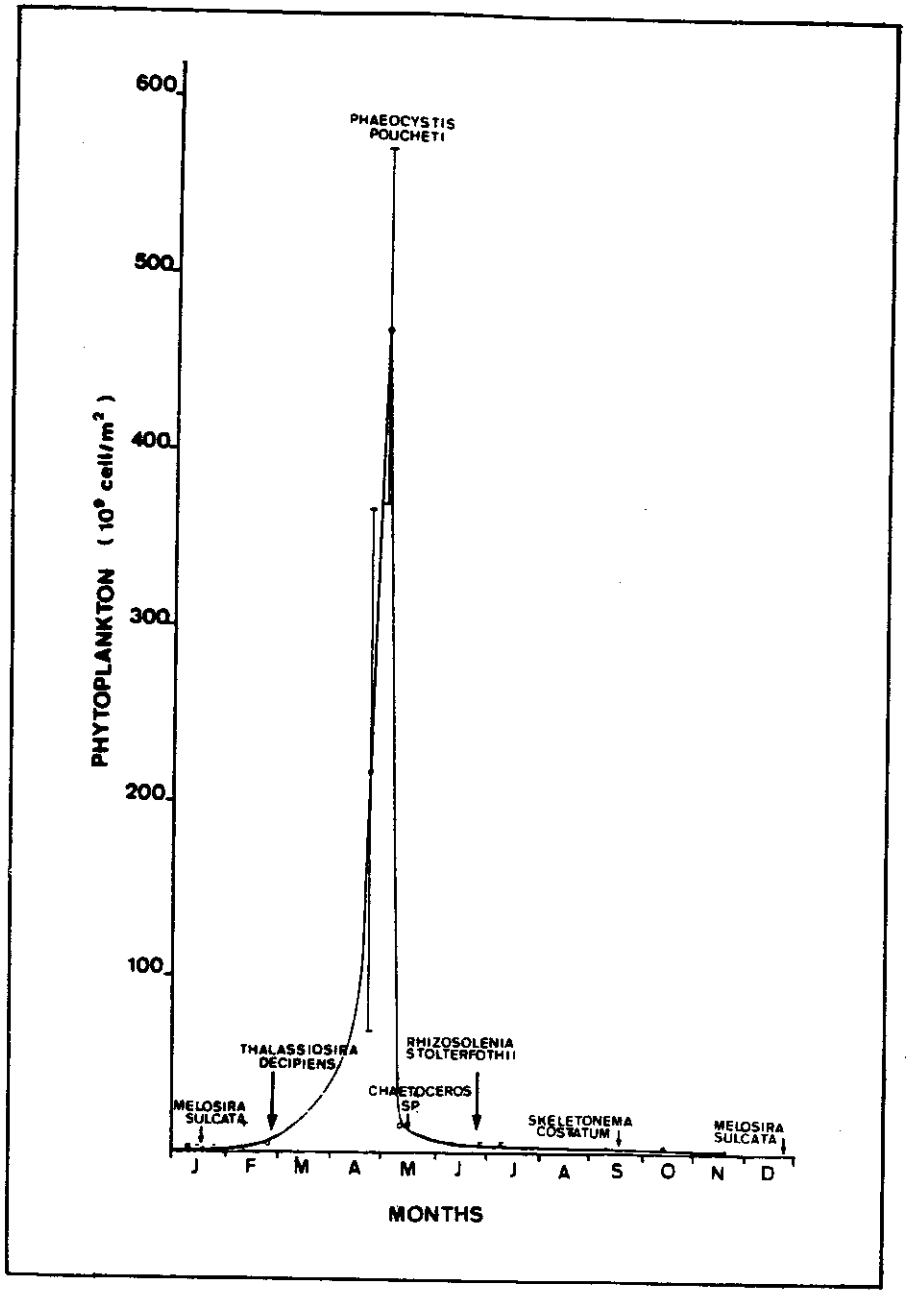


fig. 1.

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

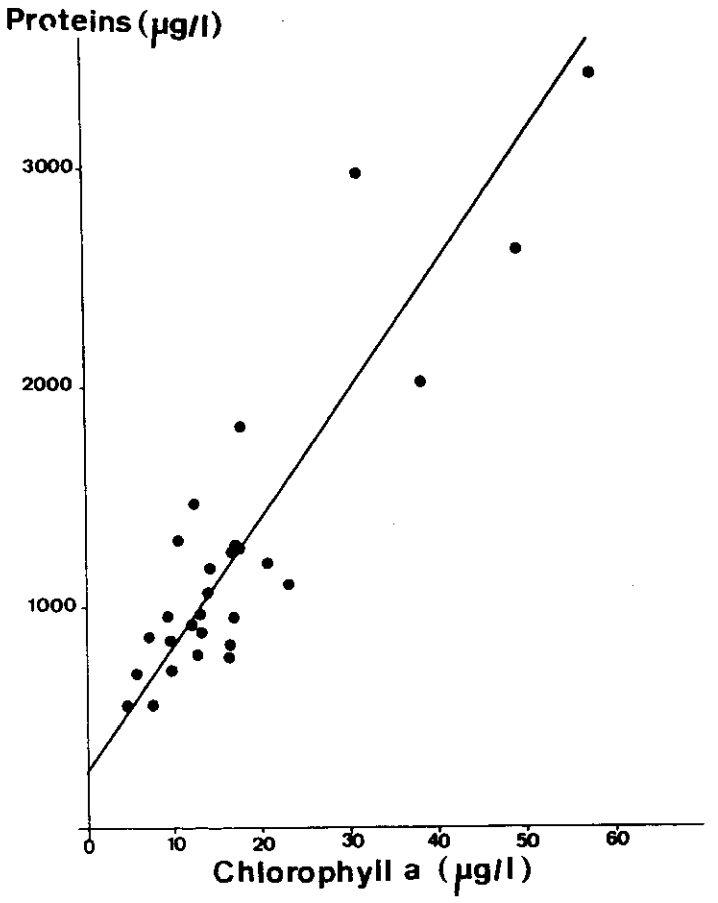


fig. 2.

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 extracellular release of oligomers (MW < 500 d) and polymers (MW > 500 d), from the classical experiment of Steemann Nielsen (1952).

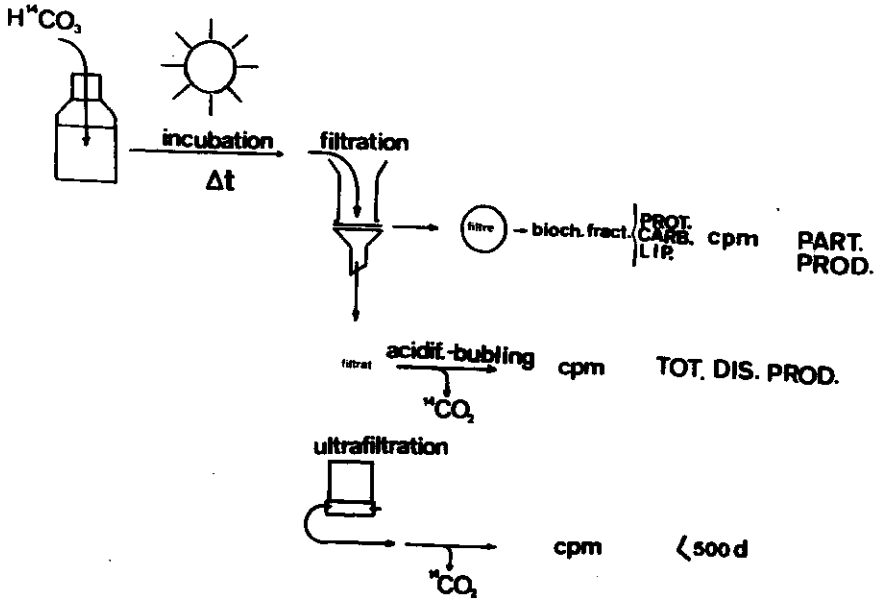


fig. 3.

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 UM05 (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).

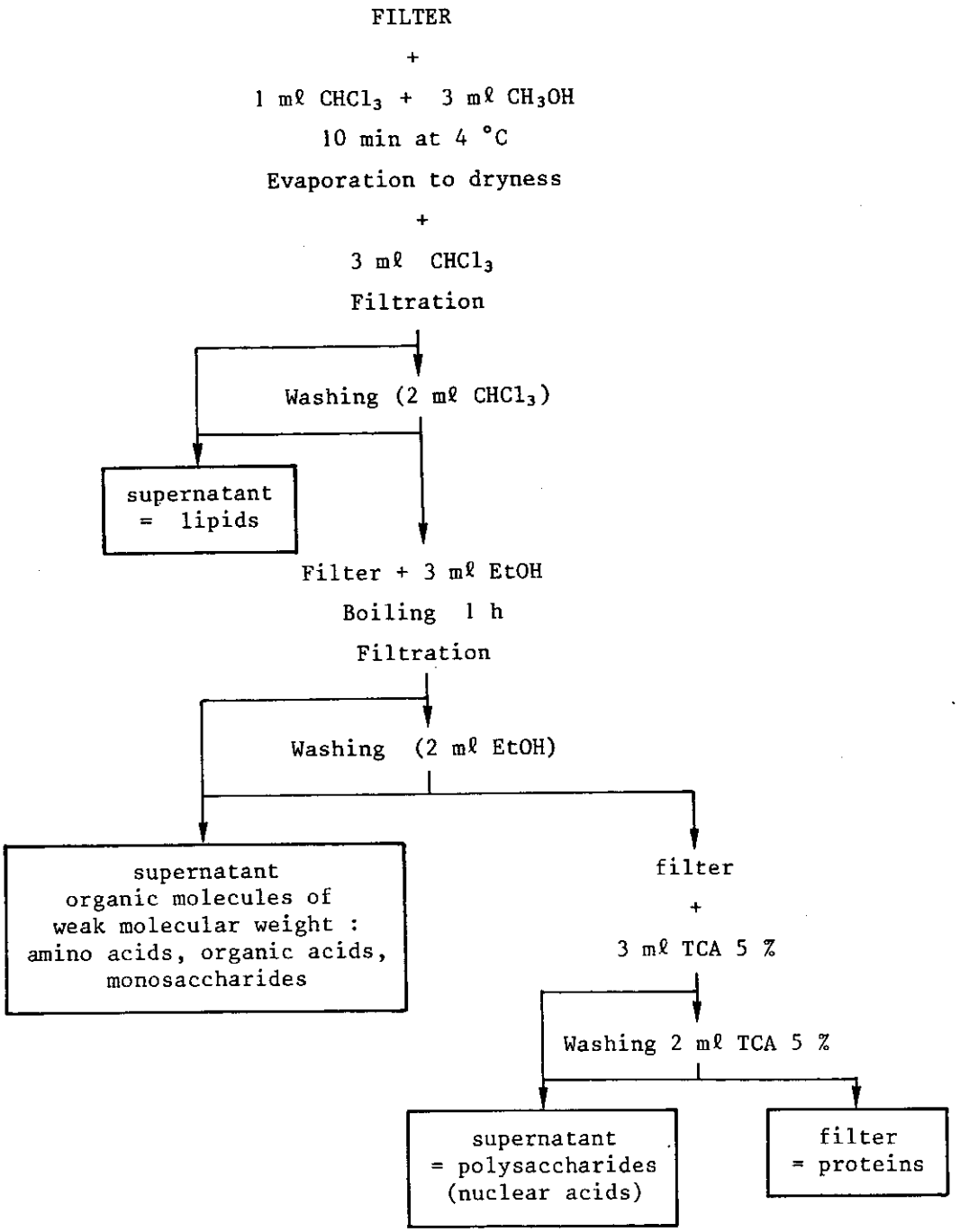


fig. 4.

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 ± 65 b = 272 ± 308	r = 0.86 a = 63 ± 34 b = 300 ± 163	r = 0.90 a = 27 ± 14 b = 8 ± 77
Spring	r = 0.89 a = 49 ± 4 b = 412 ± 87	r = 0.78 a = 27 ± 6 b = 327 ± 168	r = 0.76 a = 8 ± 3 b = 166± 81
Summer	r = 0.89 a = 60 ± 18 b = 411 ± 126	r = 0.78 a = 44 ± 21 b = 218 ± 65	r = 0.53 a = 29± 11 b = 65 ± 66
Autumn	r = 0.84 a = 62 ± 26 b = 326 ± 64	r = 0.78 a = 32 ± 15 b = 223 ± 162	r = 0.79 a = 24 ± 11 b = 63 ± 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 decipiens* *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).

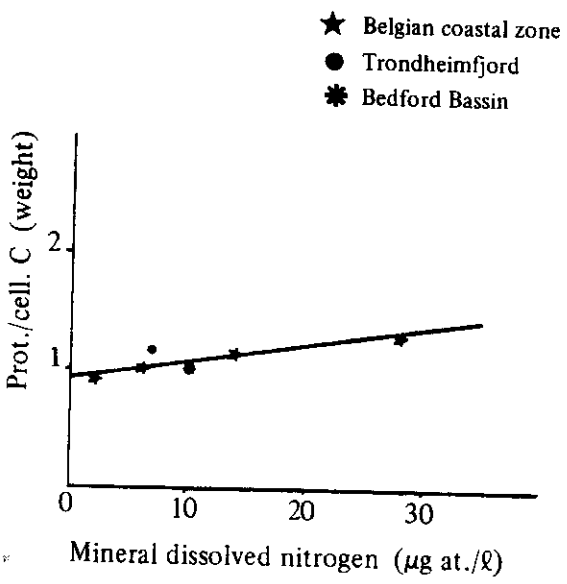


fig. 5.

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

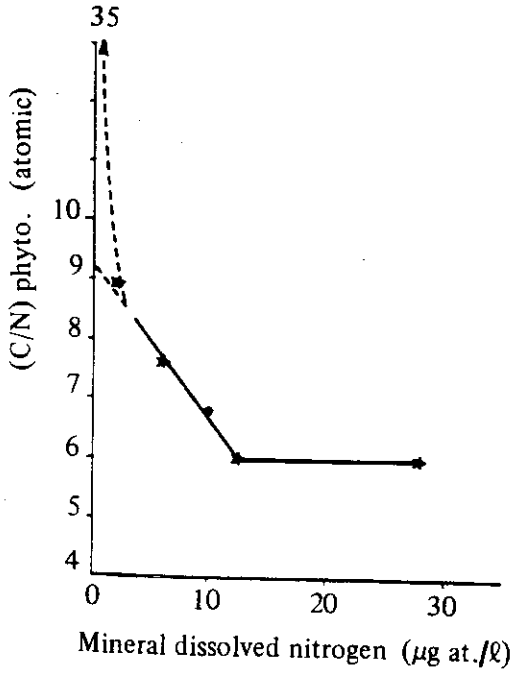


fig. 6.

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 :

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

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

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

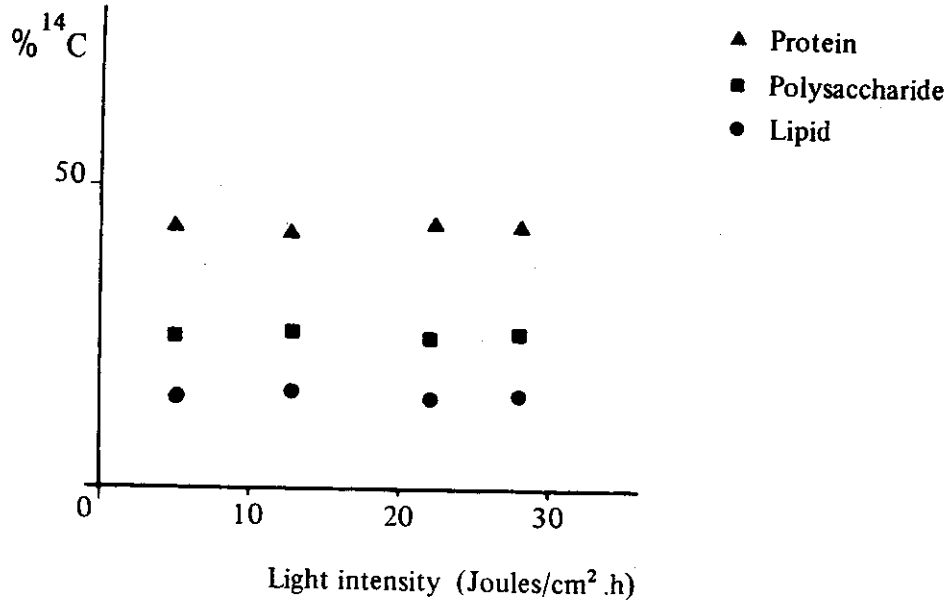


fig. 7.

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 phytoplankton 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.

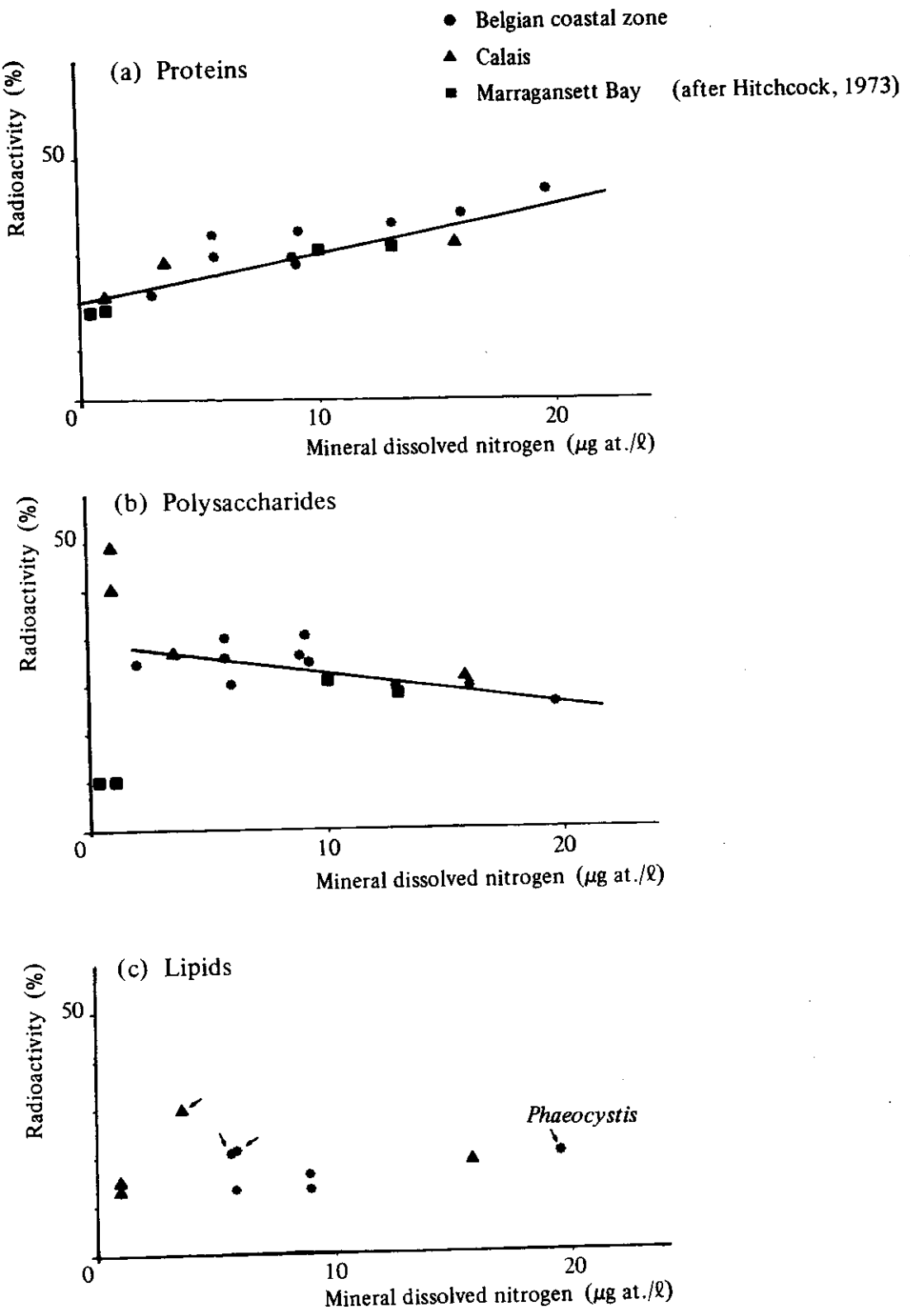


fig. 8.

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

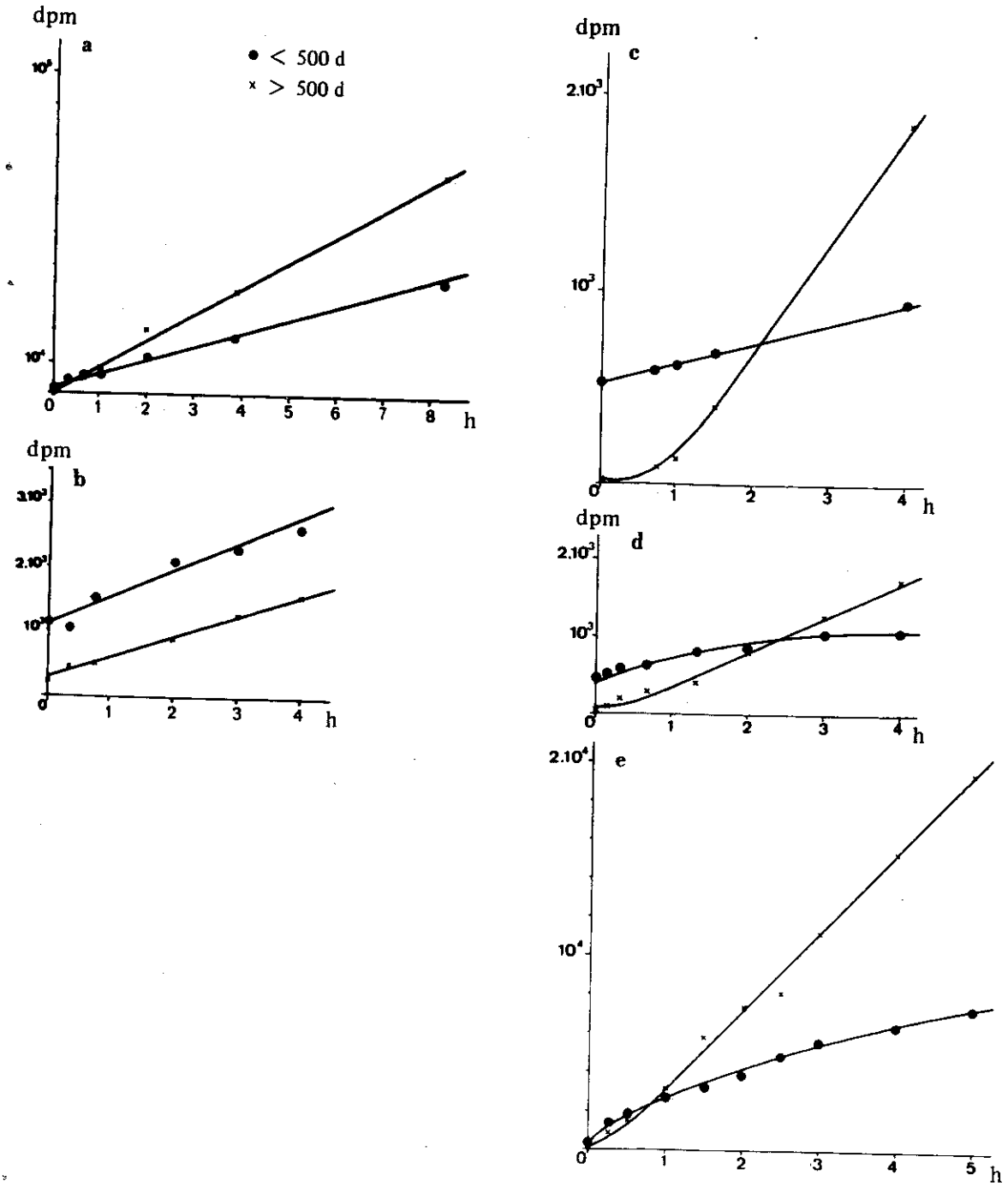


fig. 9.

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

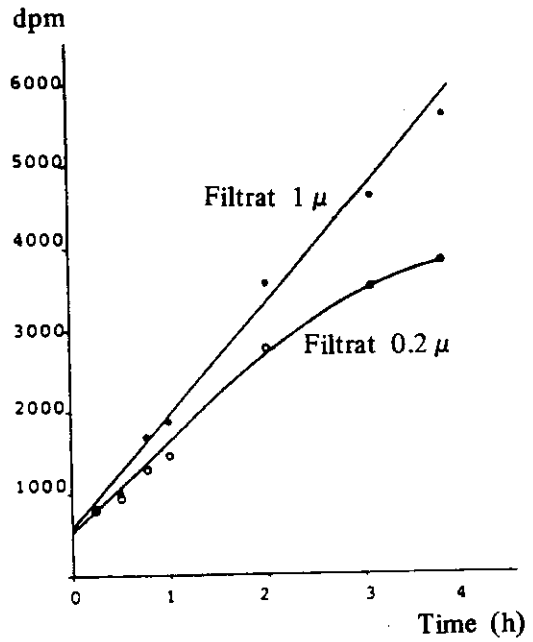


fig. 10.

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

retains bacteria although the 1 µm filtration although retaining 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 effectively 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 $1/\tau$ of phytoplankton small extracellular products in the Belgian coastal area

Datum	$1/\tau$ %/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 characterized 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 microheterotrophs* (see Billen and Somville, this volume) and have a longer residence time in sea-water.

26-06-79

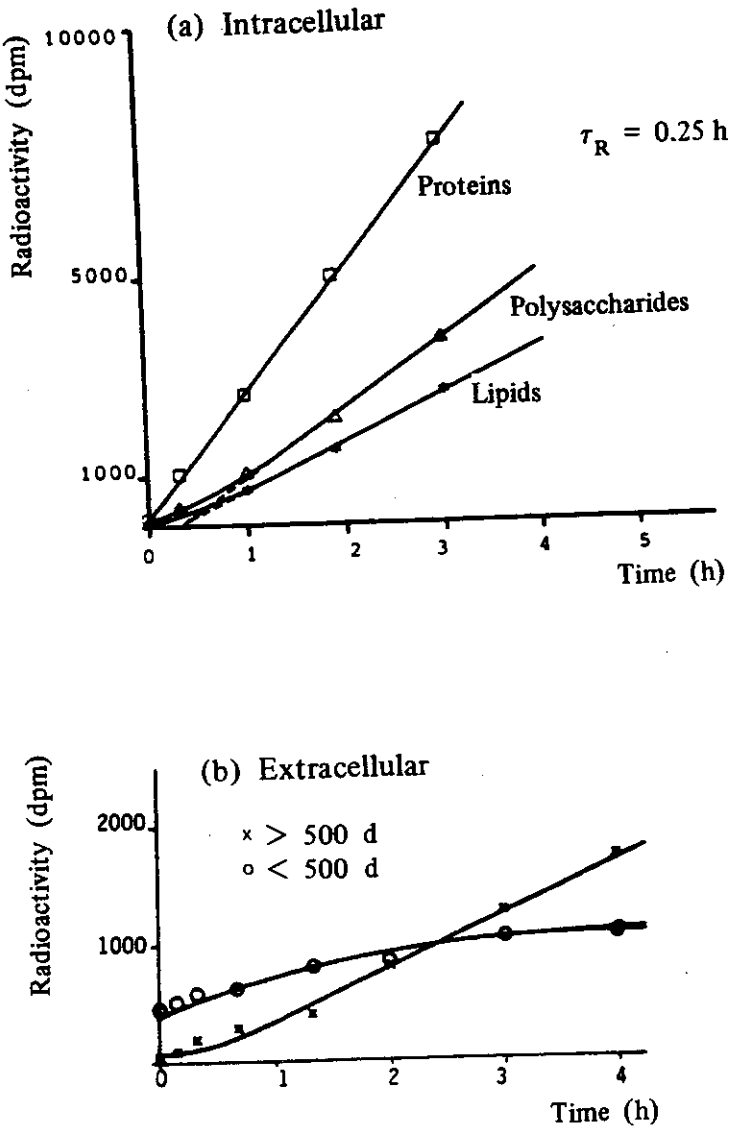


fig. 11.

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^+ µg at./l	PO_4^{3-}
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 microflagellate *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.

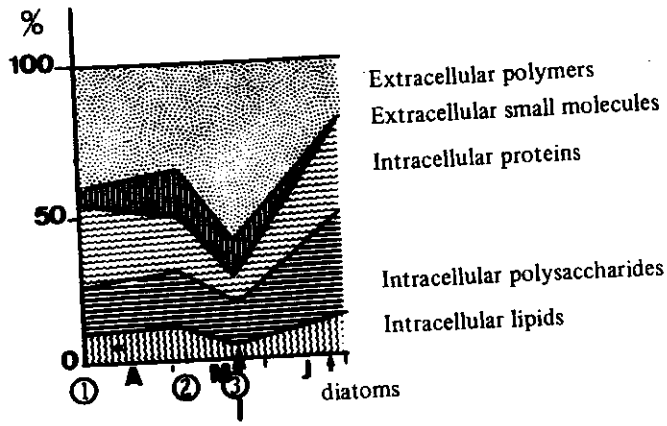


fig. 12.

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		Production		
			intracellular		extracellular
	g C/m²	%	g C/m².y	%	g C/m².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 zooplankton 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 phytoplankton 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.

References.

AL-HASSAN, R.H. and COUGHLAN, S.J., (1976). A method for the determination of glycollic acid in the extracellular products of cultured and natural phytoplancton populations. *J. Exp. Mar. Biol. Ecol.*, **25**, 141-150.

CONOVER, S.A.M., (1975). Nitrogen utilization during spring blooms of marine phytoplankton in Bedford Basin, Nova Scotia, Canada. *Mar. Biol.*, **32**, 247-261.

GUILLARD, R.R.L. and HELLEBUST, J.A., (1971). Growth and the production of extracellular substances by two strains of *Phaeocystis poucheti*, *J. Phycol.*, **7**, 330-338.

HANDA, N., (1969). Carbohydrates metabolism in the marine diatom *Skeletonema costatum*, *Mar. Biol.*, **4**, 208-214.

HAUG, A., MYKLESTAD, S. and SAKSHAUG, E., (1973). Studies on the phytoplankton ecology of the Trondheimsfjord. I. The chemical composition of phytoplankton populations. *J. Exp. Mar. Biol. Ecol.*, **11**, 15-26.

ITURRIAGA, R. and HOPPE, H.G., (1977). Observations of heterotrophic activities on photoassimilated organic matter. *Mar. Biol.*, **40**, 101-108.

LANCELOT, C., (1979). Gross excretion rates of natural marine phytoplankton and heterotrophic uptake of excreted products in the Southern North Sea, as determined by short-term kinetics. *Mar. Ecol. Prog. Ser.*, **1**, 179-186.

LANCELOT-VAN BEVEREN, C., (1980). A statistical method to estimate the biochemical composition of phytoplankton in the Southern Bight of the North Sea. *Est. Coast. Mar. Sc.*, **10**, 467-478.

LANCELOT, C., (1982). *Etude écophysiologique du phytoplancton de la zone côtière belge*. Thèse de doctorat. Université Libre de Bruxelles.

MYKLESTADT, S., (1974). Production of carbohydrates by marine planktonic diatoms. I. Comparison of nine different species in culture. *J. Exp. Mar. Biol. Ecol.*, **15**, 261-264.

MYKLESTADT, S. and HAUG., A., (1972). Production of carbohydrates by the marine diatoms *Chaetoceros affiis*. I. Effects of the concentration of nutrients in the culture medium. *J. Exp. Mar. Biol. Ecol.*, **9**, 125-136.

NALEWAJKO, C., DUNSTALL, T.G. and SHEAR, H., (1976). Kinetics of extracellular release in axenic algae and in mixed algal-bacterial cultures : significance in estimation of total (gross) phytoplankton excretion rates. *J. Phycol.*, **12**, 1-5.

RICKETTS, T.R., (1966). On the chemical composition of some unicellular algae. *Phytochem.*, **5**, 67-76.

SAKSHAUG, E. and HOLM-HANSEN, O., (1977). Chemical composition of *Skeletonema costatum* (Grev.). Cleve and *Pavlova* (monochrysis) Lutheri (Droop) as a function of nitrate, phosphate and iron-limited growth. *J. Exp. Mar. Biol. Ecol.*, **29**, 1-34.

STRICKLAND, J.D.H., HOLM-HANSEN, O., EPPLY, R.W. and LINN, R.J. (1969). The use of a deep tank in plankton ecology. I. Studies of the growth and composition of phytoplankton crops at low nutrient levels. *Limnol. Oceanogr.*, **14**, 23-24.

WATANABE, Y., (1980). A study of the excretion and extracellular products of natural phytoplankton in Lake Nakanuma, Japan. *Int. Rev. Ges. Hydrobiol.*, **65** (6), 809-834.

WIEBE, W.J. and POMEROY, L.R., (1972). Microorganisms and their association with aggregates and detritus in the sea : a microscopic study. *Mem. Ist. Ital. Idrobiol.*, **29** (suppl.), 342-352.

Part 3

UTILIZATION OF PRIMARY PRODUCTS BY ZOOPLANKTON

M.H. DARO[1], J.H. HECQ[2], M. BOSSICART[1], B. VAN GIJSEGEM and M. TACKX

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.

1. Laboratorium voor Ekologie en Systematiek, Vrije Universiteit Brussel.
2. Laboratoire de Biologie marine, Université de Liège.

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 activities. 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 juxtaposition 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

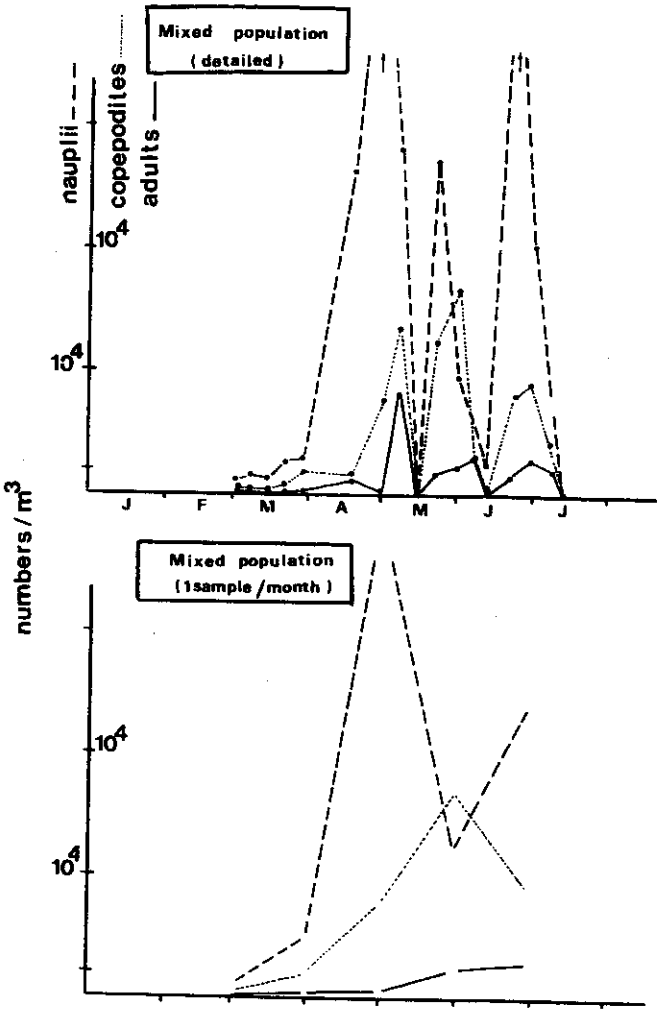


fig. 1.

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 hamatus*, 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).

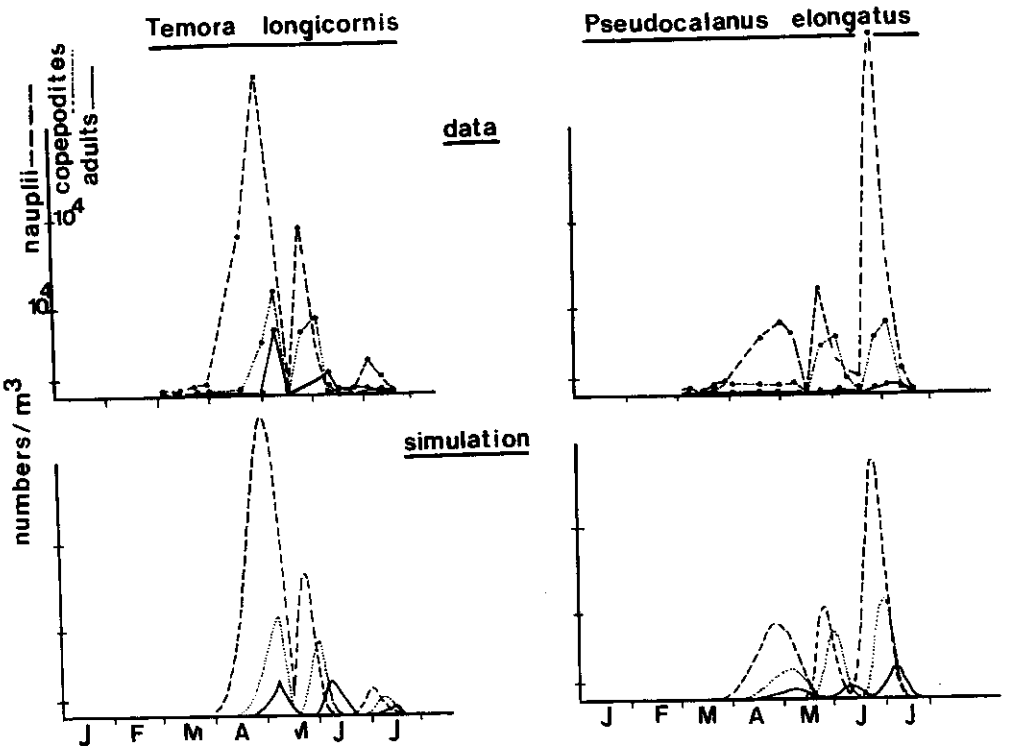


fig. 2.

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.

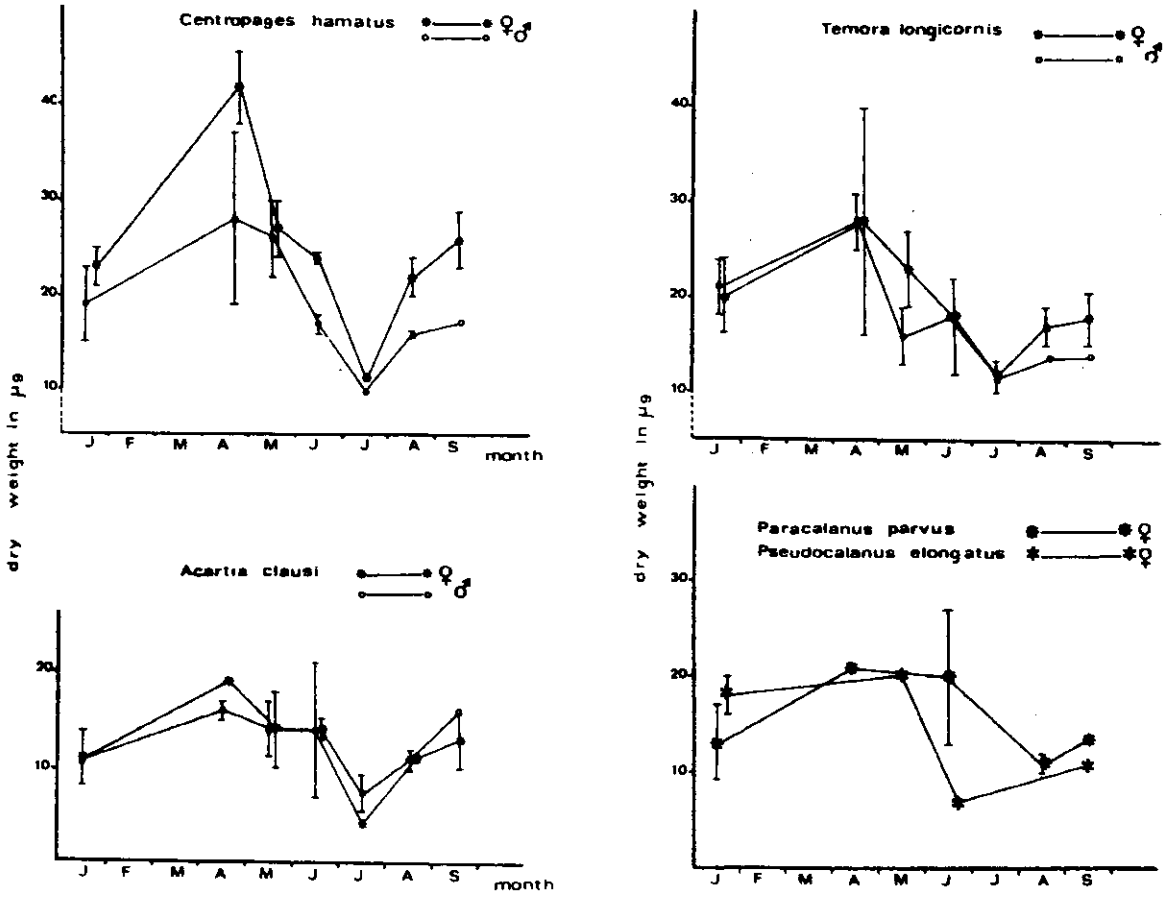


fig. 3.

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	d 85 135	d 135 160	d 155 190
biomass	144 mg C/50 dm^3	66 mg C/25 dm^3	145 mg C/35 dm^3
net prod.	28 mg C/50 dm^3	22 mg C/25 dm^3	49 mg C/35 dm^3
dead biomass	21 mg C/50 dm^3	9 mg C/25 dm^3	32 mg C/35 dm^3
Copepodites	d 90 135	d 135 165	d 160 195
biomass	91 mg C/45 dm^3	124 mg C/30 dm^3	167 mg C/35 dm^3
net prod.	20 mg C/45 dm^3	27 mg C/30 dm^3	45 mg C/35 dm^3
dead biomass	17 mg C/45 dm^3	21 mg C/30 dm^3	34 mg C/35 dm^3
Adults	d 80 135	d 125 155	d 140 190
biomass	13 mg C/45 dm^3	24 mg C/30 dm^3	28 mg C/50 dm^3
number	2178/45 dm^3	4116/30 dm^3	4810/50 dm^3
number ♀	1605/45 dm^3	3033/30 dm^3	3544/50 dm^3
number viable nauplii	47468/m^3	52722/m^3	86982/m^3

Temora longicornis

1978	1st generation	2nd generation	3rd generation
Nauplii	d 85 135	d 135 160	d 165 200
biomass	217 mg C/90 dm^3	45 mg C/25 dm^3	12 mg C/35 dm^3
net prod.	30 mg C/50 dm^3	6 mg C/25 dm^3	2 mg C/35 dm^3
dead biomass	43 mg C/50 dm^3	4 mg C/25 dm^3	1 mg C/35 dm^3
Copepodites	d 100 135	d 135 165	d 180 200
biomass	133 mg C/35 dm^3	55 mg C/30 dm^3	10 mg C/20 dm^3
net prod.	31 mg C/35 dm^3	11 mg C/30 dm^3	5 mg C/20 dm^3
dead biomass	15 mg C/35 dm^3	9 mg C/30 dm^3	2 mg C/20 dm^3
Adults	d 85 135	d 120 170	d 150 205
biomass	42 mg C/50 dm^3	11 mg C/50 dm^3	2 mg C/55 dm^3
number	13166/50 dm^3	3298/50 dm^3	632/55 dm^3
number ♀	6558/50 dm^3	1649/50 dm^3	316/55 dm^3
number viable nauplii	179108/m^3	23685/m^3	7133/m^3

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.

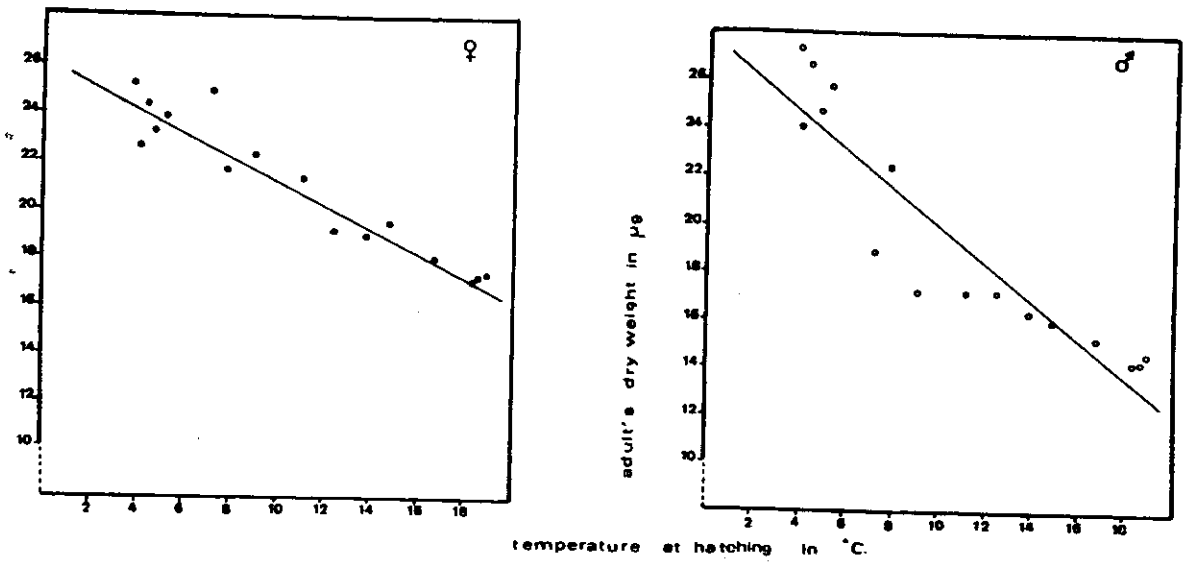


fig. 4.

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

Pseudocalanus elongatus and *Temora longicornis*

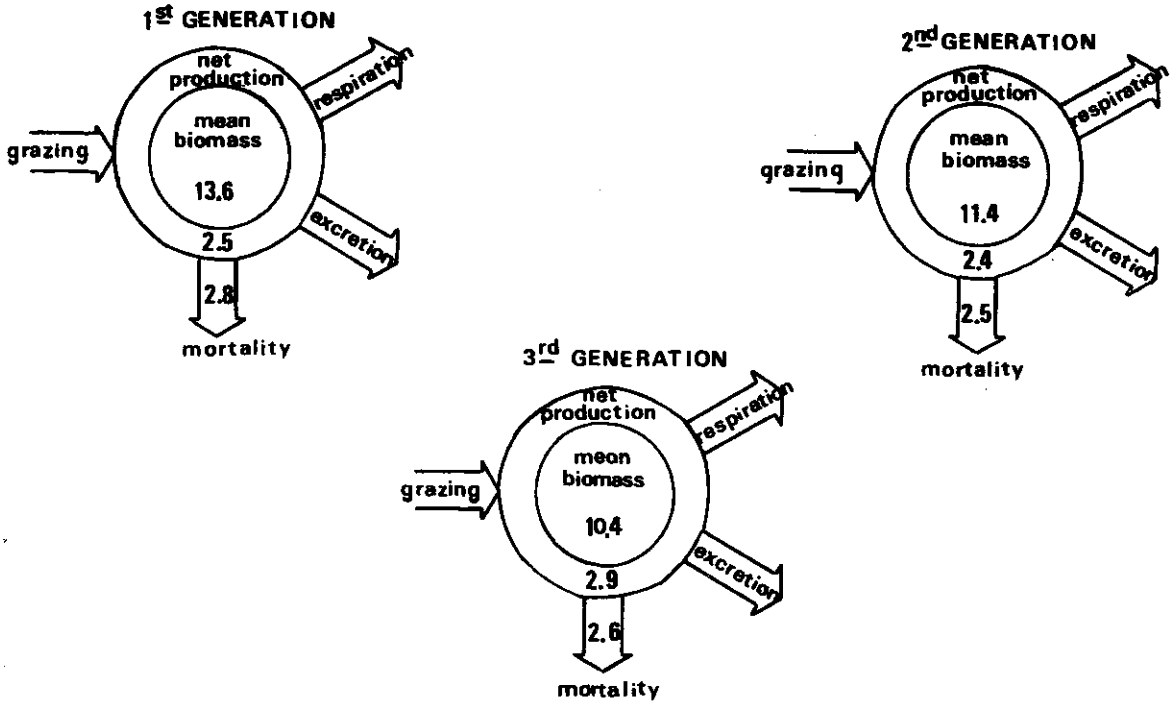


fig. 5.

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 pouchetti*, 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.

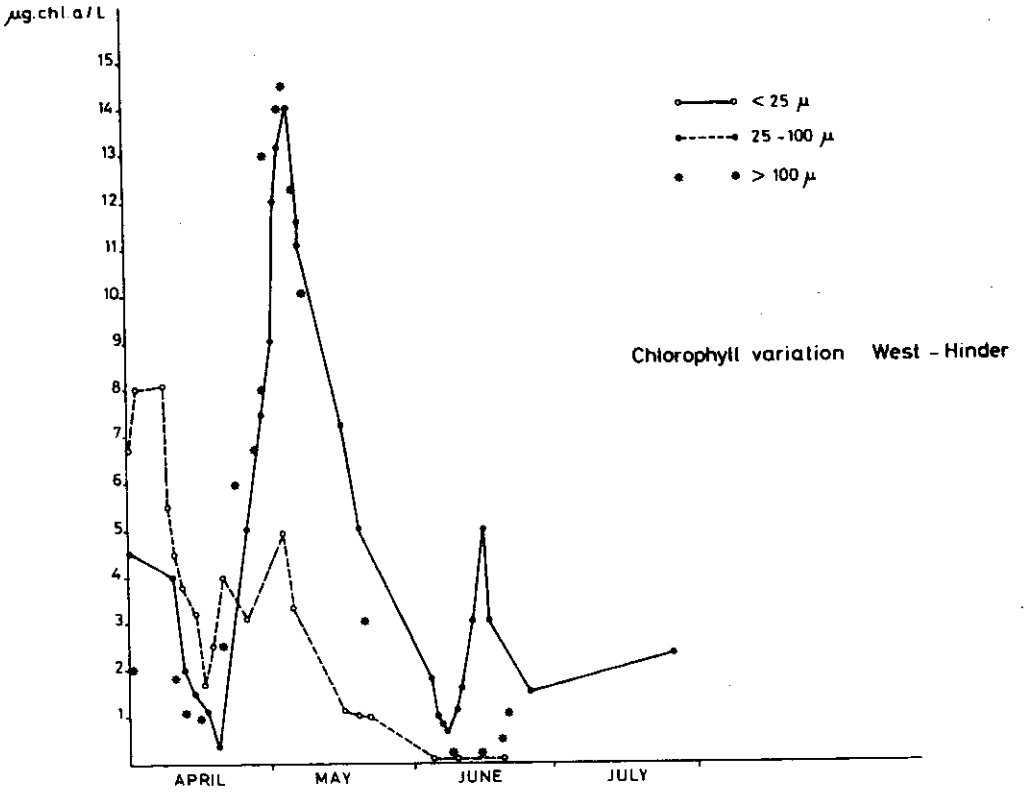


fig. 6.

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.

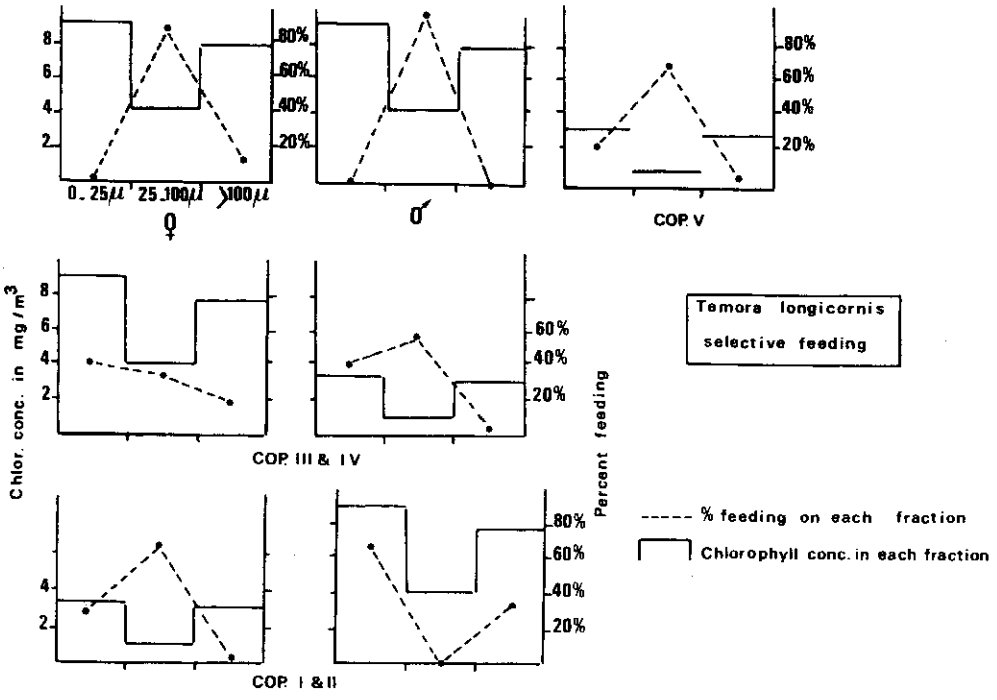


fig. 7.

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

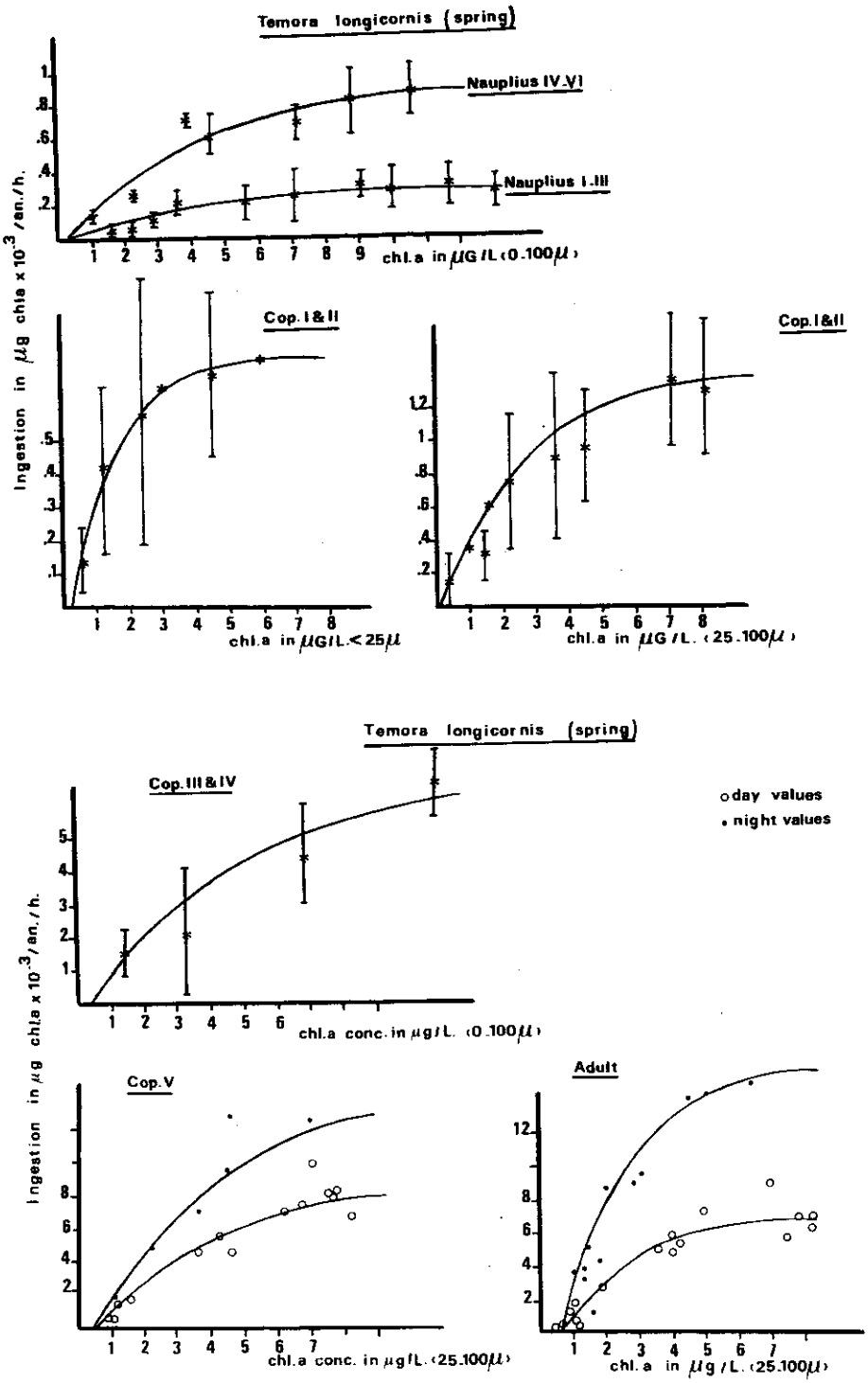


fig. 8.

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.

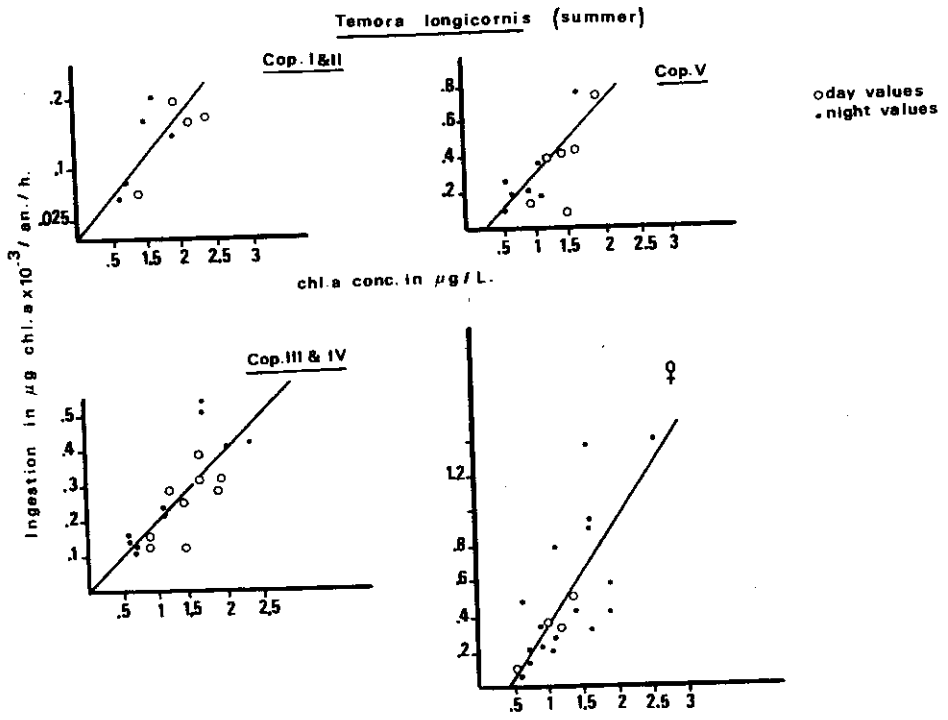


fig. 9.

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 nutrition decrease has negative consequences not only on the production (as well for what concerns biomass as for egg production) 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

$Ih = .7\ p - .03$ copepodites I

$Ih = .12\ p - .05$ copepodites II

Ih = .13 p — copepodites III
Ih = .29 p - .17 — copepodites IV
Ih = p - .52 — copepodites V
Ih = .48 p — 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.

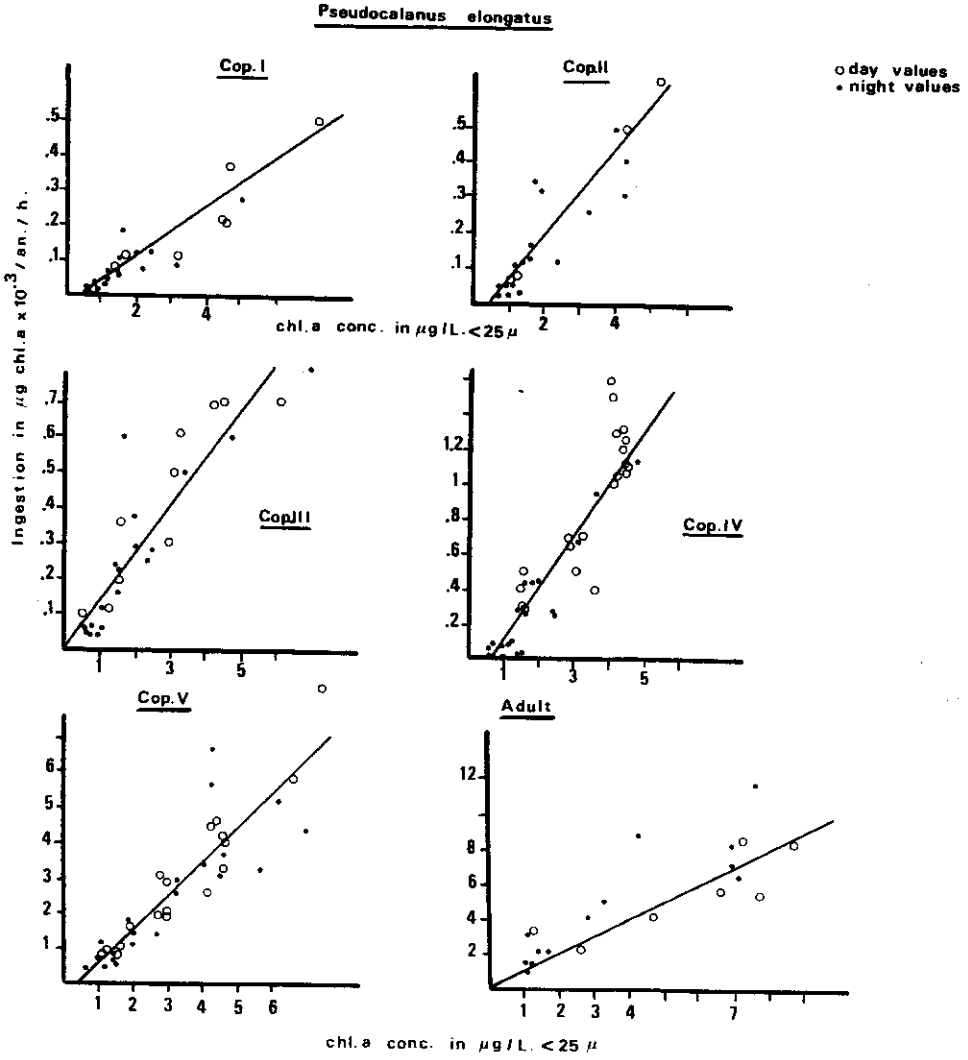


fig. 10.

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

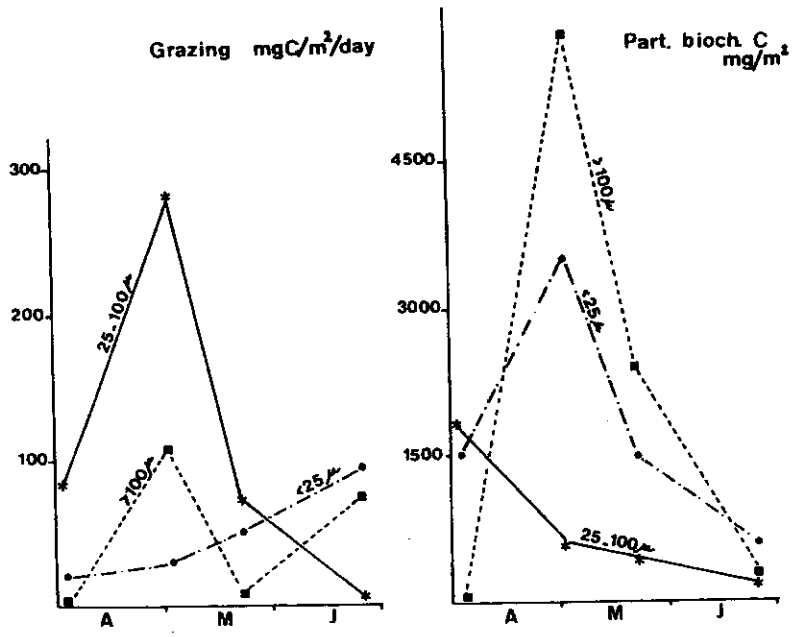


fig. 11.

Total grazing for the whole zooplankton community expressed in mg C/m^2.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 migrations. 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 consider that in the beginning of May the biomass of this fraction has a particulate production of 250 mg C/m^2.day, while the grazing represents a higher value (300 mg C/m^2.day). This remark must only be taken as an indication, because based on a rough calculation : 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

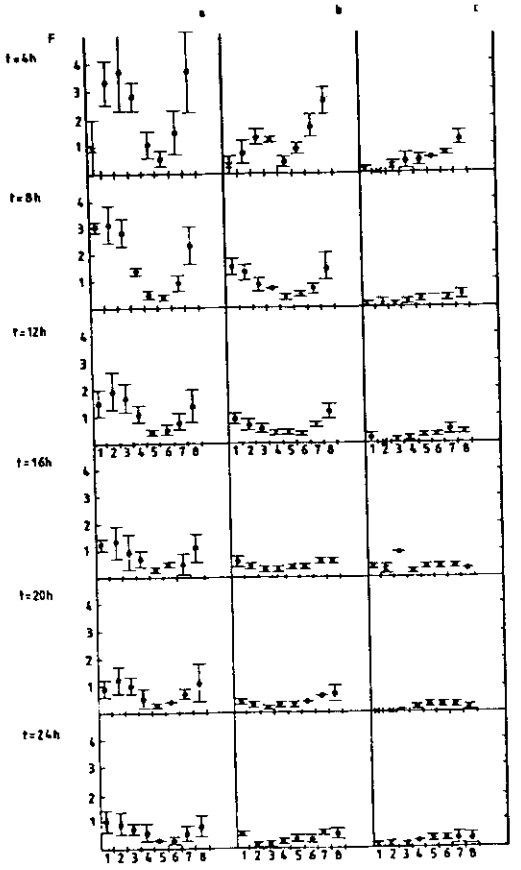


fig. 12.

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 longicornis*. 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).

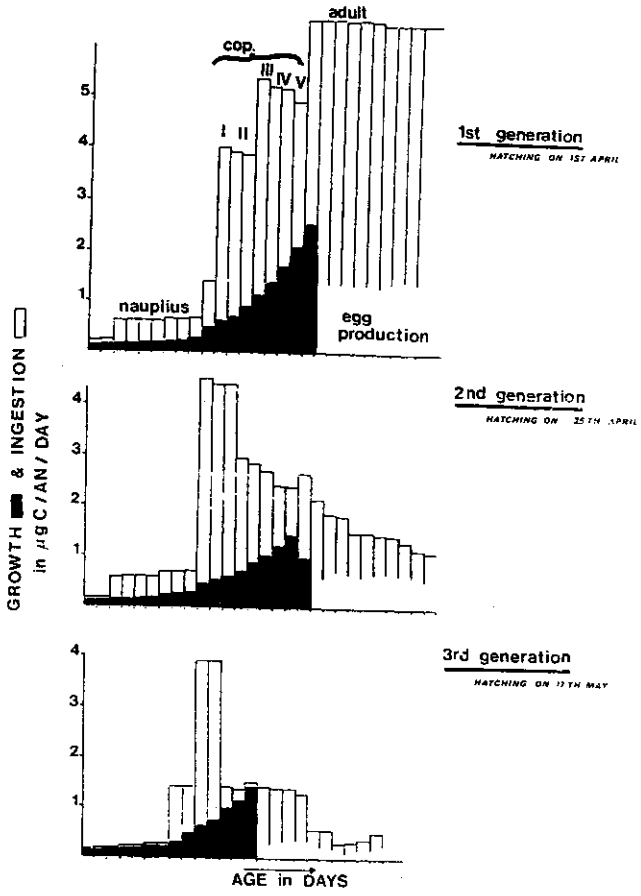


fig. 13.

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 satisfies 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 responsible 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.

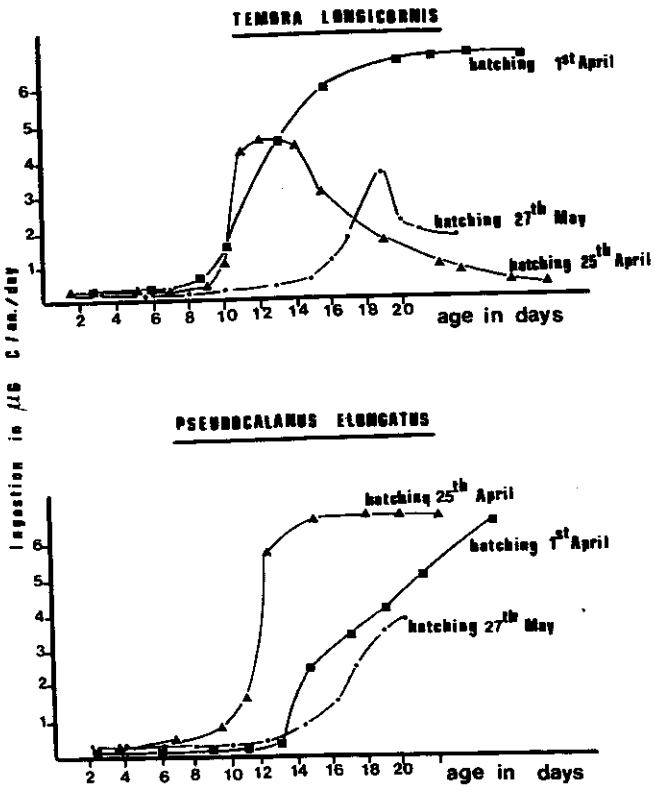


fig. 14.

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 concentrations.

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 distribution 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.

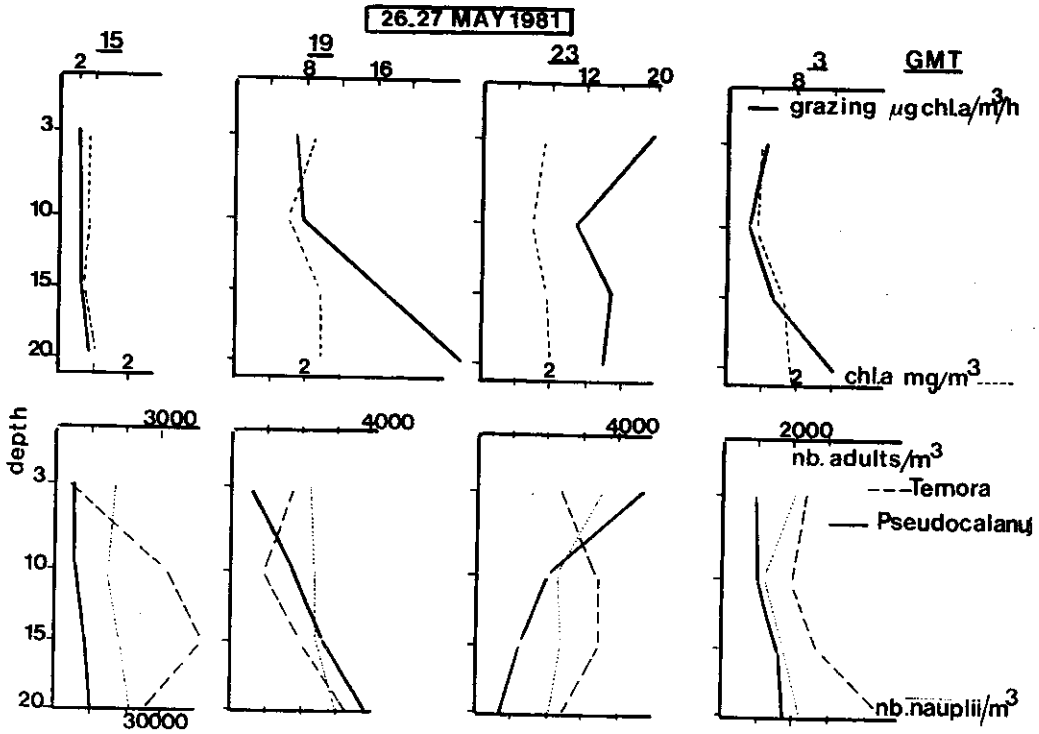


fig. 15.

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).

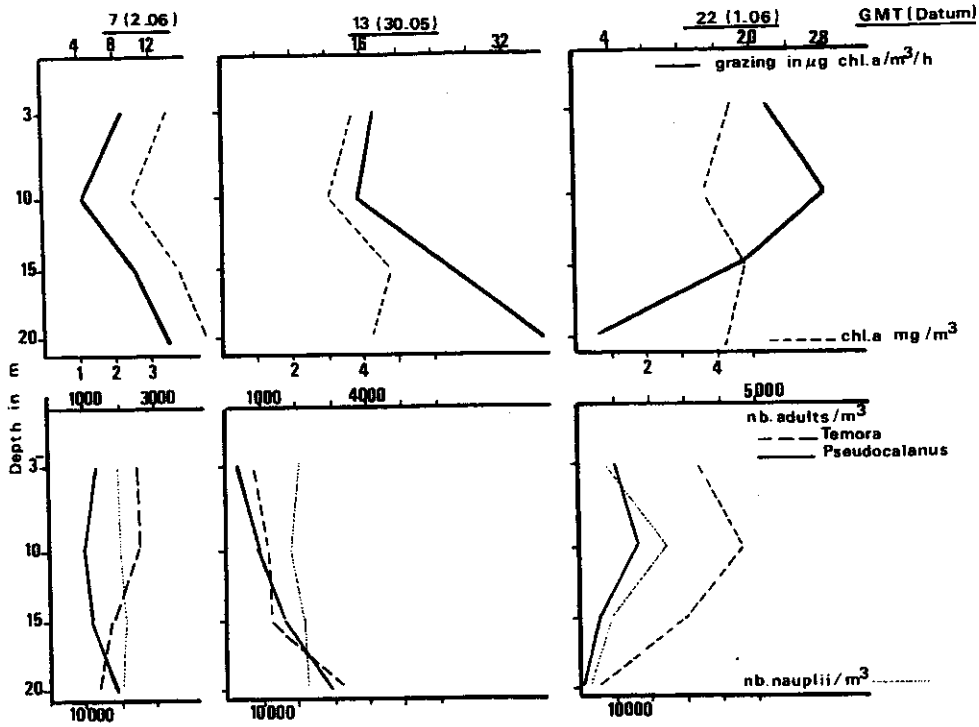


fig. 16.

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 conditions, 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)

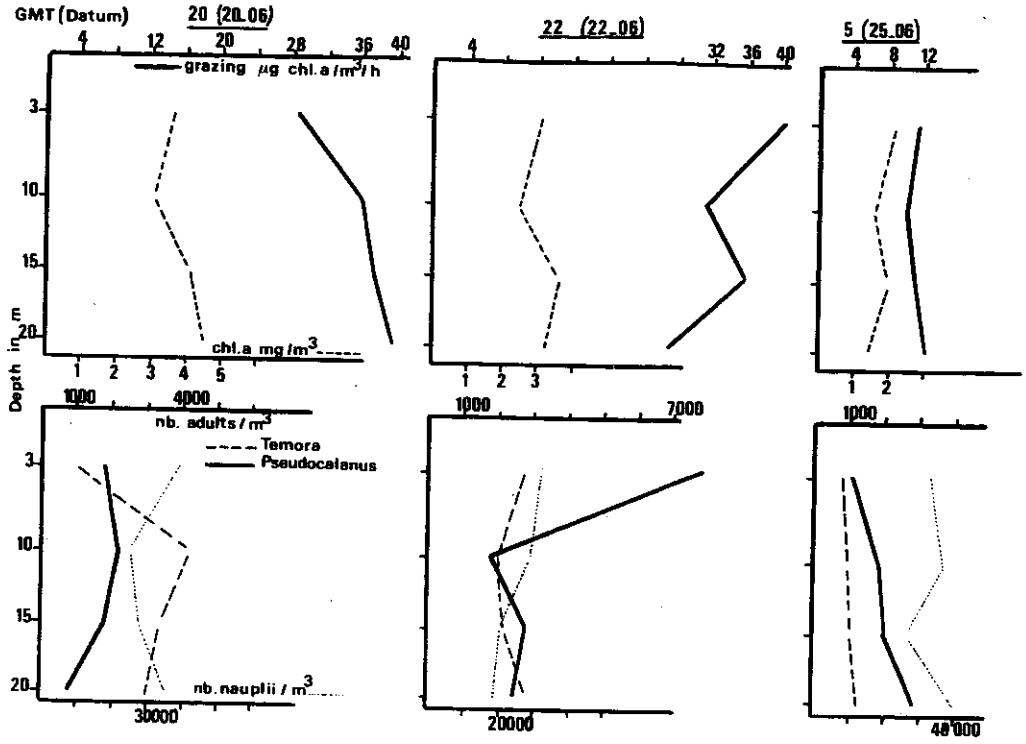


fig. 17.

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 phytoplankton 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

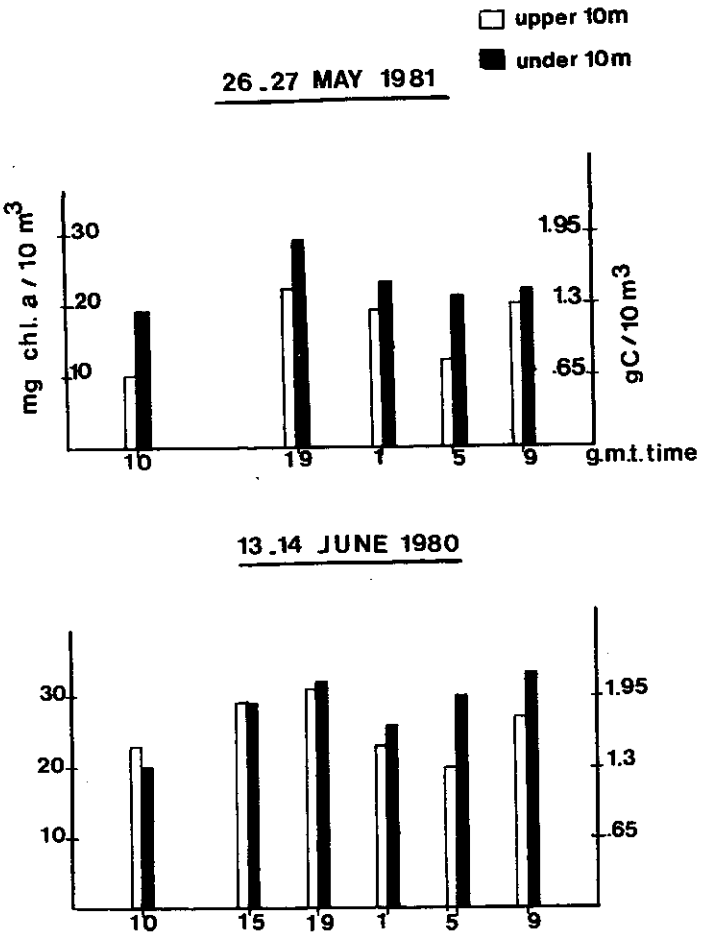


fig. 18.

Particulate organic matter expressed in chl/10 m^3 and in g C/10 m^3 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 zooplankton 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 m^3	Phaeo. in mg/10 m^3	% active chlor. within the tot. pigm.	Total pigm. in mg chl *a*/10 m^3 and in %		Phaeo. in mg/10 m^3 and in %		% active chlor. within the tot. pigm.
22-05	9	15	5	67	24.4	62	6	55	75
	19	13.2	6.7	49	15.5	54	6.7	50	54
24-05	17.30	8.2	3.5	57	8.4	51	6	63	51
26-05	10.30	10.3	6.7	35	19	65	9.6	54	49
	15.30	9.6	3.6	63	10.3	52	5	58	51
	19	22.4	9.8	56	28.7	56	13.7	58	52
	23	19.6	8.3	58	23.6	55	11.9	59	50
27-05	3	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
	17	28.3	13.0	52	30.2	52	12.5	48	59
27-06	17	12.0	6.6	48	12.4	50	5.2	44	58
28-06	6.30	8.7	3.8	56	7.1	45	3.4	47	52

References.

BOYD, C.M., (1976). Selection of particles sizes by filter feeding copepods a plea for reason. *Limnol. and Oceanogr.*, **21** (1), 175-180.

DARO, M.H., (1978). A simplified C method for grazing measurements on natural planktonic populations. *Helgol. wiss. Meeresunters*, **31**, 241-248.

DARO, M.H., (1980). Field study of the diel feeding of a population of *Calanus finmarchicus* at the end of a phytoplankton bloom. *Meteor. Forsch. Ergebnisse*, 22 (A), 123-132.

DARO, M.H. and VAN GIJSEGEM, B., (1982). Ecological factors affecting five dominant copepods in the Southern Bight of the North Sea, as regards their seasonal variations of weight. Sympos. on productivity of coastal and shelfseas of North Atlantic, Kiel, March 1982.

DEEVEY, G.B., (1960). Relative effects of temperature and food on seasonal variations in length of marine copepods in some Eastern American and Western European waters. *Bull. Bingham Ocean Coll.*, **XVII**, 54-85.

DEEVEY, G.B., (1964). Annual variations in length of copepods in the Sargasso Sea off Bermuda. *J. Mar. Biol. Ass. U.K.*, **44**, 589-600.

DEEVEY, G.B., (1966). Seasonal variations in length of copepods in South Pacific New Zeeland waters. *Aust. J. Mar. Freshw. Res.*, **17**, 155-168.

FROST, B.N., (1972). Effects of size and concentration of food particles on feeding behaviour of the marine planktonic copepod *Calanus pacificus*. *Limnol. and Oceanogr.*, **17**,805-815.

GAULD, D.T., (1966). The swimming and feeding of planktonic copepods, *in* Some contemporary studies in marine science (H. Bornes, ed.), Allen and Var., pp. 313-334.

KURATA, H., (1962). Studies on the age and growth of crustacea. *Bull. Hokkaido Reg. Fish. Res. Lab.*, **24**, 1-115.

LAUR, R.K. and FROST, B.W., (1976). Model of copepod filtering response to changes in size and concentration of food. *Limnol. and Oceanogr.*, **21** (4), 490-501.

LANCELOT, C., (1980). A statistical method to estimate the biochemical composition of phytoplankton in the Southern Bight of the North Sea. *Estuar. Coast. Mar. Sci.*, **10**, 467-478.

LEHMAN, J.T., (1976). The filter-feeder as an optimal forager and the predicted shapes of feeding curves. *Limnol. and Oceanogr.*, **21** (9), 501.

MILLER, C.B., JOHNSON, J.K. and HEINLE, D.R., (1977). Growth rules in the genus *Acartia*. *Limnol. and Oceanogr.*, 22, 326-335.

MOMMAERTS, J.P. and BOSSICART, M., (1979). A mathematical model of the population dynamics of the dominant copepods species in the Southern Bight of the North Sea. 1977-1978. ICES Report C.M. 1979/L24, Biological Oceanography Committee.

MULLIN, M.M., (1963). Some factors affecting the feeding of marine copepods of the genus *Calanus*. *Limnol. and Oceanogr.*, **8**, 239-250.

NIVAL, P. and NIVAL, S., (1976). Particle retention and efficiencies of an herbivorous copepod *Acartia clausi* (adult and copepodite stages) : effects on grazing. *Limnol. and Oceanogr.*, **21**, 24-38.

O'CONNORS, H.B., SMALL, L.F. and DONAGHAY, P.L., (1976). Particle size modification by two size classes of the estuarine copepod *Acartia clausi*. *Limnol. and Oceanogr.*, 21, 300-308.

PAFFENHOFER, G.A. and KNOWLES, (1978). Feeding of marine planktonic copepods on mixed phytoplankton. *Mar. Biol.*, **56**, 143-152.

*POULET, S.A., (1978). Comparison between five coexisting species of marine copepods feeding on naturally occuring particulate matter. *Limnol. and Oceanogr.*, **23** (6), 1126-1143.

Part 4

UTILIZATION OF PRIMARY PRODUCTS BY PLANKTONIC AND BENTHIC BACTERIA

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

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

1. Laboratoire d'Océanographie, Université libre de Bruxelles.

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.

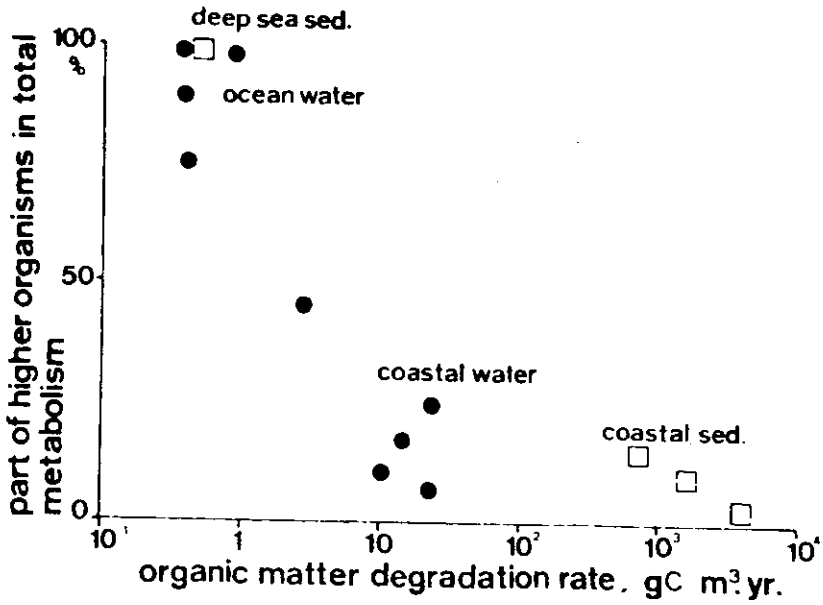


fig. 1.

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 proteolytic 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 indirectly 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.

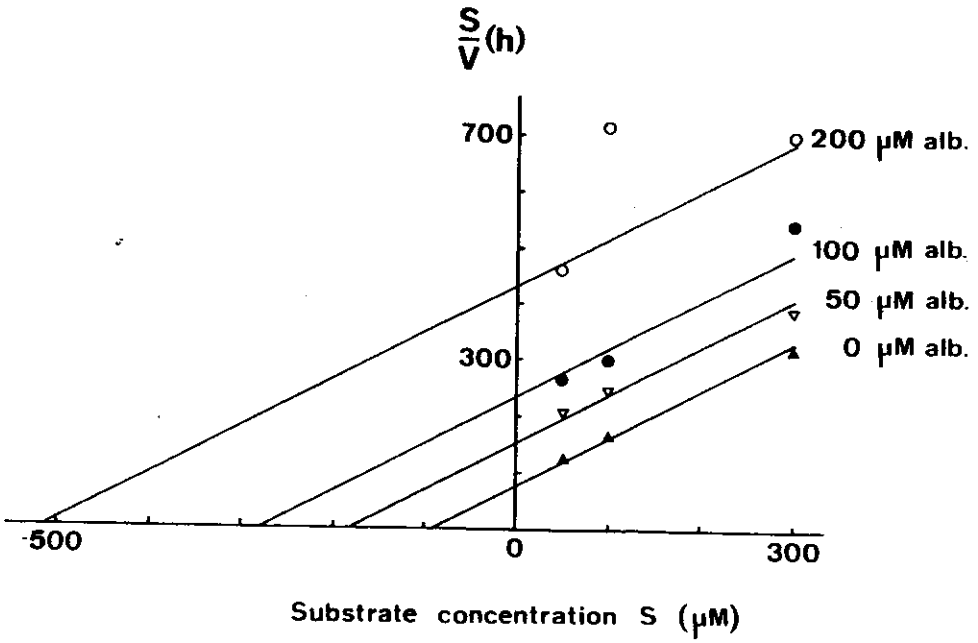


fig. 2.

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

This indicates that the enzymes responsible for LLβN hydrolysis 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 hypereutrophic, as in the heavily polluted Scheldt estuary. In some cases, the relative rate of amino-acid utilization has been simultaneously 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.

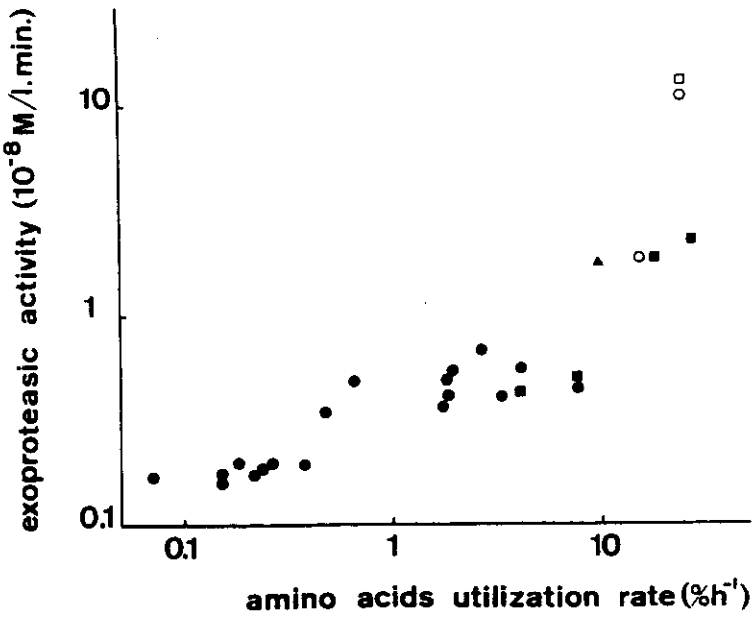


fig. 3.

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.

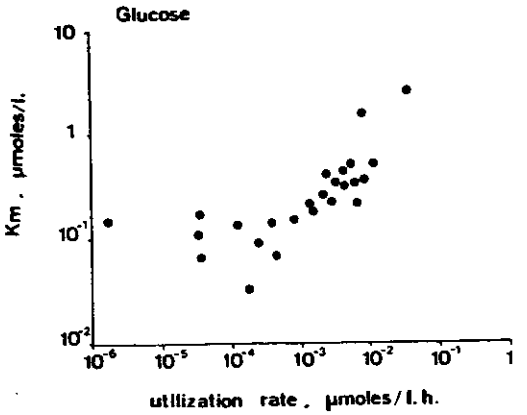


fig. 4.

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).

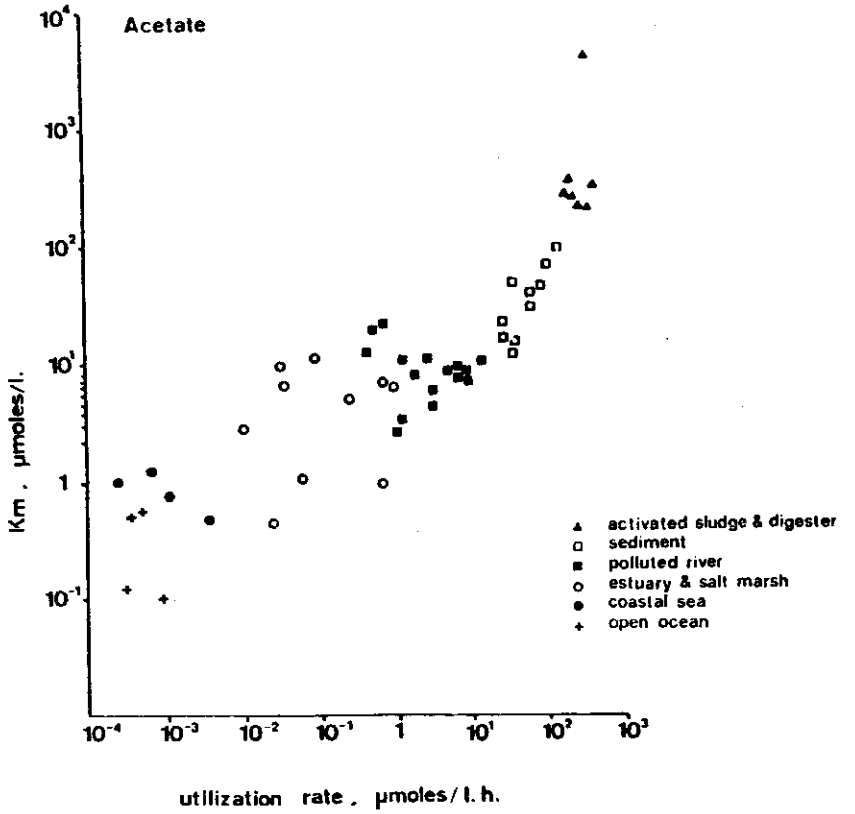


fig. 5.

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).

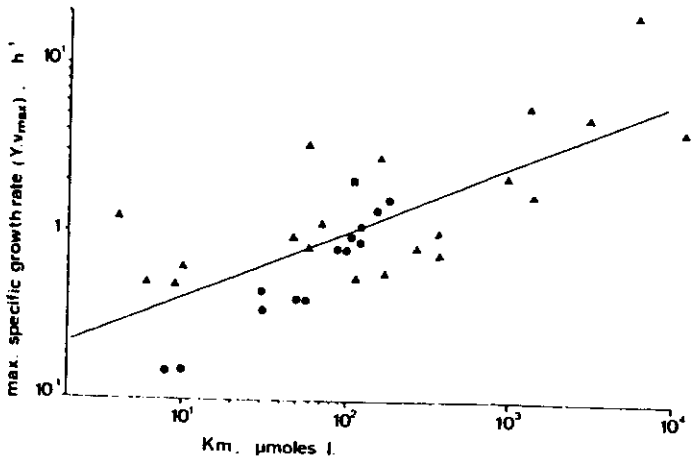


fig. 6.

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).

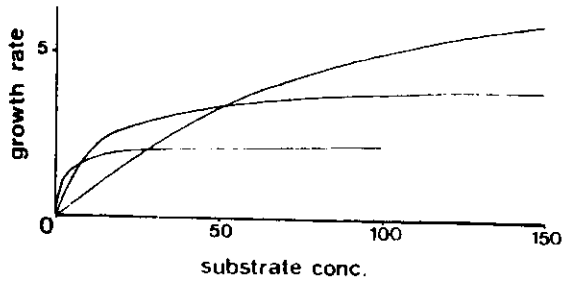


fig. 7.

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 :

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

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 :

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

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

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

$$(5) \qquad B = \frac{Y}{k_d}\ P$$

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 concentration of their direct substrates at a stationary state.

The validity of relation (4) for organic substrates predominantly used by bacteria can be tested with experimental concentration 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

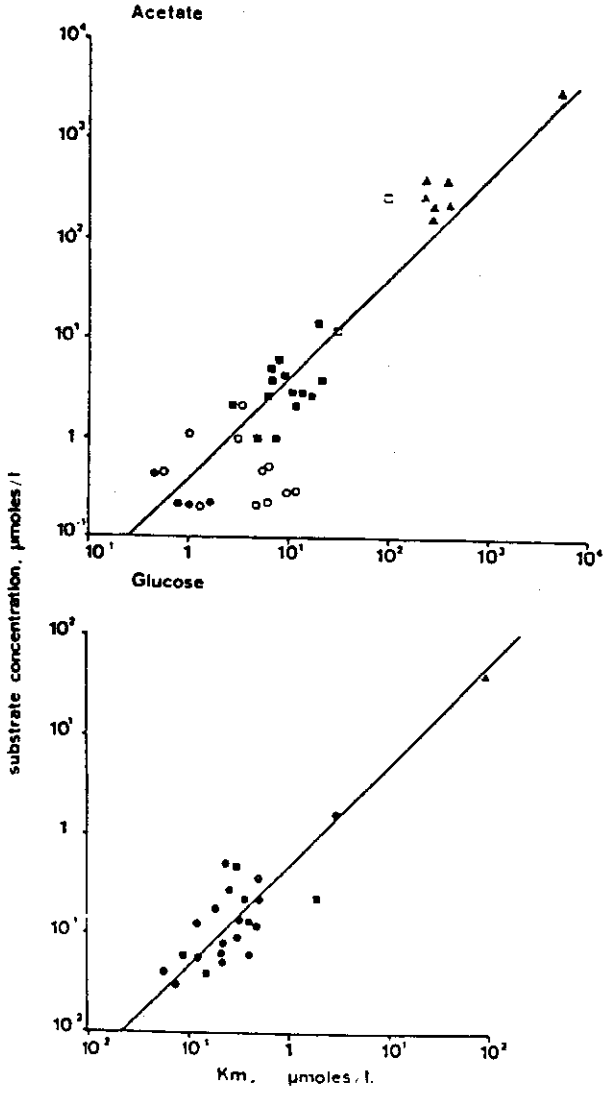


fig. 8.

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 deposition 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. Organic 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 oxidizing 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 equilibrium, including nitrogen species, which is rather unrealistic. Moreover, only one mixing coefficient for both solid and dissolved 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.

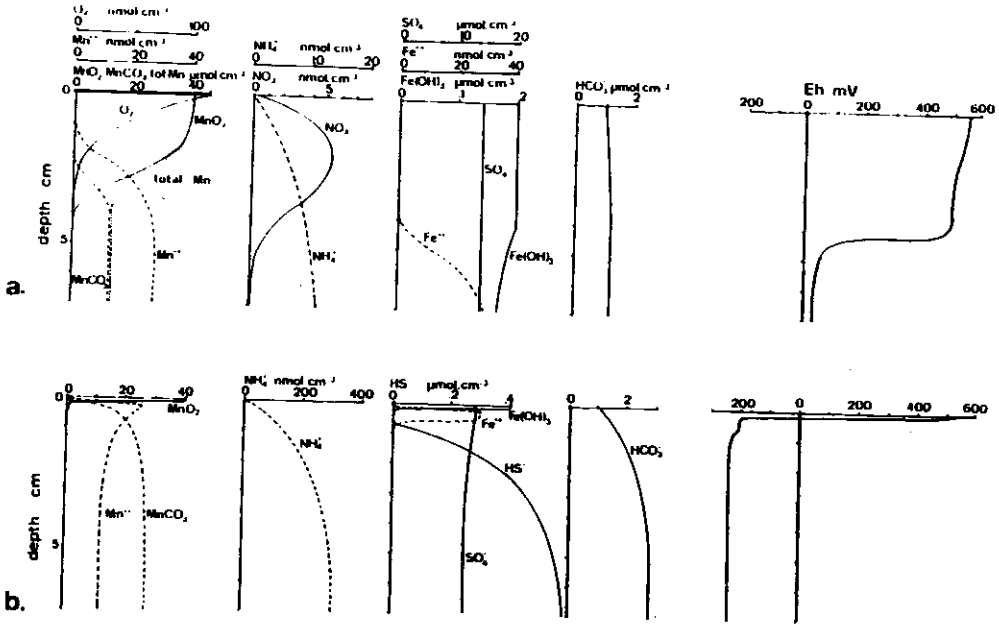


fig. 9.

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). Nevertheless, 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 sedimentary 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 depositing 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

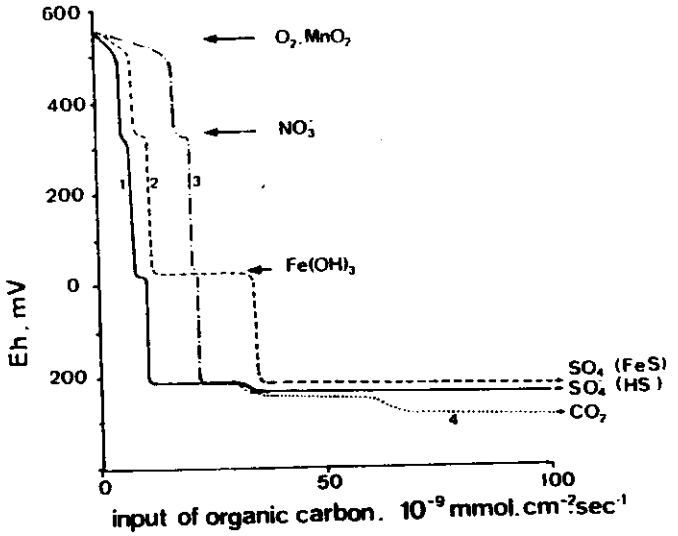


fig. 10.

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 activity, 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

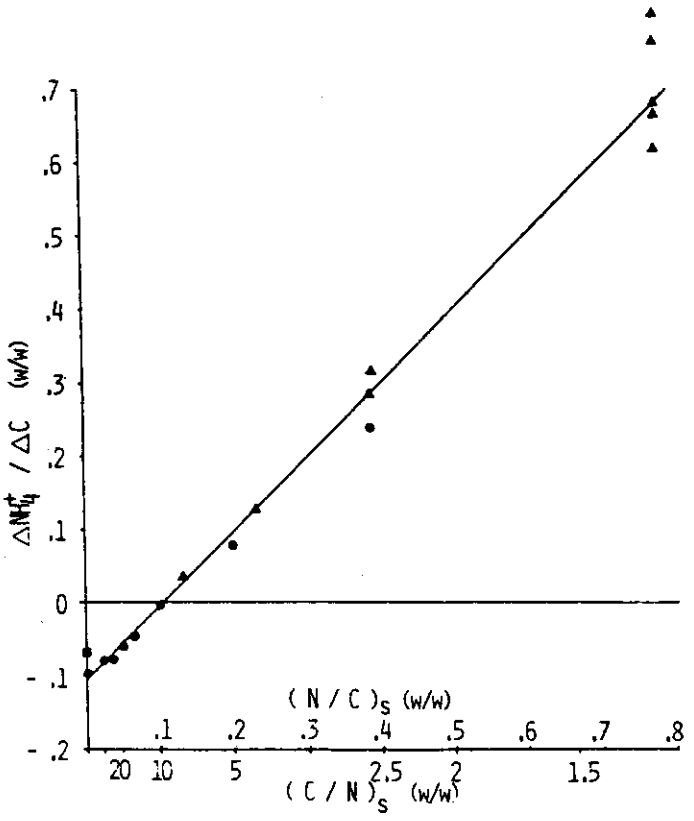


fig. 11.

Release or uptake of ammonia per unit carbon taken up by microbiological communities of marine environments, 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 mineralizers 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 heterotrophic 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 mucopolysaccharides 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 mineralization.

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 ammonification 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

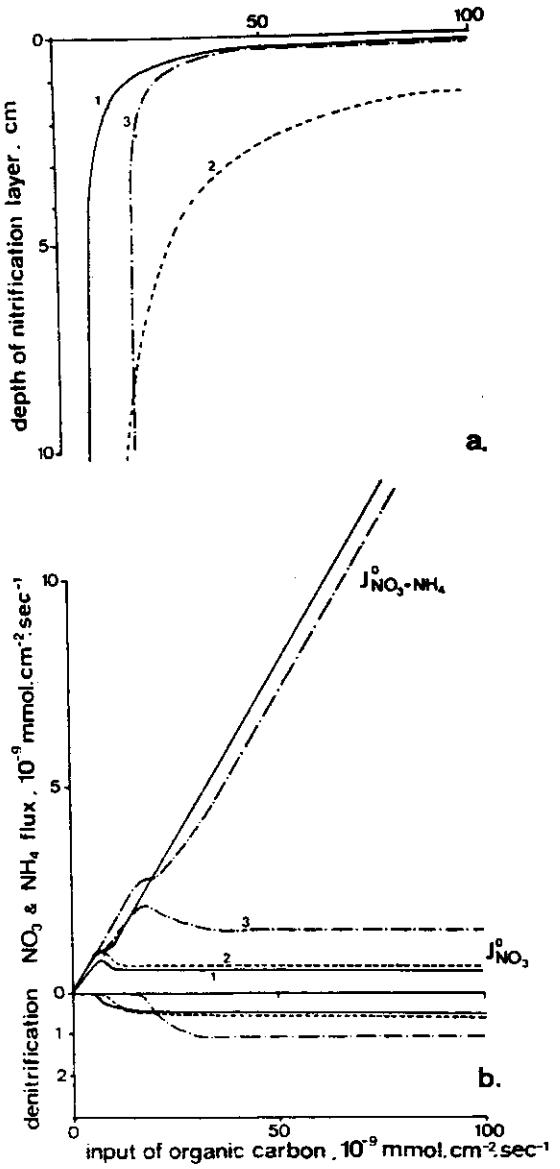


fig. 12.

Model of nitrogen recycling in marine sediments as a function of the input of organic material, for different 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 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 prevailing 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. Paradoxically 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

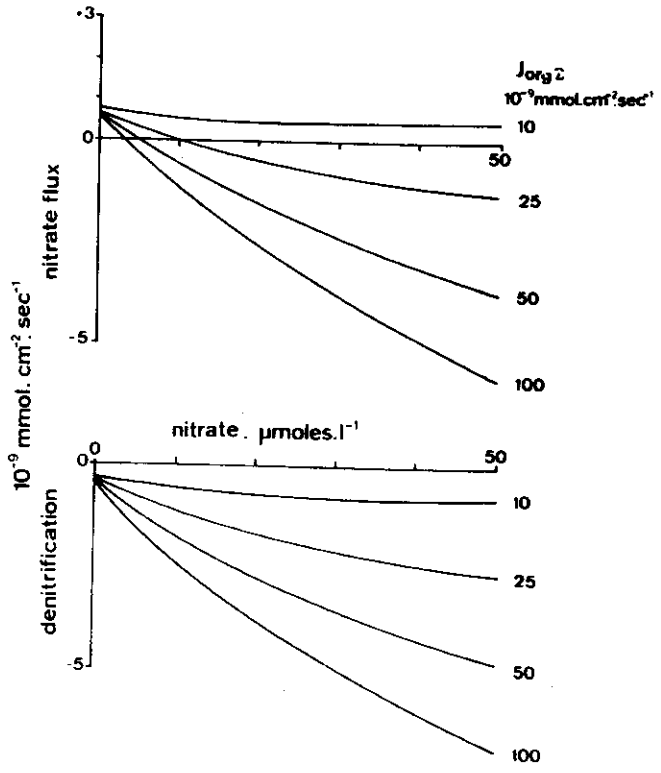


fig. 13.

Effect of nitrate concentration in the overlying water on the flux of nitrate across the sediment-water interface 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).

References.

ADAM, Y., BAEYENS, W., MOMMAERTS, J.P., PICHOT, G., (1981). Mathematical modelling of recent sedimentology in the shallow waters along the Belgian coast, in J.C.J. Nihoul (Ed.), *Ecohydrodynamics*, Elsevier, pp. 333-349.

AZAM, F. and HODSON, R.E., (1977). Size distribution and activity of marine micro-heterotrophs. *Limnol. Oceanogr.*, **22**, 493-501.

BAGANDER, L.E. and NIEMISTO, L., (1978). An evaluation of the use of redox measurements for characterising recent sediments. *Est. Coast. Mar. Sci.*, **6**, 127-134.

BILLEN, G., (1975). Nitrification in the Scheldt estuary (Belgium and The Netherlands). *Est. Coast. Mar. Sci.*, **3**, 79-89.

BILLEN, G., BOSSICART, M., DARO, M.H., DE BOEVER, R., DE CONINCK, L., GOVAERE, J., HECQ, J.H., HEIP, C., HOVART, P., JANSSENS, D., JOIRIS, C., MOMMAERTS, J.P., PICHOT, G., POLK, Ph., REDANT, F., THIELEMANS, L., RUNFOLA, Y., VAN BEVEREN, C., VAN DAMME, O. et VANDEVELDE, J., (1976). In J.C.J. Nihoul et Ph. Polk (Eds), *Trofische ketens en cyclus der nutrienten.* cyclus der nutrienten. Prog. Nat. R et D, Environnement Eau. Projet Mer. Rapport final, vol. 8. Service du premier ministre, Programmation de la Politique scientifique, Bruxelles.

BILLEN, G., (1978). The dependence of the various kinds of microbial metabolism on the redox state of the medium, in *Biogeochemistry of estuarine sediments*, Proc. of a UNESCO/SCOR Workshop, UNESCO, Paris, pp. 254-261.

BILLEN, G. and VERBEUSTEL, S. (1980). Distribution of microbial metabolisms in natural environments displaying gradients of oxidation-reduction conditions. Colloque international du CNRS sur la Biochimie de la matière organique à l'interface eau-sédiment marin. Marseille, France, 25-27 avril 1979.

BILLEN, G., JOIRIS, C., WIJNANTS, J. and GILLAIN, G., (1980). Concentration and microbiological utilization of small organic molecules in the Scheldt estuary, the Belgian coastal zone of the North Sea and the English Channel. *Est. Coast. Mar. Sci.*, **11**, 279-294.

BILLEN, G., (1982). Heterotrophic utilization and regeneration of nitrogen, in J.E. Hobbie and P.J. LeB Williams (Eds), *Heterotrophic activity in the Sea*, Plenum (in press).

CHRISTISON, A., and MARTIN, S.M., (1971). Isolation and preliminary characterization of an extracellular protease of *Cytophaga sp. Can. J. Microbiol.*, **17**, 207-1216.

DERENBACH, J.B. and LeB WILLIAMS, P.J., (1974). Autotrophic and bacterial production : fractionation of plankton populations by differential filtration of samples from the English Channel. *Mar. Biol.*, **25**, 263-269.

HOLLIBAUGH, J.T., (1978). Nitrogen regeneration during the degradation of several amino acids by plankton communities collected near Halifax, Nova Scotia, Canada. *Mar. Biol.*, **45**, 191-201.

JOIRIS, C., BILLEN, G., LANCELOT, C., DARO, M.H., MOMMAERTS, J.P., HECQ, J.H., BERTELS, A., BOSSICART, M., NIJS, J., (1981). A budget of carbon cycling in the Belgian coastal zone : relative roles of zooplankton, bacterioplankton and benthos in the utilization of primary production. 16th Eur. Mar. Biol. Symp. Texel (in press).

JOHANNES, R.E., (1968). Nutrient regeneration in lakes and oceans. *Adv. Microbiol. Sea*, **1**, 203-212.

FROELICH, P.N., KLINKHAMMER, G.P., BENDER, M.L., LUEDTKE, N.A., HEATH, G.R., CULLEN, D., DAUPHIN, P., HAMMOND, D., HARTMAN, B. and MAYNARD, V., (1979). Early oxidation of organic matter in pelagic sediments of the eastern Equatorial Atlantic : suboxic diagenesis. *Geochimica et cosmochimica Acta*, **43**, 1075-1090.

KIM, J. and ZOBELL, C.E., (1974). Occurrence and activities of cell free enzymes in oceanic environments, in R.R. Colwell and R.Y. Morita (Eds), *Effect of the Ocean Environment on Microbial Activities*. University Park Press, pp. 368-385.

LANCELOT, C., (1982). *Etude écophysiologique du phytoplankton de la zone côtière belge*. Thèse. Faculté des Sciences, Université Libre de Bruxelles, 221 pp.

MEYER-RIEL, L.A., (1981). Enzymatic decomposition of proteins and carbohydrates in marine sediments : methodology and field observations during spring. *Meeresforsch.*, **5**, 311-317.

PEARSON, T.H. and STANLEY, S.O., (1979). Comparative measurements of the redox potential of marine sediments as a rapid means of assessing the effect of organic pollution. *Mar. Biol.*, **53**, 371-379.

RITTENBERG, S.C., (1963). Marine bacteriology and the problem of mineralization, in C.H. Oppenheimer (Ed.), *Symposium on Marine Microbiology*, Thomas, Springfield, pp. 48-60.

ROTH, M., (1965). Fluorimetric assay of some peptidases, in R. Ruyssen and E.L. Vandenriesche (Eds), *Enzymes in Clinical Chemistry*, Elsevier.

SEKI, H., NAKAI, T. and OTOBA, H., (1972). Regional differences on turnover rate of dissolved materials on the Pacific Ocean at summer of 1971. *Arch. Hydrobiol.*, **71**, 79-89.

SEKI, H., NAKAI, T. and OTOBA, H., (1974). Turnover rate of dissolved materials in the Philippine Sea at winter of 1973. *Arch. Hydrobiol.*, **73**, 238-244.

SEKI, H. SHORTREED, K.S. and STOCKNER, J.G., (1980a). Turnover rate of dissolved organic materials in glacially-oligotrophic and dystrophic lakes in British Columbia, Canada. *Arch. Hydrobiol.*, **90**, 210-216.

SEKI, H., TERADA, T. and ICHIMURA, S., (1980b). Steady state oscillation of uptake kinetics by microorganisms in mesotrophic and eutrophic water masses, *Arch. Hydrobiol.*, **88**, 219-231.

SEKI, H., YAMAGUCHI, Y. and ICHIMURA, S., (1975). Turnover rate of dissolved organic materials in a coastal region of Japan at summer stagnation period of 1974. *Arch. Hydrobiol.*, **75**, 297-305.

SOMVILLE, M., (1980). *Etude écophysiologique de l'activité bactérienne dans l'estuaire de l'Escaut.* Thèse. Faculté des Sciences, Université Libre de Bruxelles.

SOMVILLE, M. and Billen, G., (1982). A method for determining exoproteasic activity in natural waters. *Limnol. Oceanogr.*, (in press).

STUMM, W., (1966). Redox potential as an environmental parameter : conceptual significance and operational limitation. *Proc. of the third International Water Pollution Research Conference*, Munich, 1, 283-308.

SUESS, E. and MULLER, P., (1980). Productivity, sedimentation rate and sedimentary matter in the oceans. II. Elemental fractionation. Coll. Int. CNRS, n° 293, Biogéochimie de la matière organique à l'interface eau-sédiment marin.

THORSTENSON, D.C., (1970). Equilibrium distribution of small organic molecules in natural waters. *Geochimica et Cosmochimica Acta*, **34**, 745-770.

WHITFIELD, M., (1969). Eh as an operational parameter in estuarine studies. *Limnol. Oceanogr.*, **14**, 547-558.

WILLIAMS, P.J. LeB, (1973). The validity of the application of simple kinetic analysis to heterogeneous microbial population. *Limnol. Oceanogr.*, **18**, 159-164.

WILLIAMS, P.J. LeB, BERMAN, T. and HOLM-HANSEN, O., (1976). Amino acid uptake and respiration by marine heterotrophs. *Mar. Biol.*, **35**, 41-47.

WRIGHT, R.T. and HOBBIE, J.E., (1966). Use of glucose and acetate by bacteria and algae in aquatic ecosystems. *Ecology*, **47**, 447-464.

DETERMINATION OF MARINE PHYTOPLANKTONIC BIOMASS

E. POST, L. GOEYENS and A. VANDENHOUDT

Laboratorium voor Analytische Scheikunde, Vrije Universiteit Brussel, Pleinlaan, 2, B-1050 BRUSSELS (Belgium)

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 inaccurate. In extreme cases the latter induce an overestimation of the concentration 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 comparing to DMSO and to 90 % acetone. Moreover, the efficiency improves by using a grinder.

1.– Introduction.

The phytoplanktonic biomass is an improtant parameter in several studies concerning natural waters (e.g. the fertility of the sea, primary production, nutrient uptake kinetics of phytoplankton, ...). 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 concentration 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 Lorenzen (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 concentration 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 photosystem 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 extraction. The extraction efficiency is inversely proportional to the ratio dry weight/weight chlorophyll *a*, by which the population 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, people 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 products when using an ultrasonic disruptor, but Yentsch and Menzel (1963) obtained the opposite results. To ensure complete extraction, 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 chlorophyll *a*' (Pennington *et al.*, 1967; Katz *et al.*, 1968). But even methanol 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 advantage 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 chloropigments 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 errors. 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 postulations of Lambert-Beer's law are not fulfilled in this case.

Comparison of direct trichromatic spectrometry and spectrometry preceded by chromatographic separation on thin-layer plates shows that chlorophyll b and c are overestimated with the classical method (Rott, 1980). Abaychi and Riley (1979) even calculated negative chlorophyll c concentrations. Also the chlorophyll *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 method, certainly when other extraction solvents than 90 % acetone 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 (Tolstoy, 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 degradation 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 measurements, 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. Therefore, 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/GL[[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 mixture of methanol, acetone and water in the ratio 75/22/3 (v/v). Pump-pressure was nearly constant at 96 bars. The sample was introduced to the column by mean of an automatic injector, Micromeritics 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 diethylether, spectroscopic grade. The exact concentration was estimated 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 OS). 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 purchased 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 consisted 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 graduated centrifuge tube.

Further work was done as follows :

2.5.1.– *Extraction with 90% acetone.*

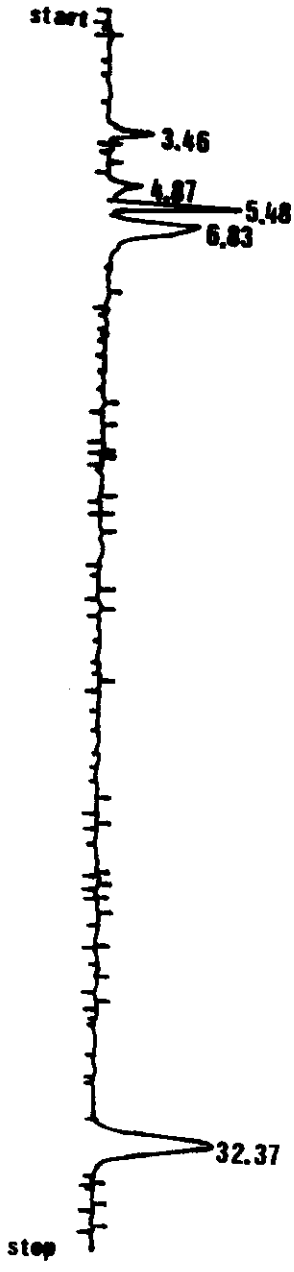
- (Strickland and Parsons, 1968) 10 mℓ of acetone were added and shaken vigorously. Digestion was done during 22 hours in an ice-box (4°C). After the sample had come to room temperature in the dark, it was centrifuged for 10 min at 3 000 g .
- (SCOR/UNESCO, 1966) 3 mℓ of acetone were added to the filter 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 transferred into 90 % acetone after drying with a rotavapor at a temperature of 20°C . For chromatographic analyses the methanol extract 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

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 centrifuged. 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 µg/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.

References.

ABAYCHI, J.K. and RILEY, J.P., (1979). The determination of phytoplankton pigments by high-performance liquid chromatography, *Anal. Chim. Acta*, **107**, 1-11.

BALLESTER, A. and PLANA, A., (1973). Analisis fluorimétrico continuo de las chlorofilas fitoplanctonicas, *Afinidad*, **30**, 787-793.

BAZZAZ, M.B. and REBEIZ, C.A., (1979). Spectrofluorometric determination of chlorophyll(ide) a and b and phaeophytin (or pheophorbide) a and b in unsegregated pigment mixtures, *Photochem. Photobiol.*, **30**, 709-721.

BOTO, K.G. and BUNT, J.S., (1978). Selective excitation fluorometry for the determination of chlorophylls and pheophytins, *Anal. Chem.*, **50**(3), 392-395.

BRAUMANN, T. and GRIMME, L.H., (1979). Single-step separation and identification of photosynthetic pigments by high-performance liquid chromatography, *J. Chromatogr.*, **170**, 264-268.

BROWN, L.M., HARGRAVE, B.T. and MacKINNON, M.D., (1981). Analysis of chorophyll a in sediments by high-pressure liquid chromatography, *Can. J. Fish. Aquat. Sci.*, **38**, 205-214.

BURNISON, B.K., (1980). Modified dimethyl sulfoxide (DMSO) extraction for chlorophyll analysis of phytoplankton, *Can. J. Fish. Aquat. Sci.*, **37**, 729-733.

DALEY, R.J., GRAY, C.B.J. and BROWN, S.R., (1973a). A quantitative semi-routine method for determining algal and sedimentary chlorophyll derivatives, *J. Fish. Res. B. Can.*, **30** (3), 345-355.

DALEY, R.J., GRAY, C.B.J. and BROWN, S.R., (1973b). Reversed-phase thin-layer chromatography of chlorophyll derivatives, *J. Chromatogr.*, **76**, 175-183.

ESKINS, K., SCHOFLIED, C.R. and DUTTON, H.J., (1977). High-performance liquid chromatography of plantpigments, *J. Chromatogr.*, **135**, 217-220.

ESKINS, K. and HARRIS, L., (1980). High-performance liquid chromatography of etioplast pigments in red kidney bean leaves, *Photochem. Photobiol.*, **33**, 131-133.

EVANS, N., GAMES, D.E., JACKSON, A.H. and MATLIN, S.A., (1975). Applications of high-pressure liquid chromatography and field desorption mass spectrometry in studies of natural porphyrins and chlorophyll derivatives, *J. Chromatogr.*, **115**, 325-333.

FALKOWSKI, P.G. and SUCHER, J., (1981). Rapid, quantitative separation of chlorophylls and their degradation products by HPLC, *J. Chromatogr.*, **213**, 349-351.

GARSIDE, L. and RILEY, J.P., (1969). A thin-layer chromatographic method for the determination of plant pigments in sea-water and cultures, *Anal. Chim. Acta*, **46**, 179-191.

GOEYENS, L., POST, E., DEHAIRS, F., VANDENHOUDT, A. and BAEYENS, W., (1982). The use of high-pressure liquid chromatography with fluorimetric detection for the measurements of chlorophyll a concentration in natural extracts of chloropigments and their degradation products, *Int. J. Environ. Anal. Chem.*, (in press).

HISCOX, J.D. and ISRAELSTAM, G.F., (1979). A method for the extraction of chlorophyll from leaf tissue without maceration, *Can. J. Bot.*, **57**, 1332-1334.

HOLM-HANSEN, O., LORENZEN, C.J., HOLMES, R.W. and STRICKLAND, J.D.H., (1965). Fluorometric determination of chlorophyll, *J. Cons. perm. int. Explor. Mer.*, **30** (1), 3-15.

HOLM-HANSEN, O. and RIEMANN, B., (1978). Chlorophyll a determination : improvements in methodology, *Oikos*, **30**, 438-447.

HUSSAINY, S.U., (1973). Some difficulties in the determination of photosynthetic pigments in inland waters, *Austr. Soc. Limnol. Bull.*, **5**, 26-28.

IRIYAMA, K., YOSHIURA, M. and SHIRAKI, M., (1978). Micro-method for the qualitative and quantitative analyses of photosynthetic pigments using high-performance liquid chromatography, *J. Chromatogr.*, **154**, 302-305.

JEFFREY, S.W., (1981). An improved thin-layer chromatographic technique for the marine phytoplankton pigments, *Limnol. Oceanogr.*, **26** (1), 191-197.

KATZ, J.J., NORMAN, G.D., SVEC, W.A. and STRAIN, H.H., (1968). Chlorophyll diastereo-isomers. The nature of chlorophylls a and b and evidence for bacterio-chlorophyll epimers from proton magnetic resonance studies, *J. Am. Chem. Soc.*, **90** (24), 6841-6845.

LORENZEN, C.J., (1966). A method for the continuous measurements of *in vivo* chlorophyll concentration, *Deep-Sea Res.*, **13**, 223-227.

LORENZEN, C.J., (1967). Determination of chlorophylls and pheopigments : spectrophotometric equations, *Limnol. Oceanogr.*, **12**, 343-346.

MARKER, A.F.H., (1972). The use of acetone and methanol in the estimation of chlorophyll in the presence of phaeophytin, *Freshwat. Biol.*, **2**, 361-385.

MARKER, A.F.H., (1980). A note on the extraction of chlorophyll from benthic algea using methanol, *Ergeb. Limnol.*, **14**, 88-90.

MARKER, A.F.H., NUSCH, E.A., RAI, H. and RIEMANN, B., (1980). The measurements of photosynthetic pigments in freshwaters and standardisation methods : conclusions and recommandations, *Ergeb. Limnol.*, **14**, 91-106.

MORETH, C.M. and YENTSCH, C.S., (1970). The role of chlorophyllase and light in the decomposition of chlorophyll from marine phytoplankton, *J. Exp. Mar. Biol. Ecol.*, **4**, 238-249.

MOSS, B., (1967). A spectrophotometric method for the estimation of percentage degradation of chlorophylls to pheopigments in extracts of algae, *Limnol. Oceanogr.*, **12**, 335-342.

NUSH, E.A., (1980). Comparison of different methods for chlorophyll and phaeopigment determination, *Ergeb. Limnol.*, **14**, 14-36.

PARSONS, T.R. and STRICKLAND, J.D., (1963). Discussion of spectro-photometric determination of marine pigments with revised equations for ascertaining chlorophylls and carotenoids, *J. Mar. Res.*, **21**, 155-163.

PENNINGTON, F.C., STRAIN, H.H., SVEC, W.A. and KATZ, J.J., (1967). Preparation and properties of 10-hydroxychlorophyll a and b, *J. Am. Soc.*, **89** (15), 3875-3880.

PRENZEL, U. and LICHTENHALER, H.K., (1979). Separation of prenyllipids by HPLC, in L.A. Appelquist and C. Liljenberg (Eds), *Advances in the Biochemistry and Physiology of Plant Lipids*, (part 3), Elsevier, Biomedical Press, pp. 319-235.

RAI, H., (1980). Comparison between trichromatic spectrophotometric equations : sources of error, *Arch. Hydrobiol.*, **88** (4), 514-517.

REBEIZ, C.A., BAZZAZ, M.B. and BELANGER, F., (1978). The separation of chlorophyll and phaeophytins by reversed-phase, *Chrom. Rev.*, **4** (2), 8-9.

REBEIZ, C.A., BELANGER, F., FREYSSINET, G. and SAAB, D.G., (1980). The occurrence of several novel chlorophyll a and b chromaphores in higher plants, *Biochim. Biophys. Acta*, **590**, 234-247.

RIEMANN, B., (1978). Carotenoid interference in the spectrophotometric determination of chlorophyll degradation products from natural populations of phytoplankton, *Limnol. Oceanogr.*, **23** (5), 1059-1066.

SCHOLZ, B. and BALLSCHMITER, K., (1981). Preparation and reversed-phase high-performance liquid chromatography of chlorophylls, *J. Chromatogr.*, **208**, 148-155.

SCOR/UNESCO, (1966). Determination of photosynthetic pigments in sea-water, in *Mongr. Oceanogr. Methodol.*, 1, 1-69.

SHOAF, W.T. and LIUM, B.W., (1976). Improved extraction of chorophyll a and b from algae using dimethyl sulfoxide, *Limnol. Oceanogr.*, **21** (6), 926-928.

SHOAF, W.T. and LIUM, B.W., (1977). The quantitative determination of chlorophylls a and b from freshwater algae without interference from degradation products, *J. Res. U.S. Geol. Survey*, **5** (2), 263-264.

SHOAF, W.T., (1978). Rapid method for the separation of chlorophylls a and b by high-pressure liquid chromatography, *J. Chromatogr.*, **152**, 247-249.

SLOVACEK, R.E. and HANNAN, P.S., (1977). *In vivo* fluorescence determinations of phytoplankton chlorophyll a, *Limnol. Oceanogr.*, **22** (5), 919-924.

STRANSKY, H., (1978). Die quantitative Bestimmung von Chloroplastenpigmenten im picomol-Bereich mit Hilfe einer isochratischen HPLC-Methode, *Z. Naturforsch. C. Biosci.*, **33** (C), 836-840.

STRICKLAND, J.D.H. and PARSONS, T.R., (1968). Pigment Analysis, in *A practical Handbook of Seawater Analysis* edited by *Fish. Res. B. Can.*, Ottawa, pp. 185-206.

STRICKLAND, J.D.H., (1968). Continuous measurement of *in vivo* chlorophyll; a precautionary note, *Deep-Sea Res.*, **15**, 225-227.

SUBBA RAO, D.V. and PLATT, T., (1969). Optimal extraction conditions of chlorophylls from cultures of five species of marine phytoplankton, *J. Fish. Res. B. Can.*, **26** (6), 1625-1630.

TOLSTOY, A., (1977a). Chlorophyll a as a measure of phytoplankton biomass, *Acta Universitatis Upsaliensis*, **416**, 30 pp.

TOLSTOY, A., (1977b). *Methods of determining chlorophyll a in phytoplankton*, SNV PM 831/NLU Rapport, 91, Uppsala (Sweden), 62 pp.

TOLSTOY, A. and TOTH, I., (1980). Bacteriochlorophyll d and its interference on determination of chlorophyll a, *Arch. Hydrobiol.*, **89** (1/2), 160-170.

WEBER, A. and WETTERN, M., (1980). Some remarks on the usefulness of algall carotenoids as chemotaxonomic markers, in F.C. Czygan (Ed.), *Pigments in Plants*, Gustav Fisher Verlag, Stuttgart - New York, 2nd ed., pp. 104-116.

YENTSCH, C.S. and MENZEL, D.W., (1963). A method for the determination of phytoplankton chlorophyll and phaephytin by fluorescence, *Deep-Sea Res.*, **10**, 221-231.

NUTRIENT UPTAKE BY MARINE PHYTOPLANKTON

L. GOEYENS, E. POST, A. VANDENHOUDT, M. DECLERCK and W. BAEYENS

Laboratorium voor Analytische Scheikunde, Vrije Universiteit Brussel, Pleinlaan, 2, B-1050 BRUSSELS (Belgium)

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

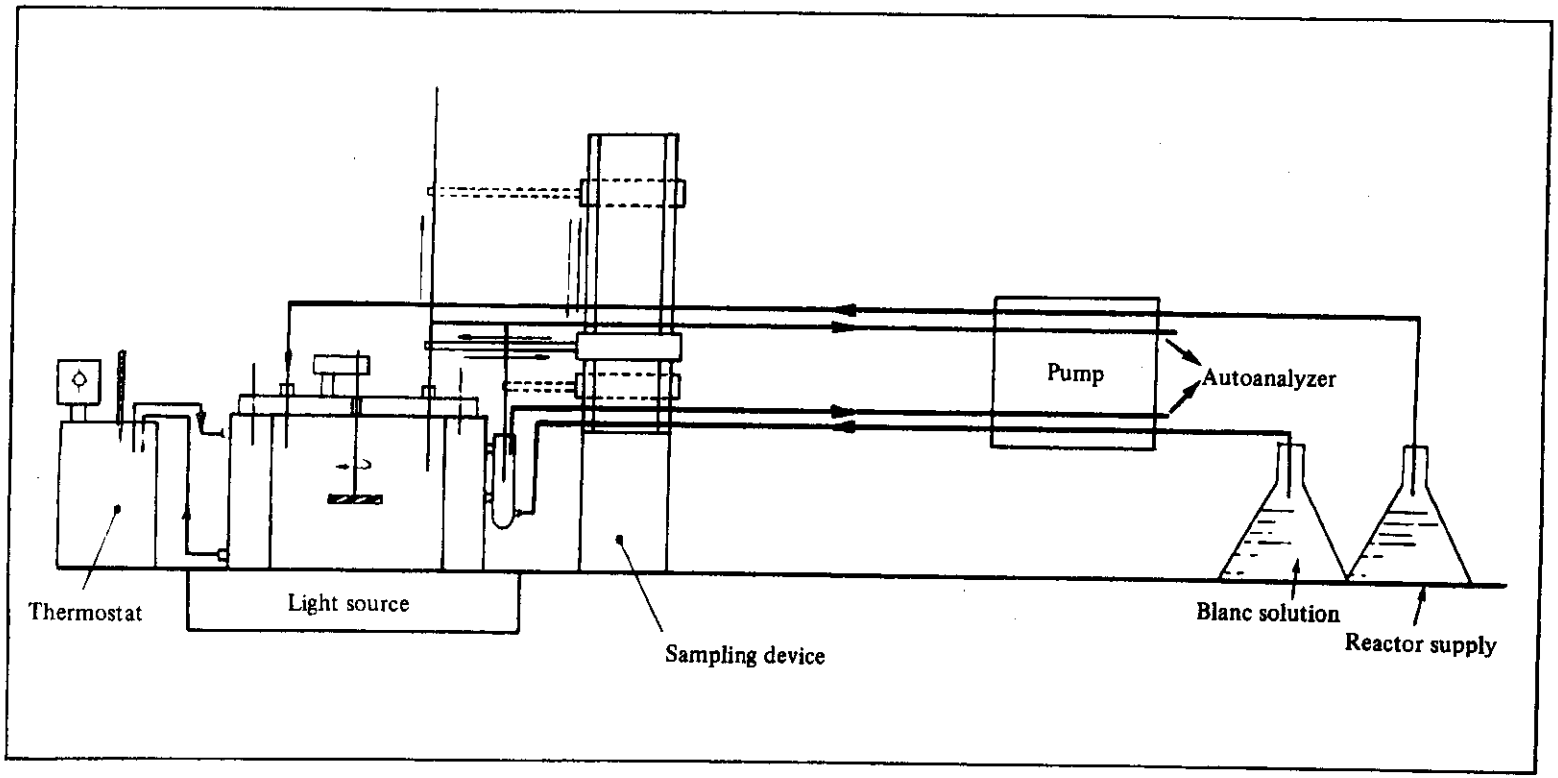


fig. 1.

Scheme of the reactor

tubing, alternatively samples reactor solution and a blank solution (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 advantage 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 zooplankton 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* concentration 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 conservation :

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

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

$$(2) \qquad \text{Uptake} = Q\,C_{in} - Q\,C_{out} - V\frac{dC}{dt},$$

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.

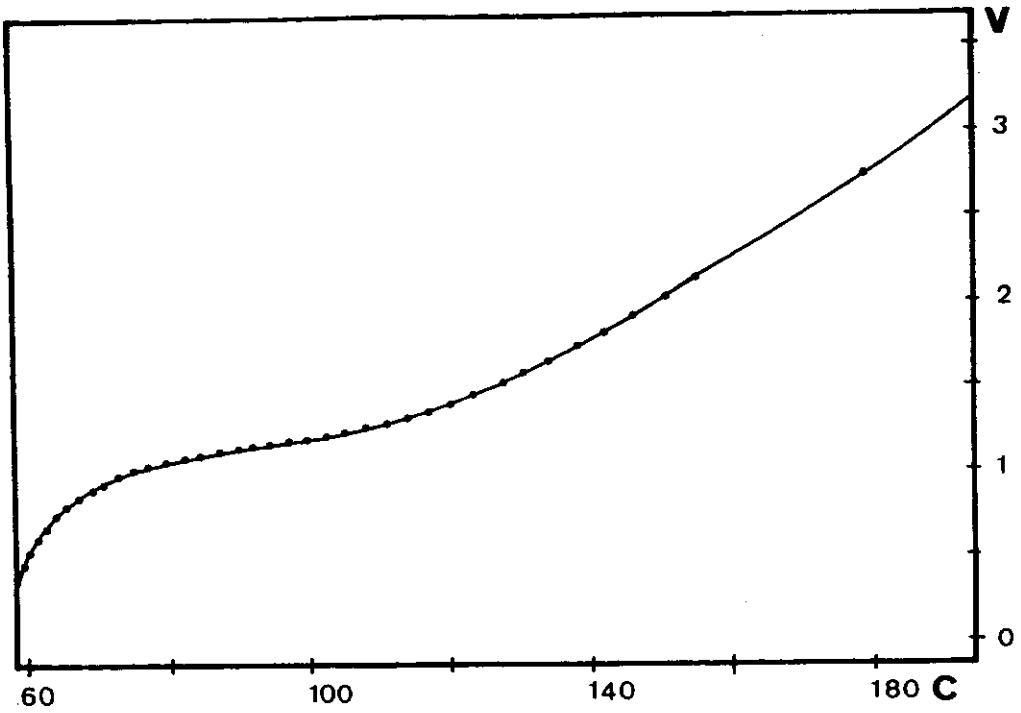


fig. 2.

Uptake rate versus concentration
(experiment 1, part 1)

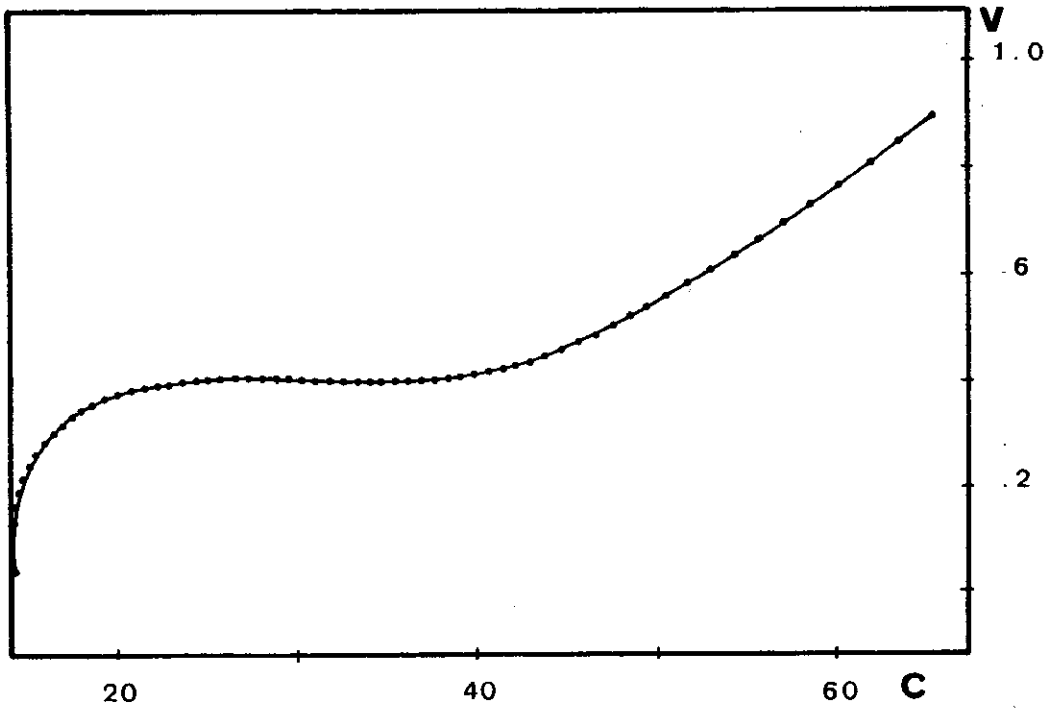


fig. 3.

Uptake rate versus concentration
(experiment 1, part 2)

For this experiment an evolution of the substrate concentration 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 realized.

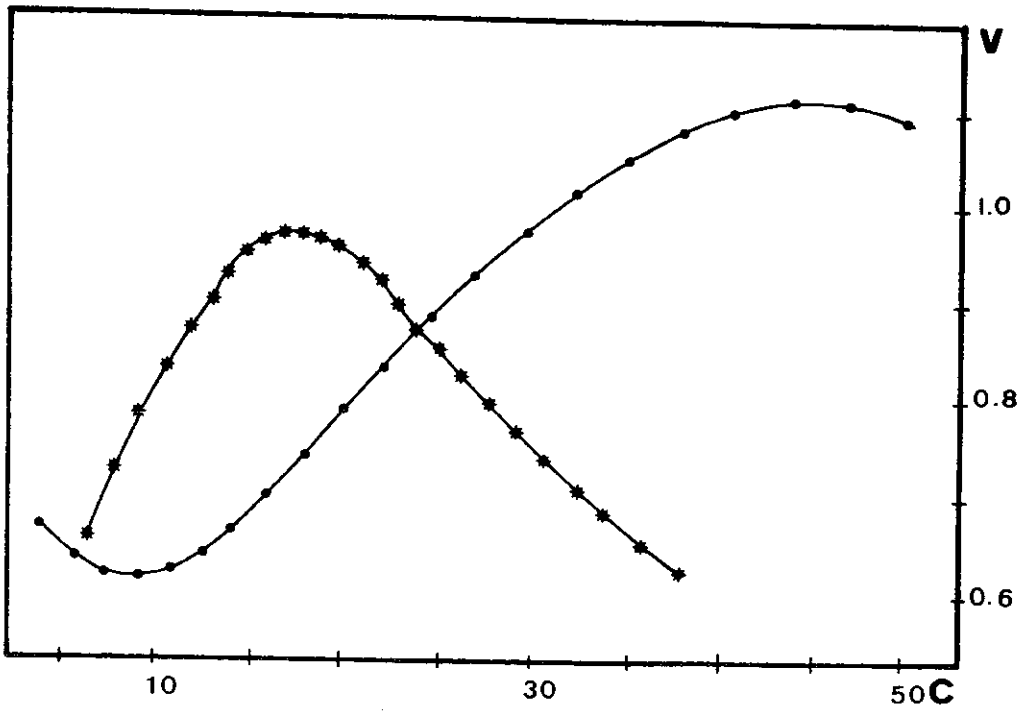


fig. 4.

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 :

$$V_{max} = 38.9 \times 10^{-3} \text{ µg N/min.(µg chlor. } a/\ell)^{-1}$$

for the first experiment, where the species *Skeletonema* was dominant, and

$$V_{max} = 11.8 \times 10^{-3} \text{ µg N/min.(µg chlor. } a/\ell)^{-1}$$

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).

Acknowledgements.

We are grateful to the members of the Management Unit of the North Sea and Scheldt estuarium and to the Belgian Navy for the logistic support. By the realization of this work we especially thank Dr. J.P. Mommaerts for the species determinations.

References.

BURMASTER, D.E., (1979). The unsteady continuous culture of phosphate-limited *Monochrysis lutheri* (Droop) : experimental and theoretical analysis, *J. exp. mar. Biol. Ecol.*, **39**, 167-186.

BURMASTER, D.E. and CHISHOLM, S.W., (1979). A comparison of two methods for measuring phosphate uptake by *Monochrysis lutheri* (Droop) grown in continuous culture, *J. exp. mar. Biol. Ecol.*, **39**, 187-202.

BUTTON, D.K., (1978). On the theory of control of microbial growth kinetics by limiting nutrient concentrations, *Deep-Sea Res.*, **25**, 1163-1177.

CARPENTER, E.J. and GUILLARD, R.R.L., (1971). Intraspecific differences in nitrate half-saturation constants for three species of marine phytoplankton, *Ecology*, **52**, 183-185.