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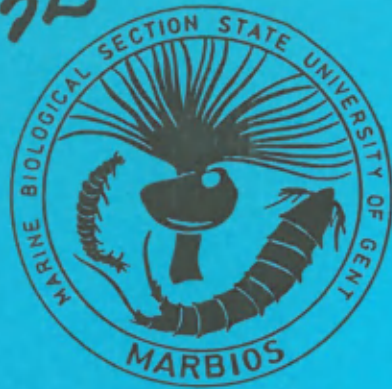
MARBIOS

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AT-Rapport
Rudy L. HERMAN

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GECONCERTEERDE ONDERZOEKSAKTIES
OCEANOGRAFIE

ECOLOGY, ECOTOXICOLOGY AND SYSTEMATICS

OF MARINE BENTHOS

Rudy L. HERMAN

ECOLOGY, ECOTOXICOLOGY AND SYSTEMATICS

OF MARINE BENTHOS

Promotoren :

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INTRODUCTION

This volume presents the final reports on four years of research in the Concerted Actions Oceanography partim Benthos by the Marine Biology Section of the Institute of Zoology of the State University of Gent. These Concerted Actions were financed by the Belgian Ministry of Scientific Policy. The volume contains ten reports that represent the activities in the Marine Biology Section over the last four years.

First of all, an important effort has been devoted to the continuation of our studies on ecological monitoring of pollution using structural characteristics of benthic communities. Long-term time series of ecological parameters are valuable and necessary but require a consistent and important effort, which is sometimes hard to realize for a research group depending on short-term contracts. Though the rationale and methodology for such studies have been the subject of much debate over the last years and some approaches are critisized for their lack of explanatory and, perhaps, predictive power, these methods represent the only ecological tool available to monitor the 'state' of an ecosystem on the long term and this is of great importance to man. It must be emphasized that only long term monitoring using a standardized methodology is really worthwhile. Examples of this type of results are shown in the reports on the effects of sand and gravel exploitation on macrofauna in the Belgian North Sea area and on the evolution of meiofauna diversity in the polluted Belgian coastal waters.

Though ecological monitoring of benthic communities is usually done using macrofauna, it has been our experience from many years of research in the North Sea and the Delta area in The Netherlands that the meiofauna, especially the nematodes and harpacticoid copepods, can be a very valuable tool as well. Meiofaunal populations and communities are much less variable spatially, due e.g.to the absence of pelagic larvae. However, meiofauna diversity is high and represents numerous taxonomic problems. For this reason taxonomic research on nematodes and harpacticoid copepods is still being continued in the Marine Biology Section (see list of publications).

In order to gain more insight in energy flow through benthic systems we have started a study on the ecology of gobies (Pomatoschistus spp.) in subtidal areas along the Belgian coast. Gobies are the most numerous fish in this area and are a clear link between benthos and larger, commericial fish. Two species are especially important and show interesting segregation in feeding and breeding activities that may be interpreted as a way to avoid competition. This programme is in cooperation with the State Fisheries Institute in Ostend and it is our hope that research on fish biology will become more important in the future.

During the last years the study of a number of problems has been transferred from the field to the laboratory. The first concerns the evaluation of the energy flow through benthic communities on the basis of structural characteristics such as density and biomass of the populations. This was necessary at a time when next to nothing was known on productivity of meiofauna and logistic limitations on sampling on sea left us no possibility to go further. We have now a much better idea about the energy flow in general and the productivity of nematodes and harpacticoid copepods in particular. Consequently, the usefulness of a structural approach based on field data only, has reached its limit, as exemplified by the report on nematode productivity. Furthermore, we have now at our disposal the research vessel Belgica which is much better equiped than previous vessels and will permit more experimental work: an example of this new orientation is the report on grazing of nematodes on bacteria.

A second series of problems concerns ecotoxicology. In the framework of a CEC-project we have developed a standardized ecotoxicological test for a marine nematode. The standardization concerns the composition of the medium and the bacterium used as food. This test has subsequently been used in acute toxicity tests for a whole number of toxicants including heavy metals, pesticides, PCB, TiO_2-waste etc. Acute toxicity tests, although useful in particular contexts, are less valuable when the ecological consequences of pollutants have to be predicted. We are currently testing a series of parameters with potential value as indicators of sublethal long-term toxicity and we hope to extend this research to microcosm and mesocosm experiments.

During the last year the series of biochemical, physiological and histological tests developed in England to measure stress in the blue mussel Mytilus edulis has been brought to our laboratory with promising results. We are currently engaged in studying Scope for Growth as an indicator of stress. Several other parameters to measure stress have been tested of which the Adenylate Energy Charge seemed to be most promising, but results with the brackish water polychaete Nereis diversicolor in the heavily polluted Western Scheldt estuary were not very encouraging

Finally, I want to mention two programmes which have been developed in other contexts: the studies on meiofauna of Posidonia, Cystoseira and sediments of the Bay of Calvi in Corsica and the Mediterranean deep-sea studies which are financed by the Belgian National Fund for Scientific Research.

As a whole, the developments in the past four years have been promising. The Marine Biology Section is now well established as a leading centre of benthic research, especially in the field of meiobenthos, in the world. However, it must be stated that the level of activity on macrofauna research has been declining and the departure of several researchers in the past few years has not yet been adequately compensated . This is unfortunate since many international developments involve the study of macrobenthos especially. On the whole the main threat to the Marine Biology Section is the fact that only its director is tenured, a situation that is incompatible with the present level of research activity .

Oceanography in Europe is in an accelerating state of development and international cooperation will become a main item in the future. The Marine Biology Section is presently engaged in the COST 647 project of the CEC, is a Founding Member of the European Association of Marine Science and Technology initiated by the Council of Europe and is the centre of a Twinning Project of Marine Benthos of the CEC; its director is chairman of the Benthos Ecology WG of the International Council for the Exploration of the Sea and coordinator of the North Sea Benthos Survey which will be without doubt influential in promoting international cooperation and especially standardization and intercalibration of methodology in Europe. At the same time projects with developing countries in the field of oceanography are now started in Belgium and the CEC and projects with Kenya, Algeria and Mauritania are currently active. We are thus in the ambiguous situation that more and more projects are being developed internationally but national funding and, most important, employment of skilled and valuable scientists, is at risk. This remains a constant problem and a real challenge for the future.

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ECOLOGY AND SYSTEMATICS

OF MARINE BENTHOS

INTRASPECIFIC VARIATION IN <u>SABATIERIA</u>-SPECIES

FROM THE SOUTHERN BIGHT OF THE NORTH SEA

by

31310

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ABSTRACT

The intraspecific variation of <u>Sabatieria</u> species from the Southern Bight of the North Sea is discussed.

A redescription (or amplification) is provided for following species : <u>Sabatieria celtica</u> Southern, 1914, <u>S. longispinosa</u> Lorenzen, 1972, <u>S. punctata</u> (Kreis, 1924).

Following taxonomic changes are proposed : <u>Sabatieria strigosa</u> Lorenzen, 1972 is synonymized with <u>S. celtica</u> Southern, 1914 ; <u>Sabatieria mortenseni</u> (Ditlevsen, 1921), <u>S. breviseta</u> (Stekhoven, 1935) and <u>S. vulgaris</u> sensu Gerlach (1965) and sensu Riemann (1966) (nec de Man, 1907) are synonymized with <u>S. punctata</u> (Kreis, 1924).

INTRODUCTION

Biological species are in practice defined as a cluster of phenetically similar individuals ; the inference that a phenetic cluster is a biological species can be viewed as a prediction that stands or falls with increased data (Hull, 1968, 1971).

Marine nematode species are often characterized and defined by diagnostic features which are valid for the few specimens on which the species diagnosis is based. The examination of large nematode populations from the Southern Bight of the North Sea shows that the diagnostic characters proposed for some species are not constant at all. This large intraspecific variation will be illustrated by a few species of the genus <u>Sabatieria</u>. The variation is especially large in <u>S. celtica</u> and <u>S. punctata</u>.

Nematologists should be stimulated to examine an increased number of data from a larger number of specimens ; nowadays, the tendency exists that each nematologist creates his own species (at least for some genera) in his area of investigation.

MATERIAL AND METHODS

From 1972 onward, a bulk of samples was collected in the Southern Part of the North Sea revealing a lot of nematodes to examine.

Description of the area and general characteristics of the nematode assemblages are given Herman <u>et al.</u> (1985).

Methods and abbreviations are as mentioned previously (Vincx, 1983).

<u>Sabatieria celtica</u> Southern, 1914 (Figs 1-2)
     syn.n. <u>S. strigosa</u> Lorenzen, 1972

Material

Tens of males, females and juveniles.

Locality

Southern Bight of the North Sea ; common ; abundance of the
species more than 10% in sites with median grain size of
the sand fraction between 150-250 um and with 1-20% silt.
56 localities (Fig.1). Collected between 1972 and 1985.

Measurements

$$\vardelta_1 : \frac{-\quad 135\quad 157\quad 315\quad M\quad 2010}{17\quad 35\quad 35\quad 39\quad 46\quad 39}\ 2150 \qquad (\text{slide no.10110})$$

    a= 46.7 b= 6.8 c= 15.4 c'= 3.6 spic= 40 μm
    14 p.s.

$$\vardelta_2 : \frac{-\quad 160\quad 174\quad 424\quad M\quad 2338}{19\quad 39\quad 41\quad 52\quad 44\quad 44}\ 2510 \qquad (\text{slide no.10111})$$

    a= 48.3 b= 5.9 c= 14.6 c'= 3.9 spic= 63 μm
    20 p.s.

$$\varphi_1 : \frac{-\quad 100\quad 106\quad 264\quad 1318\quad 2491}{19\quad 35\quad 36\quad 39\quad 51\quad 40}\ 2690 \quad (\text{slide no.10112})$$

    a= 52.7 b=10.2 c= 13.5 c'= 5.0 V= 49%

Others :

	Males (n= 10)	Females (n= 10)
L :	2100-2890	2200-2980
a :	46.3-68.9	45.7-57.0
b :	8.9-11.5	10.0-12.2
c :	14.0-17.5	14.7-16.3
c':	3.6-4.5	4.4-5.3
spic :	30-65 μm	V : 47-50%

Description

<u>S. celtica</u> has already several times been described in an
accurate way (Lorenzen, 1972 ; Boucher, 1976 ; Platt,
1984). These authors also discuss the large variability in
morphometric data (i.e. body length ; length of cephalic
setae ; diameter of the amphideal fovea ; spicule length ;
number of preanal supplements). A closely related species
is <u>S. strigosa</u> Lorenzen, 1972 of which only males were
described because this species was in all characters (i.e.

length of the cephalic setae, size of the amphideal fovea, habitus (a-index) and shape of the tail), except for the length of the spicules and number of preanal supplements, nearly identical with S. celtica.

Differences according to Lorenzen (1972) :

S. celtica	S. strigosa
L : 1800-3150 µm	1660-2120 µm
spic. length 38-61 µm	28-31 µm
number p.s. 15-22	9-12

The extremely high variability of the spicule length in S. celtica is correlated with the body length ; i.e. longer animals have longer spicules.

In different samples of the Southern Bight of the North Sea a Sabatieria species close to S. strigosa with 14 p.s. (rarely 12 and 13 p.s.) occurs and the length of the spicules ranges between 40-43 um. It occurs together with Sabatieria species which resemble more S. celtica because of the presence of 18-21 p.s.

However, the specimens with 14 preanal supplements have characters which fall within the range of the S. celtica except that some specimens (not all) show a lateral differentiation in the cuticle in which the coarser dots in the lateral field are arranged in nearly longitudinal rows from the end of the pharyngeal region on.

Therefore we consider S. strigosa synonymous with S. celtica.

Sabatieria longispinosa Lorenzen, 1972 (Figs 1, 3)

Material

Three males, one female, ten juveniles.

Locality

Southern Bight of the North Sea. Fine-medium sand with less than 2% silt. Six localities (Fig.1). Collected between 1972 and 1984.

Measurements

$$\sigma_1 : \frac{-\quad 135\quad 165\quad 237\quad M\quad 3518}{13\quad 24\quad 26\quad 30\quad 35\quad 26}\ 3700\ \text{(slide no.10113)}$$

a= 105.7   b= 15.6   c= 20.3   c'= 7.0   spic= 44 µm

(other males and female are too much curved to be measured in an accurate way).

Description

Riemann (1966), Lorenzen (1972) and Platt (1984) gave accurate descriptions of <u>Sabatieria longispinosa</u>. Additional morphological features are :
The North Sea specimens are longer, have elongated spiral amphideal foveas (3 3/4 turns) which are ventrally wound ; ± 90% of the c.b.d. ; the cuticle is punctated ; dots are arranged in transverse rows ; sublateral modified punctations are present in the pharyngeal region and the anterior part of the intestine ; these punctations consist of transverse slits which connect two cuticular points of the same row. The amphideal fovea is bordered posteriorly by such modifications (cfr. Fig.3B).
The three North Sea males have seven preanal supplements ; the three supplements close to the cloaca are smaller and associated by a long ventral seta.

<u>Sabatieria punctata</u> (Kreis, 1924) (Figs 1, 4)
    syn.n.   <u>S.   mortenseni</u> (Ditlevsen, 1921)
                <u>S.   breviseta</u> (Schuurmans Stekhoven, 1935)
                <u>S.   vulgaris</u> sensu Gerlach (1965) and sensu
                   Riemann (1966) nec (de Man, 1907)

Material

Several males, females and juveniles.

Locality

Southern Bight of the North Sea. Sublittoral fine to coarse sand with silt content between 5 and 90%. Abundance of the species more than 10% (even 95% in some coastal stations) with median grain size of the sand fraction between 100-250 um and more than 10% silt. 34 localities mainly in the coastal region (Fig.13). Collected between 1972 and 1985.

Measurements

$$\sigma_1 : \frac{-\quad 95 \quad 118 \quad 176 \quad M \quad 1398}{14 \quad 33 \quad 35 \quad 36 \quad 44 \quad 43} \ 1560 \ \text{(slide no.10114 ;}$$
Fig.4A, F)

a= 35.5   b= 8.9   c= 9.6   c'= 3.8   spic. 49 µm   5 p.s.

$$\sigma_2 : \frac{-\quad 102 \quad 116 \quad 172 \quad M \quad 1669}{14 \quad 24 \quad 24 \quad 28 \quad 31 \quad 31} \ 1810 \ \text{(slide no.10115 ;}$$
Fig.4B, G)

a= 58.4   b= 10.5   c= 12.8   c'= 4.5   spic= 48 µm   6 p.s.

$$\sigma_3 : \frac{-\quad 115 \quad 131 \quad 192 \quad M \quad 1351}{14 \quad 36 \quad 37 \quad 39 \quad 39 \quad 37} \ 1490 \ \text{(slide no.10116 ;}$$
Fig.4C, H)

a= 38.2   b= 7.8   c= 10.7   c'= 3.8   spic= 54 µm   5 p.s.

$$\vec{\male}_4 : \frac{-\quad 94\quad 104\quad 157\quad M\quad 1056}{12\quad 23\quad 24\quad 26\quad 26\quad 32} 1180 \text{ (slide no.10117 ; Fig.4D, I)}$$

a= 36.9   b= 7.5   c= 9.5   c'= 3.8   spic= 41 µm   7 p.s.

$$\vec{\male}_5 : \frac{-\quad 95\quad 113\quad 180\quad M\quad 1590}{13\quad 35\quad 36\quad 41\quad 48\quad 44} 1740 \text{ (slide no.10118 ; Fig. 4E, J)}$$

a= 36.3   b= 9.7   c= 11.6   c'= 3.4   spic= 45 µm   8 p.s.

$$\female_1 : \frac{-\quad 120\quad 136\quad 214\quad 857\quad 1439}{17\quad 40\quad 42\quad 44\quad 52\quad 35} 1595$$

a= 30.7   b= 7.5   c= 10.2   c'= 4.5   V= 54%

Others :

	Males (n= 25)	Females (n= 10)
L :	1095-2400	1300-1690
a :	22.0-43.0	27.6-34.8
b :	7.2-10.5	7.5-8.6
c :	10.0-15.9	10.1-12.1
c':	3.1-4.5	4.1-5.2
spic:	37-52 um	V : 49-55%

Description

Males. Body cylindrical with rounded head and a cylin-
droconical tail with a swollen tip.
     Cuticle punctated ; in the pharyngeal and caudal re-
gion of some specimens, the punctation is present in
transverse rows and coarse dots ar separated by rows of
smaller dots (Fig.4B, D) ; in this case, no lateral dif-
ferentiation is present ; in other specimens, the annula-
tion is more regular and with or without (Fig.4A, C, E)
lateral differentiation. In the mid-body region the
difference between the three types of cuticular pattern is
no longer present.
     Somatic setae short and arranged in four sublateral
rows throughout the body. Two pairs of sublateral cervical
setae (3-5 um long) are situated posterior to the amphideal
fovea ; in some specimens some of these setae are lacking
(cfr. Fig.4A-D). The six internal labial papillae are
small but always obvious. The six external labial
sensillae are longer (2 um) and situated at the level of
the base of the buccal cavity. The four cephalic setae
(4-5 um long ; i.e. 20-30% of the c.b.d.) are situated at
the anterior border of the amphideal fovea. Amphideal
fovea spiral, describes 3 1/4-3 3/4 turns (i.e. 60-90% of
c.b.d.) and is ventrally wound.
     Nerve ring at 54-61% of the pharyngeal length.
Ventral pore at 63-68% of the pharyngeal length. In all
specimens examined, the "supplementary lateral cells" of the
excretory system (as described by Jensen, 1979) are situated
in the neighbourhood of the ventral gland.

Buccal cavity small and cup-shaped ; a small dorsal tooth-like structure is mostly present at its base.

Muscular pharynx with a weakly developed bulb (cfr. figures in Platt, 1984, 1985). Diorchic with opposite testes ; the anterior one at the left, the posterior one at the right of the intestine.

Paired spicules of equal size and curved, 41-54 um long or 1.0-1.6 a.b.d. The proximal part is broader, with sometimes a weakly developed capitulum (obvious in Fig. 4I); the inner side of the capitulum is connected with the distal tip of the spicule by a weakly cuticularized median lamella which is thickened in its proximal part. The gubernaculum surrounds the distal end of the spicule and has two dorsocaudally or caudally orientated apophyses (20-24 um long) ; the apophyses are interconnected at their distal part by a strongly sclerotized median part, the cuneus. The cuneus has a caudally directed bar extending from its dorsal end. The apophyses may have a ventrally curved bend which is obvious only in some views (cfr. Fig.4H-G).

The muscles of the spicular apparatus are well developed (cfr. Fig.4K, L) ; the protractor of the spicule consists of two parts : one part extends from the ventral side of the capitulum to the subventral body wall and the other part extends from the median cuticularized lamella of the spicule to the dorsal side of the gubernacular apophysis. The retractor of the spiculum extends from the capitulum to the dorsolateral body wall ; in Fig.4K, another muscle, extending from the median part of the spicule to the dorsolateral body wall is represented (this muscle is not always obvious).

The protractors of the gubernaculum extends from the proximal ventral part of the apophyses to the subventral body wall ; the retractors consist of two pairs : one situated between cuneus and dorsolateral body walls ; and the other is orientated caudally from the dorsal proximal tip of the apophyses to the dorsolateral body walls.

The number of preanal supplements varies from 5-8 ; 211 males were examined : 24 have five preanal supplements ; 167 males have six p.s. ; 16 males have seven p.s. and four males have eight p.s. Supplements 1 and 2 (most close to the anus) are more distant from each other than 3, 4, 5 or 6 more anteriorly located supplements.

The tail is cylindro-conical with a swollen tip ; the length of the cylindrical part between the swollen tip and the conical anterior part of the tail varies from almost nothing to 20 um. Several subventral somatic setae are located on the tail ; the cell bodies of the three caudal glands are restricted to the tail.

Females : morphological variation in the females is not as large as in the males.

Only characters which were considered diagnostic will be discussed here. For a general description of the females we refer to Platt (1984) for the description of Sabatieria breviseta and Sabatieria punctata (see also discussion).

The females have a smaller amphid (2-3 turns or 50% of the c.b.d.) ; the cuticular pattern consists of an irregular punctations which may show in some specimens a slight lateral differentiation in that the points in the lateral field are somewhat larger (this differentiation only occurs in the pharyngeal and caudal region only).

Discussion

In the Southern Bight of the North Sea, the Sabatieria-
population of the "pulchra-group" (as defined by Platt,
1985) is characterized by species which have the features
of four (five?) Sabatieria-species, described earlier ;
i.e. S. breviseta (Stekhoven, 1935), S. clavicauda
(Filipjev, 1918), S. punctata (Kreis, 1924), S. vulgaris
(de Man, 1907) and S. pulchra (Schneider, 1906). Recent-
ly, Platt (1985) considered three of these as valid species
: S. breviseta, S. punctata and S. pulchra. He synony-
mized S. clavicauda and S. vulgaris with S. pulchra
(Schneider, 1906) (as proposed earlier respectively by
Gerlach (1965) and Riemann (1970)).
      The difference between the sympatric species S. punc-
tata and S. breviseta is determined by Platt (1984) as
follows : characters which are "conspiciously different"
are : cuticular pattern, male amphid size and relative
development of the supplements ; minor differences have S.
punctata and S. breviseta in following characters : the
length of the cephalic setae (0.4 c.b.d. for S. punctata
and 0.3 h.d. for S. breviseta), slightly less curved and
shorter spicules (1.3 c.b.d. vs. 1.5-1.6 c.b.d. as arc),
less conspicuous median piece, slimmer tail and a different
orientation of the ovaries to the gut (based on 1 and 2
females resp.). Both species have six preanal supplements.
      Fig.4 illustrates that a different combination of
diagnostic characters between the several specimens exists
in one population of Sabatieria species of the "pulchra-
group". Therefore S. breviseta is synonymized with S.
punctata. S. vulgaris sensu Gerlach (1965) and sensu
Riemann (1966) are also synonymous with S. punctata.
Riemann (1966) also discussed the variability in some
characters (amphid size, cuticle, ...) of 'his' S. vulga-
ris ; most of the males have 6-7 p.s. (one specimen with 5
and one with 8). Specimens described by Gerlach (1965)
have also six p.s. and large amphids as most of the
specimens considered as S. punctata now.
      The original description of S. vulgaris (de Man,
1907) is very similar with the descriptions of S. pulchra
and, therefore, the two species are considered synonymous
as stated already by Platt (1985).
      Even though, problems do exist about the identity of
the different species in the 'pulchra'-group.
      S. propisinna Vitiello, 1976 and S. pisinna Vitiel-
lo, 1970 were considered clearly different form the other
species of the group because of their small body size (i.e.
670-780 um and 657-777 um resp.). The difference between
these two species is discussed by Vitiello (1976).
      The intraspecific variation described for S. morten-
seni (Ditlevsen, 1921) corresponds with the variation
encountered in S. punctata. Therefore we consider S.
mortenseni (type material disappeared) synonymous with S.
punctata.
      S. pulchra (Schneider, 1906) appears to be a true
brackish water (mesohaline) species (cfr. Jensen, 1979)
which shows in its habitat an intraspecific variation of
less than 10% of 11 adult characters. The number of pre-
anal supplements shows little variation in specimens from
the type locality (Jensen, 1979) : 118 ind. have eight
supplements, 106 ind. have seven supplements, three ind.
have 5, 6 or 9 supplements. In the Dievengat (a polyhaline
pond near the Belgian coast), S. pulchra has individuals

with 7 and 8 preanal supplements (17 males with 7 and 3
males with 8 supplements) (Smol, pers.com.).

The marine S. punctata has mostly 5-6 preanal sup-
plements. However, the differences between females of the
two species S. punctata and S. pulchra are not obvious at
all.

But, as in many other nematode genera, true brackish-
water species do exist and perhaps that following dif-
ferences with the marine S. punctata are diagnostic ; S.
pulchra has : two turns in the amphideal fovea ; a very
slender tail end with a cylindrical part that ends in a
clearly delineated swollen tip.

Because of minor differences, the species of the S.
pulchra-group (Platt, 1985) (except S. pisinna and S.
propisinna) may be considered as different ecophenotypes,
in which the marine and the poly-mesohaline Sabatieria
specimens are two distinct types which show only clear
variation in the number of preanal supplements. Never-
theless, the low variability in characters for S. pulchra
compared with S. punctata is striking. The two pheno-
types are found in the same geographic range but in dif-
ferent habitats and may be called therefore species with a
microallopatric distribution.

If S. pulchra and S. punctata are two different
species, it is probable that localised hybridisation or
introgression occurs regularly in some groups. Wiley
(1981) says that species which do not hybridize or intro-
gress under normal circumstances may do so in disturbed
habitats. Either may also occur under special ecological
circumstances.

The marine Sabatieria species of the "pulchra-group",
which show a large variability live in an area that is very
disturbed and loaded with a variety of pollutants (in some
sediments of this area, nematodes are the only metazoans
which can survive).

Sabatieria sp. 1 (Figs 1, 5)

Material

One male and two juveniles.

Locality

Southern Bight of the North Sea. Fine to coarse sand with
silt content between 0-20%. Three localities (Fig.1).
Collected Sep. 1978 and Apr. 1972.

Measurements

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    -    140   160   271   M    2797
▆  : ----------------------------------- 2970 (slide no.10119)
    19    22    24    27    41    41
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a= 72.4   b= 11.0   c= 17.2   c'= 4.2   spic= 84 μm

Description

Body cylindrical ; cuticular ornamentation approximately in
transverse rows ; a lateral field of coarser punctations is
present thoughout the body length. The labial sensilla are
papilliform ; the four cephalic setae are 10 um long, i.e.
53% of the c.b.d. ; amphideal fovea spiral with two turns,
ventrally wound ; 67% of the c.b.d. Four rows of somatic
setae are arranged over the whole body length.
      Nerve ring at 52% of pharyngeal length ; ventral pore
at 59% of pharyngeal length.
      Buccal cavity and pharynx typical for the genus.
      Diorchic, with outstretched testes. Spicules heavily
sclerotized, twice c.b.d., with slightly developed capi-
tulum and a short cuticularized internal projection from
the proximal end ; no median lamella absent ; the distal
end shows a triangular elevation. The gubernaculum has two
long nearly straight apophyses (41 um) which are caudally
directed. Eighteen tubular preanal supplements are weakly
sclerotized ; they are connected with glandular cells ;
last supplement provided with one seta.
      The protractors of the spicules consist of two parts ;
one from the ventral side of the capitulum to the sub-
ventral body wall ; the other is splitted into two bran-
ches, one at the outer side of the spicule between the
capitulum and the caudal end of the gubernacular apophysis
and one at the inner side of the spicule between the dorsal
side of the capitulum and the gubernacular apophysis.
      The retractor of the spicules extends between the
capitulum and the subdorsal body wall. The protractors of
the gubernaculum extend between the middle part of the
apophysis and the ventral body wall ; the retractor between
the caudal tip of the apophysis and the dorsal body wall.
      Glands (anal or cloacal) are present, one obscure the
gubernacular structure. One is situated between the distal
end of the spicules and one pair is situated at the lateral
side of the spicules. Tail is cylindro-conical, slightly
swollen at the tip and with three terminal setae.

Females not found.

Discussion

This species belongs to the "Sabatieria praedatrix-sub-
group" (cfr. Platt, 1985) which is characterized by
species with simple tubular or pore-like supplements and
straight gubernacular apophyses. As the group is currently
constituted, none have spicules with the central lamella
apart from the proximal projection and most have a cuti-
cular differentiation with large, more widely spaced dots.
      Sabatieria sp. 1 is close to S. praedatrix but
differences are : longer gubernacular apophysis, higher
a-ratio. However, also within this subgroup very little is
known about the intraspecific variability.

Sabatieria sp. 2 (Figs 1, 5)

Material

One male, one female (poorly preserved).

Locality

Southern Bight of the North Sea. Median grain size of the sand fraction is 150 um ; 5% silt. One locality (Fig.1).
Collected 4 Apr. 1972 and 26 June 1972.

Measurements

$$\sigma_1^{\prime} : \frac{-\quad 116\quad 134\quad 232\quad M\quad 2314}{15\quad 33\quad 33\quad 36\quad 48\quad 40}\ 2510\ (\text{slide no.}10120)$$

a= 52.3   b= 10.8   c= 12.8   c'= 4.9   spic= 67 µm

$$\varphi_1 : \frac{-\quad ?\quad ?\quad 262\quad 1170\quad 2068}{17\quad ?\quad ?\quad 42\quad 52\quad 44}\ 2260\ (\text{slide no.}10121)$$

a= 43.5   b= 8.6   c= 11.8   c'= 4.4   V= 51.8%

Description

General body shape similar to Sabatieria sp. 1, except the longer tail. Cuticular punctations arranged in transverse rows ; the lateral differentiation consists of coarser points. Somatic setae arranged in eight longitudinal rows. Labial sensillae papilliform ; the four cephalic setae are 7 um long, i.e. 47% of the c.b.d. Amphideal fovea spiral with 2 1/4 turns ; 8 um diameter or 50% of the c.b.d.
    Buccal cavity and pharynx typical for the genus. Nerve ring at 50% of the pharyngeal length ; ventral pore 58% of the pharyngeal length. Diorchic with outstretched testis, anterior left, posterior right of intestine.
    Spicules heavily sclerotized, 67 um long, i.e. 1.7 times a.b.d. ; a rather pronounced (13 um) internal cuti-cularized projection from the proximal end ; no central lamella developed. The gubernaculum has two long, straight, caudally directed apophyes (29 um). Anal cloacal glands present. One precloacal seta and 15 tiny tubular preanal supplements are weakly cuticularized ; each one is connected with a glandular cell. Spicular muscles not obvious.

Female : not well preserved ; detailed description not possible. Didelphic, ovaries outstretched, anterior left, posterior right of intestine.

Discussion

Sabatieria sp. 2 belongs to the 'Sabatieria praedatrix-sub-group' (Platt, 1985) ; it resembles S. praedatrix but the latter species has a different median piece of the gubernaculum.

The real identiy of Sabatieria sp. 2 can be confirmed by examination of more material. Unfortunately, this was not available.

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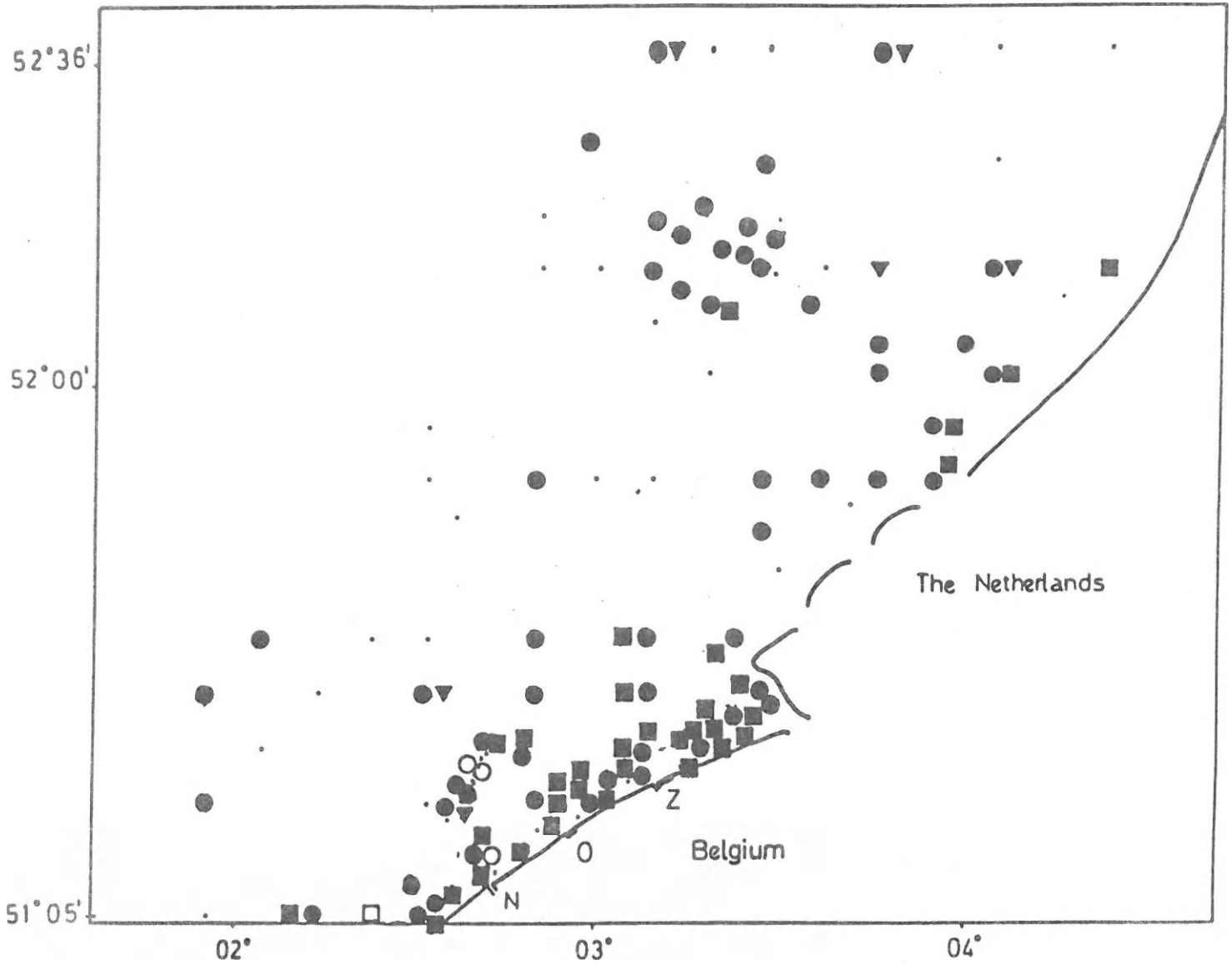
Fig.1. Distribution of <u>Sabatieria celtica</u> (●), <u>S. longi-</u>
<u>spinosa</u> (▼), <u>S. punctata</u> (■), <u>Sabatieria</u> sp.1 (o),
<u>Sabatieria</u> sp.2 (□).
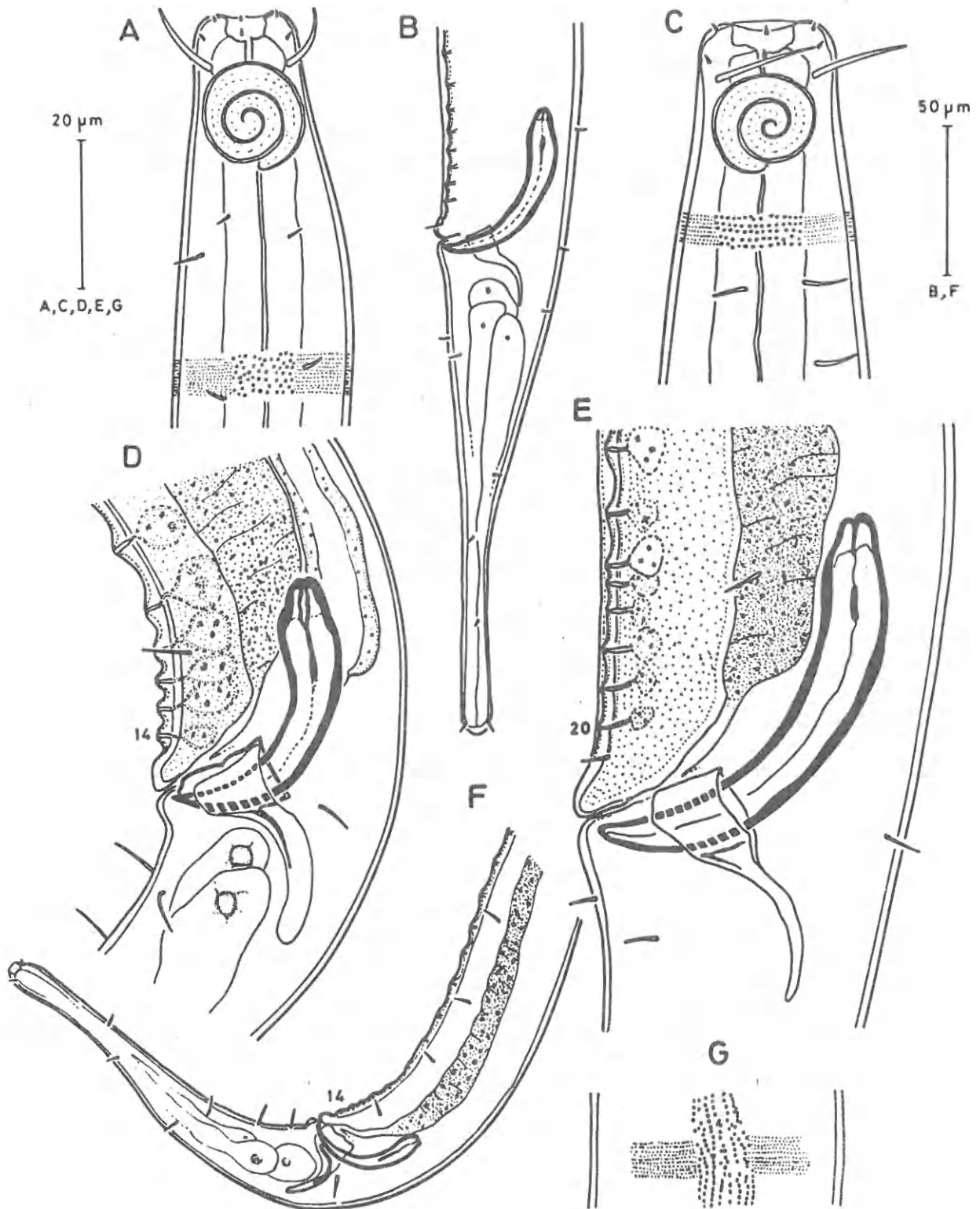
Fig.2. <u>Sabatieria celtica</u>. A. Head end ♂₁ ; B. Tail region ♂₂ ; C. Head end ♂₂ ; D. Copulatory apparatus ♂₁ ; E. Copulatory apparatus ♂₂ ; F. Tail region ♂₁ ; G. Cuticular pattern at the cardial level ♂₂.
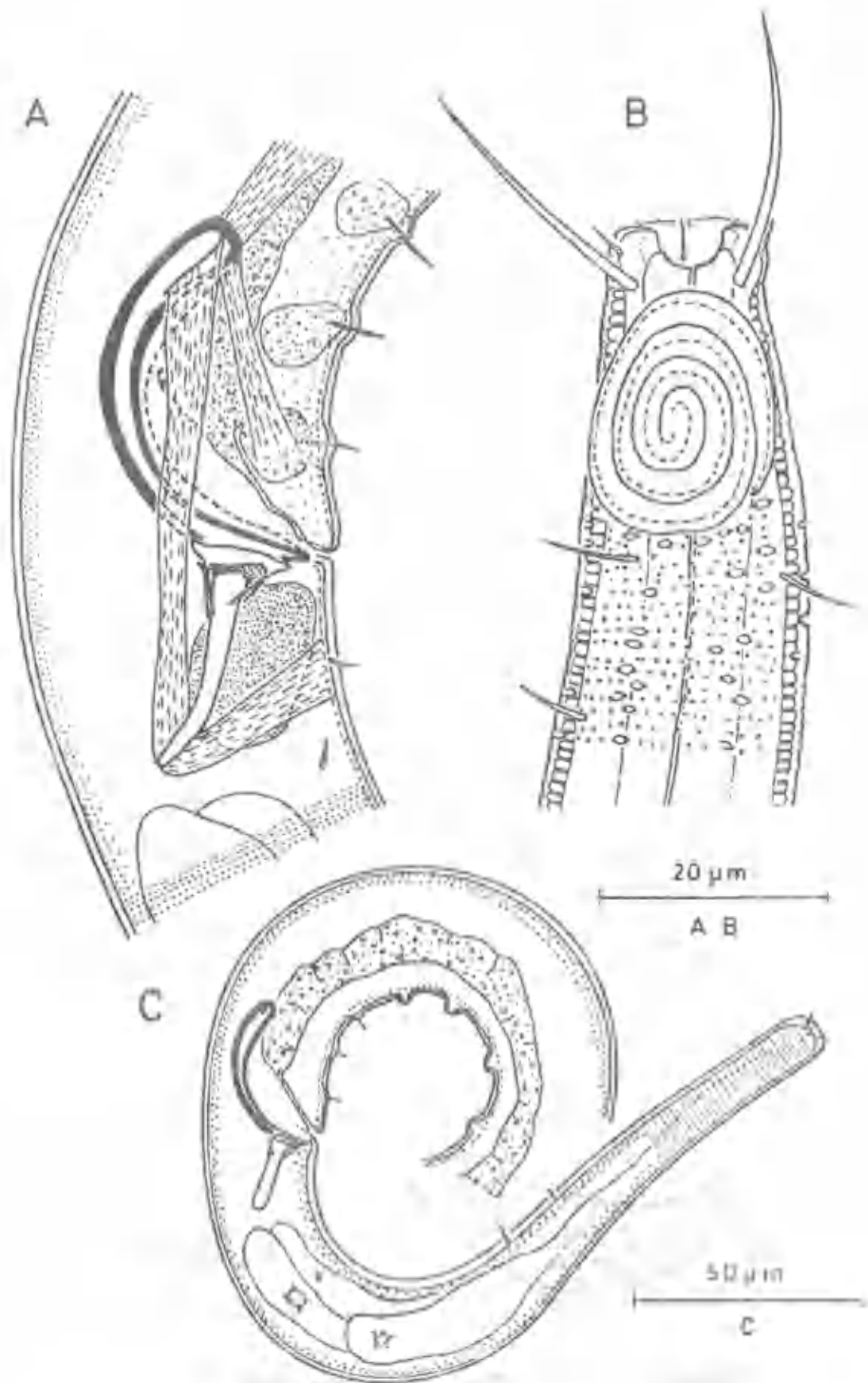
Fig.3. _Sabatieria longispinosa_ ($\vec{\sigma}_1$) : A.   Spicular appa-
ratus ; B.   Head region ; C.   Tail region.
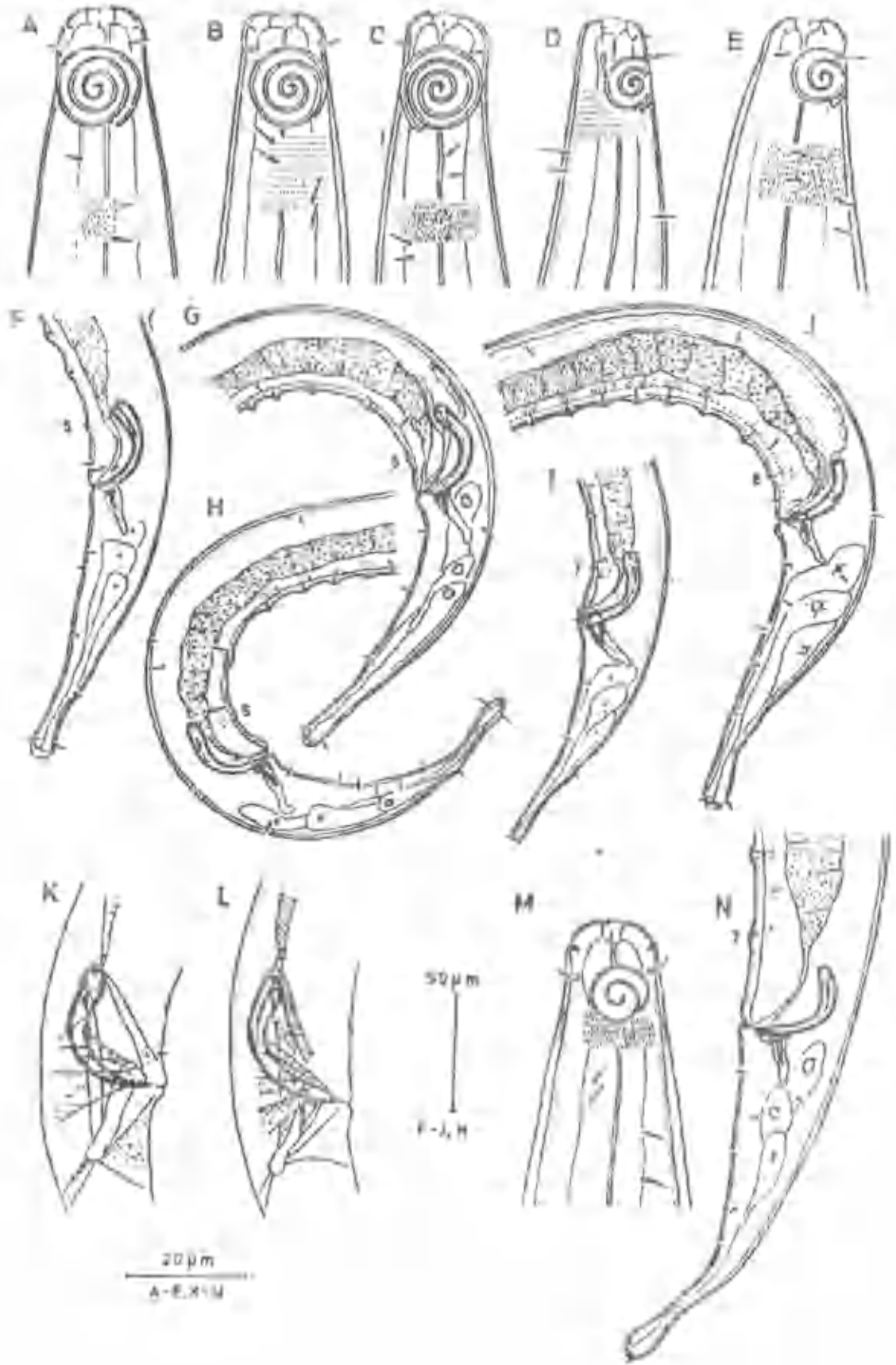
Fig.4. Sabatieria punctata. A. Head end ♂₁ ; B. Head end ♂₂ ; C. Head end ♂₃ ; D. Head end ♂₄ ; E. Head end ♂₅ ; F. Tail region ♂₁ ; G. Tail region ♂₂ ; H. Tail region ♂₃ ; I. Tail region ♂₄ ; J. Tail region ♂₅ ; K. Spicular apparatus ♂₆ ; L. Spicular apparatus ♂₇.
Sabatieria pulchra (from type locality ; provided by P. Jensen) ; M. Head end ; N. Tail region.
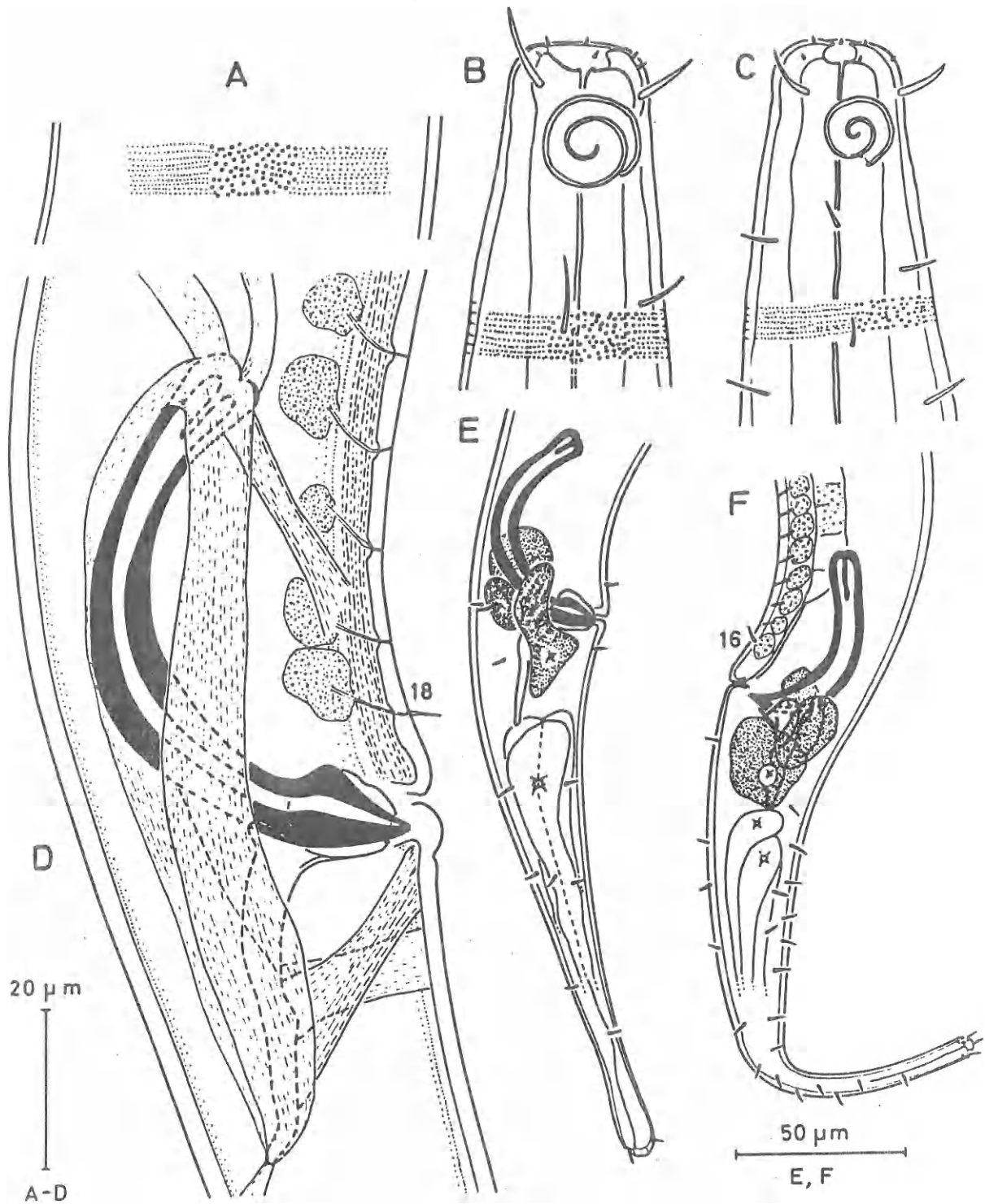
Fig.5. _Sabatieria_ sp.1. A. Cuticular pattern at the
cardial level ♂₁ ; B. Head end ♂₁ ; D. Spicular
apparatus ♂₁ ; E. Tail region ♂₁.
Sabatieria sp.2. C. Head end ♂₁ ; F. Tail region
♂₁.

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Seasonal fluctuations of Sabatieria punctata (Nematoda) in a silty-sand station off the Belgian coast.

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Abstract

The life cycle of S.punctata, the dominant nematode species in the meiobenthos off Zeebrugge(Belgian coast) was studied over a three year period. Juveniles occur throughout the year and reproduction is considered to be nearly continuous. A more active reproductive period occurs in spring from March till May, and in autummn (Sep-Oct). Density varies between 45 (Feb83) and 2083 (Nov83) ind./10 cm^2; relative abundance varies between 18.8% (Jun85) and 98.6% (Mar83).

S.punctata contributed for about 75% to the production of the whole community estimated as 22 gww/m^2.y. The yearly P/B=16.91 in 1983 for S.punctata (P/B=20 for the whole nematode community (Vranken et al.,in press).

Introduction

Sabatieria punctata (Kreis, 1924) is one of the most dominant nematode species in silty and silty-sand sediments of the sublittoral area of the northern hemisphere (Lorenzen,1974;Juario,1975;Herman et al.,1985 for ecological data). The species has been considered cosmopolit (North Sea and adjacent regions;English Channel,E- and W-coast of the USA,Brazil,Argentina, Falkland Island, Black Sea, Svalbard, Mediterranean, Antarctica, Auckland Islands).

Identification problems are very large for S.punctata and many different types have been described in the past as separate species (see Platt,1985 for a revision). However, as the intraspecific variation within the S.punctata population of the Belgian coast is quite large, Vincx (1986) decided to synonymize closely related species with the original description of S.punctata. Only one closely related species has been retained as valid,i.e. S.pulchra, the brackish water "Sabatieria" of the northern hemisphere.

A nematode community off the Belgian coast dominated by S.punctata has been followed with monthly intervals during 1983-1985. Other seasonal samplings were carried out in 1977-1979.

Material and Methods

Station 11860 (51°22'38"N, 03°18'41"E) was sampled every month in the period 1983-1985. Meiofauna was collected by subsampling a Van Veen grab (sampling area:0.10 m^2) or a modified Reineck-box (sampling area:170 cm^2) before summer 1984. From October 1984 on, meiofauna was sampled using a box-corer (sampling area:0.25m^2) from the Belgian Oceanographic Research Vessel "Belgica".

Each meiofauna sample was immediately subsampled with four plastic cores (surface:10.2 cm^2): two for faunistic analysis (fixed with 4% hot (70°C) formaldehyde), one for sedimentological analysis and one was kept for chemical analysis (heavy metals during 1983, Braeckman et al., 1984).

Sediment analysis was carried out as described in previous papers (Heip et al., 1979).

Animals were extracted by decanting the sediment a few times (on a 38 µm sieve); afterwards they were centrifuged with 50% Ludox and fresh water (method described by Heip et al., 1985). Animals, coloured with rose Bengal, were counted under a stereoscopic microscope; the first 200 nematodes were identified to species level with a Leitz Diavert (reversed) microscope. Males, females and juveniles were counted. Juveniles were measured from each month (body length and maximum body width) because it is difficult to distinguish different juvenile stages under the reversed microscope (it is not even easy with oil immersion under a normal microscope).

Results

Environmental characteristics

Sediment

The median grain size (mm), silt clay fraction and the sorting coefficient (∅) are determined for the period 1977-1983. In 1984-1985, the sediment analysis from only a few sampling dates was carried out. Table 1 shows the different sediment characteristics. Note that the median and the sorting coefficient of the last two years are only determined for the sand fraction and therefore not comparable with the earlier data. Only the silt-clay amount is comparable.

The amount of silt-clay in 1983-1985 is higher than in the earlier years. In 1983, monthly samples were analysed and in that year the median of the grain size is different between winter (Dec-Apr) and summer (May-Nov).

Temperature

Bottom temperature was measured each month (1983-1985); values are given in Table 2.

Others

Salinity, pH and O_2 at the bottom were measured only in 1985.

Month/Year	Median Grain Size (mm)	Silt-Clay Fraction (%) (< 62 um)	Sorting Coefficient (∅)
Jun 77	0.183	3.0	0.35
Sep 77	0.165	9.0	0.40
Mar 78	0.095	46.5	3.10
Apr 78	0.165	12.5	0.53
Sep 78	0.159	17.0	1.10
Dec 78	0.183	0.5	0.28
Apr 79	0.129	40.5	2.75
Jun 79	0.096	58.0	2.95
Sep 79	0.094	43.1	2.18
Jan 83	0.022	81.0	3.13
Feb 83	0.044	63.5	2.40
Mar 83	0.032	69.5	2.95
Apr 83	0.025	77.5	3.28
May 83	0.038	63.5	2.85
Jun 83	0.054	53.5	2.45
Jul 83	0.082	45.0	2.45
Sep 83	0.053	53.5	3.65
Oct 83	0.054	53.5	2.60
Nov 83	0.051	56.5	2.80
Dec 83	0.036	71.5	2.65
Apr 84	ˣ0.153	57.7	ˣ0.34
Oct 84	ˣ0.156	35.0	ˣ0.32
Apr 85 (0-5cm)	ˣ0.158	66.4	ˣ0.56
(> 5cm)	ˣ0.114	70.5	ˣ0.64

Table 1: Sediment characteristics from stations 11860 . (median and sorting coefficient for 1984 and 1985 are determined on the sand fraction only; ealier data are determined and the total sediment fraction).

	1983	1984	1985			
	T	T	T	Sal.	pH	O_2
Jan	8.4	–	3.2	33.9	7.96	15.5
Feb	5.5	6.8	0.5	30.6	8.26	–
Mar	5.4	7.8	4.2	–	–	–
Apr	7.5	7.6	7.8	32.8	8.51	9.3
May	10.1	9.6	11.9	29.8	8.48	8.4
Jun	12.3	12.3	15.2	–	–	5.5
Jul	16.8	–	–	–	–	–
Aug	–	–	17.5	32.9	8.53	8.1
Sep	17.3	–	16.0	30.0	8.12	9.6
Oct	15.3	14.8	–	–	–	–
Nov	11.9	–	7.5	32.3	8.21	9.6
Dec	9.5	10.0	–	–	–	–

Table 2: Temperature (T in °C), Salinity (Sal in ‰), pH and ppm O_2 in station 11860.

Density of the whole nematode community

The mean density values of the whole nematode
community is presented in Fig1. Differences between the
two replica's of one sample are examined by means of a one
way-anova(numbers were transformed to log10)
    Between sample(=months) variation is significantly
higher than within sample variation (F=4.966,df:26 and
27,p<0.001).
    The mean density of the total community varies between
55 ind./10 cm^2 (Feb83) and 5610 ind./10 cm^2 (Jun85). Dates
of minimum and maximum abundances together with the
relative abundance of _Sabatieria punctata_ in the community
are given in Table3.
    The minimum numbers in the first half of the year
(Jan-Jun) occur always in two periods.
    Maximal values occur in Sep83, Nov83 and Oct84 in the
second half of the year. A late maximum in 1985 was not
observed but we probably missed it because two important
autumn months were not sampled.
    The relative abundance of S.punctata within the
community ranges between 18.8% (Jun85) and 98.6% (Mar83).
    The species composition of the community is not
discussed in this report (cfr for 1983, Vincx & Heip,
1984). The very low relative abundance of _S.punctata_ in
Jun 85 is due to an explosive occurrence of juveniles of
Daptonema tenuispiculum, a species which usually
contributes a maximum of about 15-20% to the community.

Density of _Sabatieria punctata_

Between sample variance of the _S.punctata_ population
density (ad+juv) is significantly higher than within sample
variance (between replica's) (F=4.237,df=26 and 27,
p<0.001). Therefore I did not use a running mean for the
representation of the fluctuations during the three years;
also, the sample intervals are quite large for population
studies.
    Fig.1 shows the fluctuation of the total density over
the three years. Because _S.punctata_ is the dominant
species of the community, the pattern is similar to the
density pattern of the whole community.
    A maximum density peak is present in Mar83, Sep83,
Nov83, Apr84, Oct84, Feb85, Apr85 and Jun85. An absolute
maximum is found in Nov83 (2083 ind./10 cm^2). Lowest
density value occurs in Feb83(45 ind./10 cm^2); in Oct83,
Feb84, Jun84, Jan85, Mar85, May85 and AUG to Nov85 only
about 100 ind./10 cm^2 were present.
    Neither the maximum, nor the minimum values are
preceded by an important increase or decrease in temperature
(cfr. Table2).
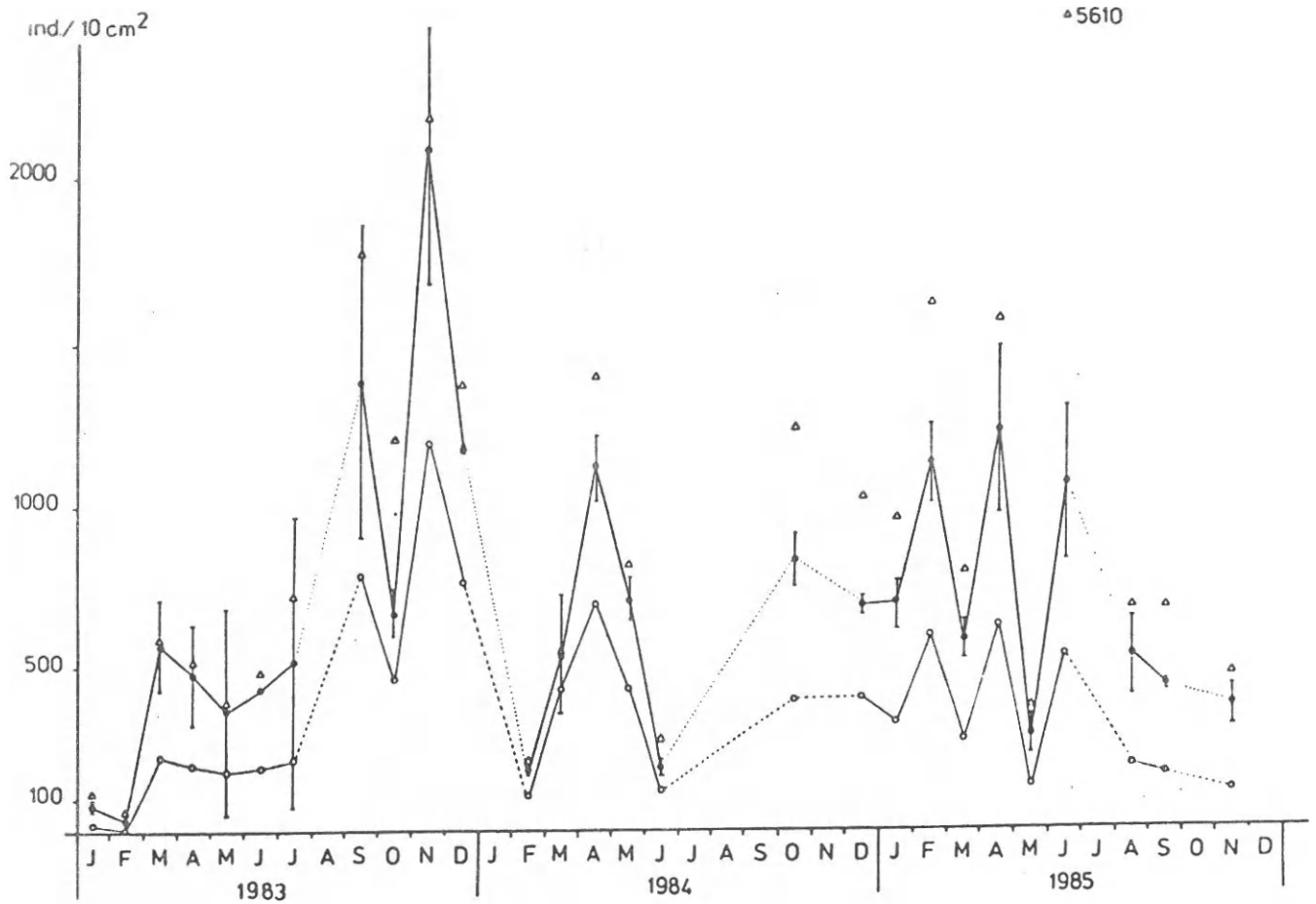
Fig.1. Density (ind./10cm^2) of the nematode community (△), of S.punctata (ad + juv) (●; ± SE) and of S.punctata (juv.(○)) over three years in station 11860.

month	minimum	%Saba	month	maximum	%Saba
Feb83	55	74.4	Mar83	582	98.6
May83	389	94.3	Sep83	1774	78.8
Oct83	1208	54.7	Nov83	2186	95.6
Feb84	202	88.7	Apr84	1400	81.0
Jun84	278	69.8	Oct84	1224	67.8
Jan85	954	72.7	Feb85	1622	69.8
Mar85	796	75.2	Apr85	1570	79.8
May85	368	78.0	Jun85	5610	18.8
Nov85	475	77.8			

Table 3 : Dates and numbers of minimum and maximum density values (ind./10cm^2) for the nematode community and rela_tive abundance of S.punctata (%Saba) within the community.

Adults

The population consists for 30.4% to 70.7% of adults; mean value over the three years is 49.4%. The sex-ratio (♀♀/♂♂) equals 1 in most periods. Females are more abundant in Feb 83, Mar83,Apr83, Sep83, Nov83, Dec83, Jun84, Jan85 and Feb85. Males are more abundant than females in Sep83, Mar84, May84, Oct84 and Dec84. The relative abundance of the males and females is shown in Fig.2.

Juveniles

On the average, 50.1% of the total population is represented by juveniles (cfr. Fig.1); values vary between 29.3% and 69.6%.

Mean densities of juveniles over three years are given in Table 4. The density pattern of the juveniles follows the increase and decrease in total density (cfr. Fig1).

Three size classes of juveniles (JuvI: less than 700 μm; JuvII: between 700-1100 μm and JUVIII: more than 1100 μm) are distinguished (cfr. Juario, 1975). The detailed composition of the population (relative abundance of JuvI, JuvII, JuvIII, ♀♀ and ♂♂) is presented in Fig.2.

The smallest juveniles were absent in Jan83, Feb83, Mar85, May85 and Jun85. They account for 20-30% of the juveniles in Mar83,Apr83,Oct83,Jun84,Oct84,Aug85 and Sep85. The largest juveniles account for 40% or more of the juveniles in Jan83, Feb83, May83, Jul83, Nov83, Feb85, Mar85, May85 and Jun85. These maxima are about one to two months after the max of the smallest juveniles. The maximum density of the smallest juveniles is 152 ind/10cm (Oct83); the max. density of the largest juveniles is 479 ind./10 cm (Nov83).

In the following table the periods are noted when the increase in relative abundance of one of the three juvenile classes exceeds or equals 10% in comparison with the previous date.

In this way, highest abundances are noted in following periods:

| JuvI: Mar83 - Apr83	JuvII: Apr83 - Jun83	JuvIII: May83
Oct83	Sep83	Oct83 - Nov83
Apr84 - Jun84	Dec83 - Mar84	(Apr84)
Oct84	May84 - Jun84	Dec84 - Jun85
Apr85 (Δ =6%)	(Dec84 - Apr85)	
Aug85 - Sep85		

Table 4. Composition of the *Sabatieria punctata* population over three years (mean values per month).
(N : ind./10 cm^2, relative abundance (%) and sex ratio (♀♀/♂♂)).

Month	N (juv)	% (juv)	N (♀♀)	% (♀♀)	N (♂♂)	% (♂♂)	N tot.	♀♀/♂♂
Jan 83	27	30.6	47	52.6	15	16.8	89	3.13
Feb 83	12	29.3	15	35.5	14	35.2	41	1.07
Mar 83	227	40.3	173	30.7	163	29.0	563	1.06
Apr 83	200	41.4	142	29.6	140	29.1	482	1.01
May 83	180	50.0	125	34.8	55	15.2	360	2.27
Jun 83	191	46.4	141	32.6	91	21.0	433	1.55
Jul 83	205	39.6	193	37.3	119	23.1	517	1.62
Sep 83	787	56.4	294	21.1	314	22.5	1395	0.94
Oct 83	455	69.6	130	19.9	69	10.6	654	1.88
Nov 83	1197	57.5	440	21.2	445	21.4	2082	0.99
Dec 83	753	64.1	210	17.9	211	18.0	1174	0.99
Feb 84	107	59.9	41	23.1	31	17.0	179	1.32
Mar 84	435	83.3	35	6.8	52	9.9	522	0.67
Apr 84	699	62.2	271	24.2	154	13.7	1124	1.76
May 84	434	61.9	101	14.4	166	23.7	701	0.61
Jun 84	124	64.0	38	19.7	32	16.3	194	1.19
Oct 84	395	48.1	193	23.5	234	28.4	822	0.82
Dec 84	398	61.3	80	12.3	172	26.4	650	0.47
Jan 85	322	46.3	188	27.1	185	26.6	695	1.02
Feb 85	589	51.9	295	26.0	252	22.2	1136	1.17
Mar 85	260	44.5	185	31.6	140	23.9	585	1.32
Apr 85	626	50.8	428	34.8	178	14.4	1231	2.40
May 85	141	49.4	97	34.2	47	16.4	285	2.06
Jun 85	525	49.2	323	30.3	219	20.5	1067	1.47
Aug 85	194	36.9	192	36.5	140	26.6	526	1.37
Sep 85	179	40.9	169	38.7	89	20.4	437	1.90
Nov 85	116	31.6	137	37.5	112	30.9	365	1.22

Fig.2. Age structure of S.punctata over three years in station 11860. Five age classes were distinguished; from the left to the right in each month: JuvI, JuvII, JuvIII, ♀♀ and ♂♂.

1983

1984

1985
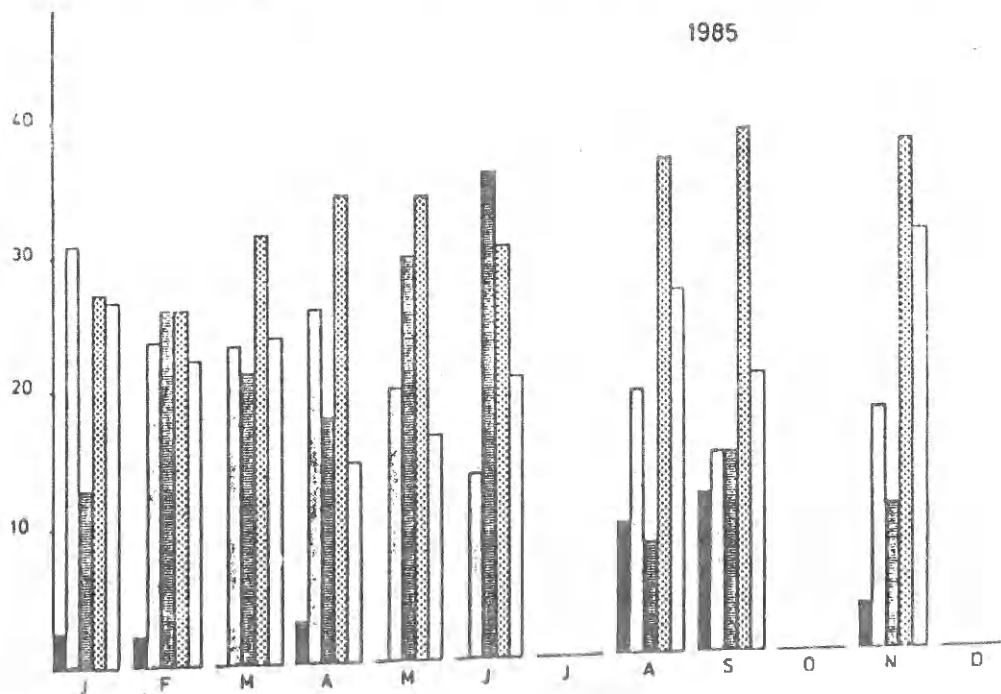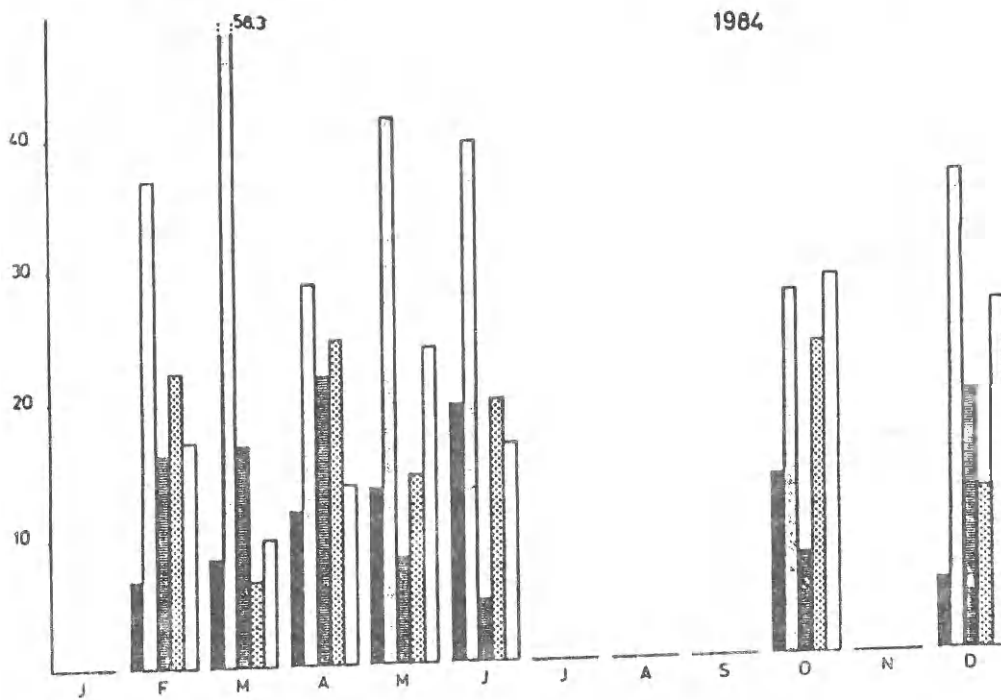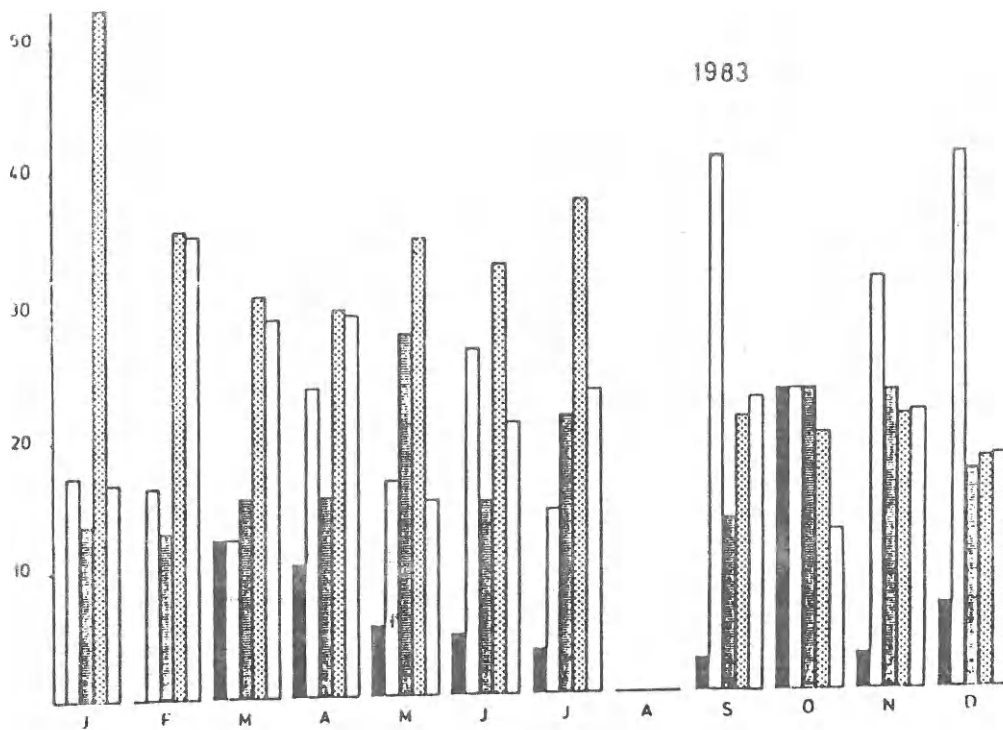
The mean body length of all the juveniles over three
years is shown in Fig.3.   The differences between the mean
values of juvenile body length per month are significantly
different,(F=2.432,df:26 and 481,p<0.001).   An a posteriori
contrast test (LSD) shows that the juveniles of Oct84,
Oct83, May84, Jun84,Apr83,Mar84,Apr84,Aug85,Sep85,Jan85 and
Nov85 are significantly smaller than in the other months
(arranged in decreasing order of difference) at the 5%
level.

The numbers (and relative abundance) of the smallest
size class of juveniles increase two times per year: the
first time in spring (March, Apr,May) and a second time in
autumn (Oct83, Aug85, Sep85); only in spring 1984 is the
increase in small juveniles not limited to one or two
months but seems to continue to a maximum value of smallest
juveniles in June84; no summer data of 1984 are available
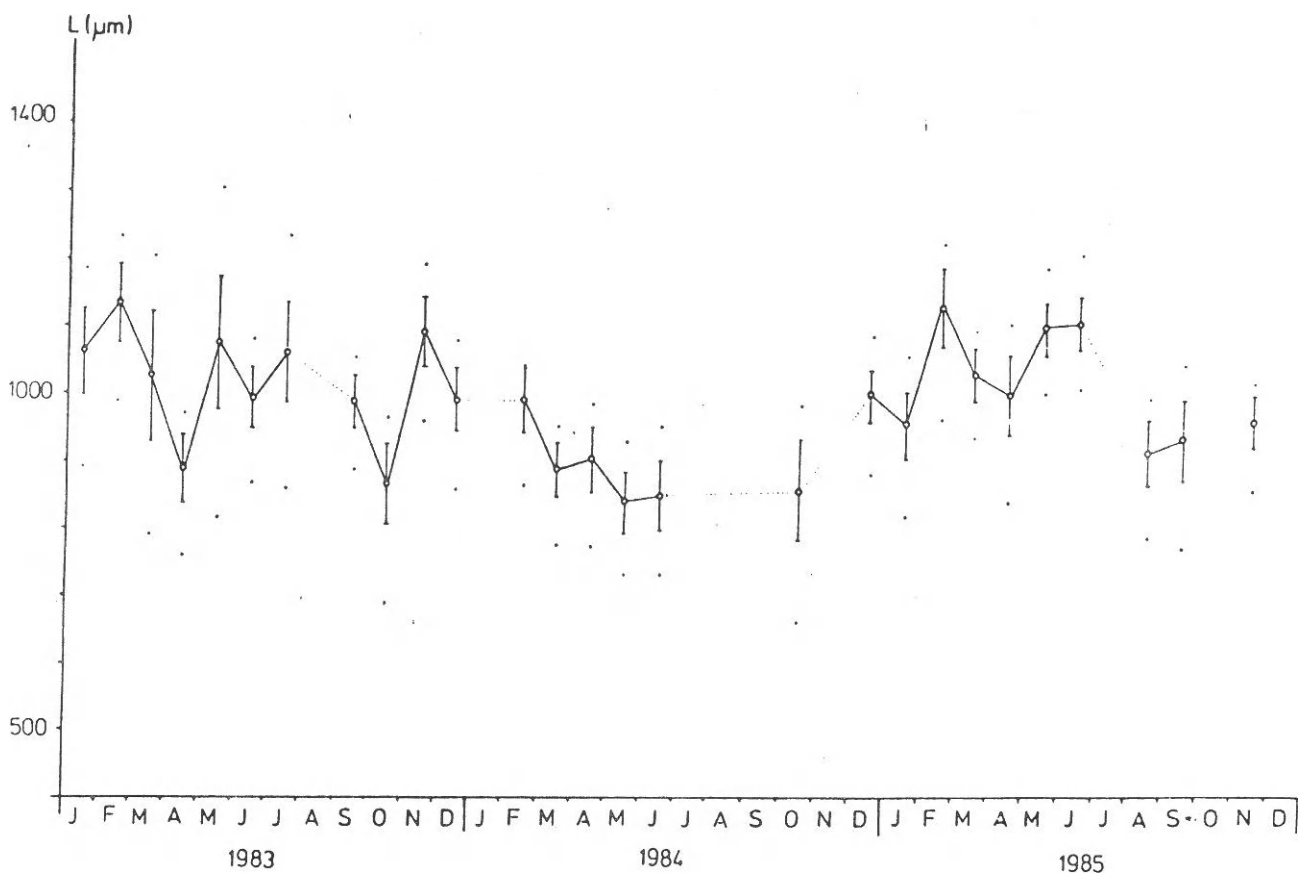but Oct84 has a fairly large amount of small juveniles too.

Fig.3. Mean body length of the juveniles of S.punctata over three years in
station 11860 (including SE and 95% confidence intervals).

The increase of the relative abundance of the smallest
juveniles coincides with a decrease in total numbers of the
population; i.e. the increase of the relative abundance of
the juvenile classes may be partly due to mortality of
adults too. This probably occurs in Oct83, Feb84, Jun84,
Aug85 and Sep85. These periods may be considered as
periods where a more distinct change of generations takes
place.

However, it is not possible from these data to predict
the exact number of generations per year. Moreover, the
interpretation is even more uncertain when we compare with
the seasonal data from 1977-1979. The total nematode
density, relative abundance of S.punctata within the
community and the age structure of S.punctata are given in
Table4. The low relative abundances in March 78 and June
79 are due to a very high number of Daptonema tenuispiculum
(comparable with Jun85).

	Dens.Nem.	%Saba	Juv	♀♀	♂♂
Jun 77	45	84.4	23.7	50.0	26.3
Mar 78	2065	8.0	50.0	37.5	12.5
Apr 78	3817	93.0	49.5	30.1	19.4
Jun 78	1910	71.0	56.3	25.4	18.3
Dec 78	855	74.0	51.4	24.3	24.3
Apr 79	2250	91.0	57.5	26.3	16.2
Jun 79	721	13.5	16.7	50.0	33.3
Sep 79	1439	52.2	37.5	29.2	33.3

Table 5. Total nematode density (ind./10 cm^2; relative abundance
of Sabatieria punctata in the community and age structure
(% Juv, ♀♀ and ♂♂) for the period 1977-1979.

Vertical distribution in the sediment

The vertical profile of Feb85 and Mar85 is examined in order to see if there is an optimum zone for the different age classes. Density changes are given in Fig.4 for the whole S.punctata population. Highest density occurs in the 3-4 cm layer with a rather abrupt decrease beneath 6 cm (Feb85); In Mar, max.density occurs in the 2-3cm layer. Abundance of S.punctata varies from 12.2% at the surface level (where the other species Ascolaimus elongatus and Daptonema tenuispiculum are abundant) to 100% from 6 cm on in Feb and from 4 cm on in Mar85.

The pattern (relative abundance of Juv,♀♀ and ♂♂) is given in Table 6. Juveniles as well as adults are present from the surface to 6-8 cm in both Feb and Mar85. Juveniles are more dominant in the upper layers; females are more dominant in the deeper layers.

We do not possess measurements of environmental parameters of the vertical profile from this period.

Production estimates of S.punctata

Production of S.punctata is calculated by the method developed by Vranken et al (in press). This method is based on a regression equation relating egg-to-egg development time Tmin to temperature (t) and adult female body wet weight(W in ug):

$$\log T_{min} = 2.202 - 0.0461t + 0.627\log W \quad (1)$$

The P/B was calculated for each month as $1/T_{min} \times D \times 3$ (D=number of days per month). Biomass structure (males,females and juveniles) is determined for each month and so the monthly production for the species is calculated. Total production for one year divided by the average biomass (ww) gives the annual P/B for the species.

Dry weight of S.punctata is determined for 150 males, females and juveniles; dry weight is 15% of the wet weight and individual ww are : males : 2.297 µg, females : 2.424 µg and juveniles 0.699 µg.

From equation (1) it is shown that the calculated Tmin for S.punctata varies between 263.0 days at $0.5°C$ and 43.3 days at $17.5°C$.

The annual P/B is not determined for 1984 because too many months (and even long periods) were not sampled.
1983 (Jan-Dec)
   Total production : 16.61 g ww/m^2/year
   Average biomass : 0.98 g ww/m^2
   P/B : 16.91
1985 (Dec84-Nov85)
   Total production : 14.17 g ww/m^2/year
   Average biomass : 1.00 g ww/m^2
   P/B : 14.17

Table 6 . Vertical distribution pattern of the age
structure of *Sabatieria punctata* in station
11860.
(N = number of specimens examined).

Feb 1985

	Juv (%)	♀♀ (%)	♂♂ (%)	
0- 1 cm	87.5	-	12.5	(N = 16)
1- 2 cm	75.6	17.0	7.4	(N = 41)
2- 3 cm	55.6	23.0	21.4	(N = 127)
3- 4 cm	38.5	35.6	25.9	(N = 174)
4- 6 cm	55.8	23.1	21.1	(N = 303)
6- 8 cm	16.7	55.6	27.7	(N = 18)
8-10 cm	-	-	-	-

Mar 1985

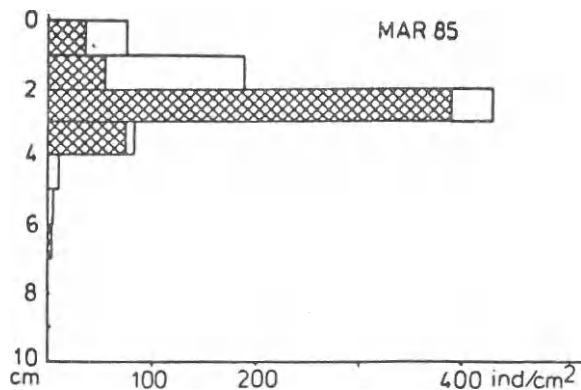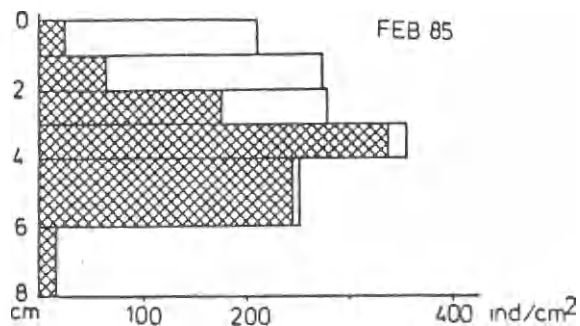|	Juv (%)	♀♀ (%)	♂♂ (%)	
0- 1 cm	100.0	-	-	(N = 5)
1- 2 cm	67.8	22.0	10.2	(N = 59)
2- 3 cm	45.3	29.2	25.5	(N = 161)
3- 4 cm	32.3	39.9	27.8	(N = 158)
4- 5 cm	44.0	36.0	20.0	(N = 25)
5- 6 cm	0	71.4	28.6	(N = 7)
6- 7 cm	60.0	-	40.0	(N = 5)
7- 8 cm	-	-	-	-
8- 9 cm	-	-	-	-
9-10 cm	-	-	-	-

Fig.4. Vertical density profile (mean density of two replica's) of the
nematode community from two months in station 11860. Shaded area
indicates the total mean density of S.punctata.

Discussion

Sabatieria punctata occurs in very high numbers in the silty sand station 11860 off Zeebrugge (Belgian coast) with a grand mean of 678 ind./10cm^2 over three years and peak values up to 2186 ind./10 cm^2(Nov83) or a relative abundance of 98.6% (Mar85) in the whole community. S.punctata is always very abundant in silty stations along the Belgian coast. Mean relative abundance of the species decreases generally from the west coast to the east coast. (cfr.Vincx et al.,1984).

In station 11860 it is obvious that the relative abundance of S.punctata (or the abundance of accompanying species) is determined by factors which are not quiteunderstood at the moment (cfr.aberrant situation in Jun85).

The life cycle can be summarized as follows: juveniles occur throughout the year and reproduction is considered to be nearly continuous. Analysis of growth or mortality of cohorts in the field has not been possible for this population. A more active reproductive period occurs in spring from March till May. Juveniles from this period probably reach adulthood two to three months later. These adults probably produce juveniles in autumn (Sep-Oct) and adults of the older generation die at this moment (?) (there is always a clear decrease in total density at that time and the decrease in adults is more pronounced than in the juveniles,cfr.Fig.1). Difference between male and female development have not been found.

From regression equation (1) it is shown that the influence of temperature on the reproductive cycle is very important. Equation (1) is mainly determined by values for opportunistic species. The nematodes from sublittoral areas are probably more conservative species and the obtained result for the annual P/B may overestimate the productivity of these species (Vranken et al., in press).Vranken & Heip (1985)found a relationship between egg weight and embryonic development at 20°C. This relationship predicted the embryonic development time of S.punctata from the Sluice Dock of Ostend exactly (prediction:9.87d; experimental:9.92d).Generation time is about 3.5 times longer than embryonic development (Vranken, unpublished results) and from this a generation time of about 35 days is predicted for 20°C. A similar value is obtained calculating Tmin from equation (1) for 20°C, i.e. 33.2d.

The annual P/B is 16.91 for 1983 and 14.17 for 1985. Vranken et al.(in press) calculated the annual P/B (1983) for the whole community : P/B = 20.

Vranken et al.(in press) calculated an annual production of 22.2 g ww/m^2.y (=1.33 g C/m^2.y) and an average biomass of 1.10 g ww/m^2(=0.66g C/m^2) for the nematodes in station 11860 for the period Jan83-Dec83. S.punctata contributed for about 75% to the production of the whole community. Ascolaimus elongatus (2.2 µg ww/ind.) and Daptonema tenuispiculum (0.9 ug ww/ind.) are the other important species of the community.

For the period 1977-1979, Heip et al.(1984) calculated the production of the nematode communities for several coastal stations. The average biomass of the nematode community for this period equals 0.15 gC/m^2 which is two times higher than for 1983. For the period 1977-1979, only the high density months were sampled, and no information was available on the fluctuation of the biomass over the year. P/B=9 (Gerlach, 1971) was used to calculate the annual production and a value of 1.37 gC/m^2.y was obtained.When we use the P/B=20, as determined by Vranken et al.(in press) for the nematode community in 1983, a value of 3 gC/m^2.y is obtained for the period 1977-1979.

Billen & Somville(1985) discussed the flux of organic material to the sediment. In shallow coastal seas, such as the Belgian shelf, up to 50% of net primary production is deposited on the sediment. Faecal pellets and zooplankton corpses only make up a small fraction of this sedimentation flux. Phytoplanktonic cells and phytoplanktonic-derived detritus constitute the bulk of the organic matter deposited on the sediment. The local distribution of the flux of sedimenting organic material in the Belgian coastal zone can be explained on the basis of a hydrodynamical model of the tidal circulation. 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 (Adam et al.,1981). The annual amount of organic carbon deposition there has been estimated as 390 gC/m^2.year, while the mean value for the Belgian coastal zone as a whole is only 160 gC/m^2.year and is 70 gC/m^2.year in the offshore area(for a review see Joiris et al., 1982).

Heip et al.(1984) found that the nematodes from station 11860 have a lower production than the nematodes in the stations along the Belgian coast. The production of the nematodes is higher in the region off Ostend, which seems to contrast with the higher amount of organic C present off Zeebrugge. However, the east coast is more loaded with pollutants than the rest of the Belgian coast (Braeckman et al.,1984).

A review of the knowledge of the seasonal cycles of marine free-living nematodes is given in Heip et al.(1985). Temperature and food are the most obvious factors explaining the density changes in marine nematodes. Deposit-feeders (as is S.punctata) tend to reach maximum numbers in autumn, winter or early spring, due to the incorporation of primary production into the sediment. From several studies (Tietjen, 1969; Skoolmun & Gerlach, 1971; Smol et al.,1981; Bouwman, 1983) it is shown that spring and summer peaks in nematode densities are common in intertidal, shallow subtidal or brackish-water areas on an annual basis; however very few is known on long-term temporal variability.

Comparing overall community densities throughout the year, no significant differences in winter and summer values can be found in the sublittoral nematode communities studied so far (e.g. Lorenzen, 1974; Juario, 1975; Boucher, 1980). Only few species show significant

differences between seasons. In most cases, the species
which show significant differences between summer and
winter are those from which enough material (specimens) is
examined.

Several authors examined the reproductivity of
S.punctata in the field.

Skoolmun & Gerlach (1971) found a density peak in
winter or spring from S.vulgaris (=syn. with S.punctata)
in an intertidal sand-flat in the Weser estuery in Germany.
Juario (1975) discussed the life cycle of S. pulchra
(=close to S.punctata) in the German Bight. The three
juvenile size classes were encountered every month; egg
deposition occurs regardless of season. The mean abundance
as well as the number of juveniles in summer and winter do
not differ significantly. Throughout the year, the
population consists for more than 50% of juveniles except
in sep (45-50%).Bouwman (1983) found no significant
differences between seasons for S.pulchra in the
Ems-Dollard estuary; reproduction is continuous and
juveniles dominated in all seasons.

Acknowledgements

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Benthic studies of the southern Bight of the North
Sea.XI. The meiofauna in the Belgian coastal waters in the
period 1980-1981.

31314

HERMAN, R.L., K. VAN HOLSBEKE AND C. HEIP.

Abstract

Density, biomass and diversity of the meiobenthic
community have been studied in six stations off the Belgian
coast in 1980 - 1981.

Three of them located east of Ostend, show a poor
meiofauna composition. In the more sandy sediments West of
Ostend a richer fauna occurs.

The mean total density of $1.2 \ 10^6.m-2$ and an average
nematode biomass of 0.17 g C.m-2 are of the same order of
magnitude as in previous studies in that area (HERMAN et al.
1984,1985).

Of the 13 meiofaunal taxa found the majority occurs in
the western zone. Detailed analysis of the harpacticoid
community demonstrates a net differentiation of the western
area from the eastern part of the coastal zone, and again
illustrates that this Belgian coastal area is impoverished
compared to off shore sediments.

Introduction

Meiobenthos of the Belgian coastal zone was sampled
seasonally from june 1977 onwards. In a first study, 18
stations were investigated over the period 1977-79 (HERMAN
et al., 1985). These stations were classified into three
groups, according to sediment type.

A second study in six selected coastal stations over
the period 1982-83 (HERMAN et al., 1984) demonstrated that
density and diversity of meiobenthic communities were much
than in 1977-79.

Whether this was simple coincidence or due to long
term fluctuations of certain parameters could partly be
explained by investigating the 1980-81 samples. Analysis
of this material provided for a continuous series of data
over more than seven years.

In this study a quantitative and qualitative analysis
of the meiofauna is done for two sand , two muddy sand and
two mud stations. A detailed analysis of the harpacticoid
fauna of the Belgian coastal zone is given.

Materials and methods.

Six coastal stations were sampled in March, May and September 1980 an in March, July and October 1981. Their localisation is show in fig. 1.

Due to logistic reasons and bad weather conditions samples were collected by subsampling a 0.1 m2 Van Veen grab, except in May 1980 and in October 1981 when samples were obtained by subsampling a 170 cm2 Reineck box corer.
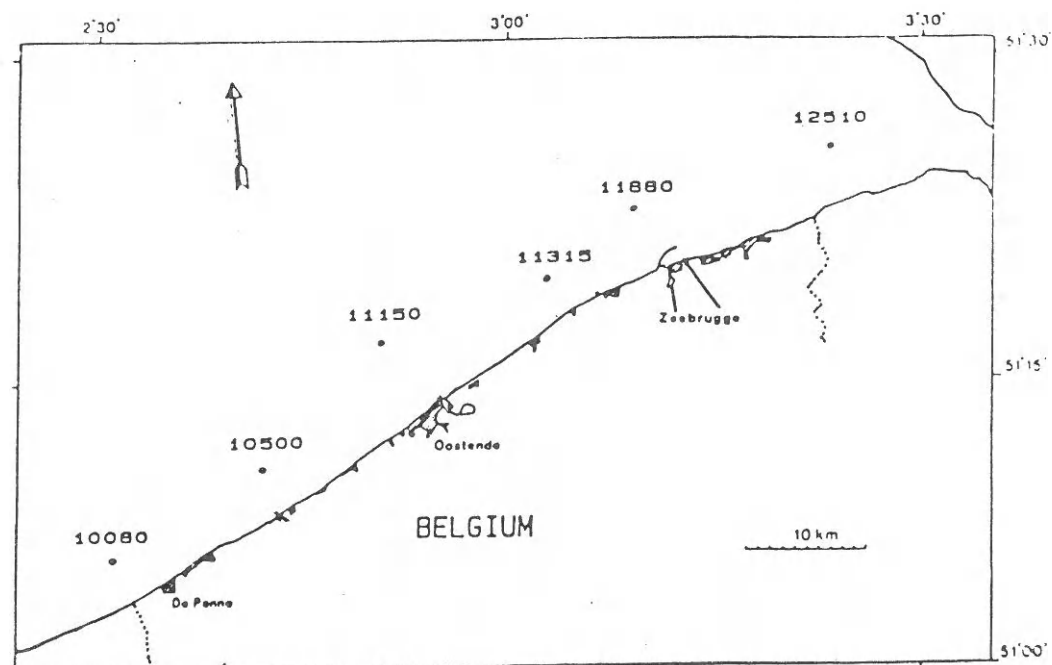
Fig. 1. Localisation of the six coastal stations.

All meiofauna sampling was done using 10.2 cm^2 plastic cores. Samples were fixed with warm formalin (70°C) to a final concentration of 4%. A supplementary core was used for sediment analysis. The methods for elutriation of the fauna, biomass and sediment analysis are described in HEIP et al., (1979). For the harpacticoid copepod community species diversity was estimated using Brillouin's formula and evennnes was calculated using the Heip-, the Fielou- and the Alatalo-index. Furthermore two multidimensional statistic analyses were applied to the Harpacticoid community : Correspondence analysis was executed following Lefebvre (1976) and cluster analysis methods as described in Legendre and Legendre (1979).

Results and discussion.

Sediment analysis

Sediment composition of the six stations is listed in table 1. The mean median grain size of the sand fraction for the coastal zone is 0.198 mm. The sediment composition is rather stable for all stations in the 1980-91 period. The mud content is the most variable component. The only very exceptional value was noted for the pure sand station 10080 where in September 1980 an aberrant value of 22.4 % mud was found, probably due to a navigation error.

Table 1.   Coordinates of the six coastal stations and sediment characteristics over the 1982-83 period, (Mean grain size of the sand fraction, mean % mud and sand).

Station	Lat. N	Long. E	Grain size Md_{mm}	% Mud $<63\mu m$	% Sand
10080	51°17'00"	02°31'00"	0.205	3.2	93.4
11150	51°16'32"	02°51'08"	0.332	1.4	96.2
10500	51°11'06"	02°42'04"	0.189	5.7	93.6
12510	51°26'58"	03°21'45"	0.185	7.1	92.8
11315	51°19'30"	03°03'00"	0.179	44.7	55.3
11880	51°22'00"	03°09'15"	0.103	81.0	18.7

Taxonomic group diversity

The meiofauna of the six coastal stations belongs to thirteen taxonomic groups (tab. 2). The most important taxa are Nematoda, Harpacticoida, Turbellaria and Gastrotricha. Less important taxa are Archiannelida, Halacarida, Tardigrada, interstitial Polychaeta, Ostracoda, Hydrozoa, Oligochaeta, Rotifera and Nemertini.

The mean number of taxa is higher than in previous studies (HERMAN et al., 1984,1985). This is demonstrated in fig. 2 where the trend values of the mean number of taxa per sample is plotted for the six coastal stations for the 1977-1983 period. The mean values for the three study periods are :

	Coastal zone	West of Ostend	East of Ostend
1977-79	3.8	4.7	2.6
1980-81	6.7	8.9	4.5
1982-83	4.4	5.8	3.0

Table 2. Composition of the meiofauna : density (N.10cm^{-2}), mean and dominance per taxon for all sampling data for the three sediment groups.

SAND STATIONS

Taxon	10080 3.80	10080 5.80	10080 9.80	10080 3.81	10080 7.81	10080 10.81	11150 3.80	11150 5.80	11150 9.80	11150 3.81	11150 7.81	11150 10.81	Gem	%D
Nematoda	188.5	513.0	8745.0	345.5	1010.0	3251.5	35.5	434.5	826.5	364.5	1383.5	244.0	1445.2	86.37
Harpacticoida	9.5	4.5	19.0	2.0	33.0	46.5	5.5	174.5	254.5	96.0	441.5	74.0	96.7	5.91
Turbellaria	10.5	13.5	13.0	20.0	10.0	27.0	10.5	23.5	33.0	18.5	55.5	19.0	21.2	1.29
Archiannelida	0.5	1.0	–	1.5	11.0	1.5	5.5	2.0	15.0	6.5	11.0	12.0	5.8	0.35
Gastrotricha	2.5	7.5	1.0	0.5	18.0	3.0	21.5	40.5	63.5	32.0	128.5	32.0	29.2	1.79
Ostracoda	0.5	1.0	–	–	1.0	–	–	5.5	42.0	–	89.0	3.5	11.9	0.73
Tardigrada	–	1.0	–	2.5	10.0	–	–	7.0	11.0	1.5	10.0	0.5	3.6	0.22
Hydrozoa	–	–	–	–	–	0.5	1.5	13.0	38.0	3.0	15.0	9.0	6.7	0.11
Halacarida	0.5	1.5	2.0	0.5	–	1.0	4.0	16.5	23.0	6.5	17.0	18.5	7.6	0.46
Oligochaeta	–	1.0	–	–	–	2.0	–	1.5	0.5	–	1.0	0.5	0.5	0.03
Nemertini	–	–	–	–	–	–	–	–	0.5	–	–	0.5	0.1	0.01
Polychaeta-meio	–	–	–	–	10.0	16.0	2.0	1.0	18.5	1.5	4.0	17.0	5.8	0.36
Rotatoria	1.5	1.5	–	1.0	–	–	–	8.0	–	–	0.5	–	1.0	0.06
Aantal individuen :	214	546	8780	374	1103	3349	86	728	1126	530	2159	431	1635	
Aantal taxa :	8	10	5	8	8	9	8	12	12	9	12	12	13	

MUDDY SAND STATIONS

Taxon	10500 3.80	10500 5.80	10500 3.81	10500 7.81	10500 10.81	12510 5.80	12510 9.80	12510 3.81	12510 7.81	12510 10.81	Gem	%D
Nematoda	832.5	2870.0	489.5	697.0	952.0	422.5	5719.0	447.5	696.0	1737.0	1466.3	95.06
Harpacticoida	15.5	12.5	13.0	19.0	4.5	1.0	3.0	3.0	9.5	2.0	8.3	0.53
Turbellaria	34.0	37.0	59.0	31.5	9.0	14.5	50.0	8.0	23.5	11.0	27.6	1.77
Archiannelida	0.5	–	0.5	–	–	–	4.0	–	–	–	0.5	0.03
Gastrotricha	29.0	15.0	51.0	60.5	21.0	1.5	21.0	4.0	8.5	8.0	22.0	1.40
Ostracoda	1.0	–	–	–	–	–	–	–	–	–	0.1	0.01
Tardigrada	2.5	2.5	87.0	8.5	0.5	7.0	–	–	19.5	–	12.8	0.82
Hydrozoa	1.0	–	–	–	–	–	–	–	–	–	0.1	0.01
Halacarida	0.5	0.5	–	0.5	1.0	–	3.0	0.5	1.0	2.0	0.9	0.06
Oligochaeta	–	0.5	–	3.0	–	–	2.0	–	–	1.0	0.7	0.04
Nemertini	–	–	–	–	–	1.0	–	–	–	–	0.1	0.01
Polychaeta-meio	4.5	1.5	3.5	0.5	–	0.5	28.0	–	2.0	0.5	4.1	0.26
Rotatoria	0.5	–	–	–	–	–	–	–	–	0.5	0.1	0.01
Aantal individuen :	922	2940	704	821	988	448	5830	463	760	1762	1564	
Aantal taxa :	11	8	7	8	6	7	8	5	7	8	13	

MUD STATIONS

| Taxon	11315 5.80	11315 9.80	11315 3.81	11315 7.81	11315 10.81	11880 3.80	11880 5.80	11880 9.80	11880 3.81	11880 7.81	11880 10.81	Gem	%D
Nematoda	225.0	626.0	1272.0	434.5	310.5	19.5	473.5	18.5	247.5	603.0	62.5	390.2	88.88
Harpacticoida	–	1.0	0.5	468.0	0.5	7.0	2.5	0.5	–	5.0	–	44.1	10.04
Turbellaria	–	15.0	10.5	6.5	2.5	1.5	3.5	0.5	1.0	3.0	0.5	4.0	0.92
Gastrotricha	–	–	0.5	–	–	–	–	–	–	–	–	0.0	0.01
Halacarida	–	–	–	3.5	–	–	0.5	0.5	–	1.5	–	0.5	0.12
Polychaeta-meio	–	–	–	0.5	–	–	–	–	–	–	–	0.0	0.01
Rotatoria	–	–	–	–	0.5	–	–	–	–	–	–	0.0	0.01
Aantal individuen :	225	642	1284	913	314	28	480	20	249	613	63	439	
Aantal taxa :	1	3	4	5	4	3	4	4	2	4	2	7	
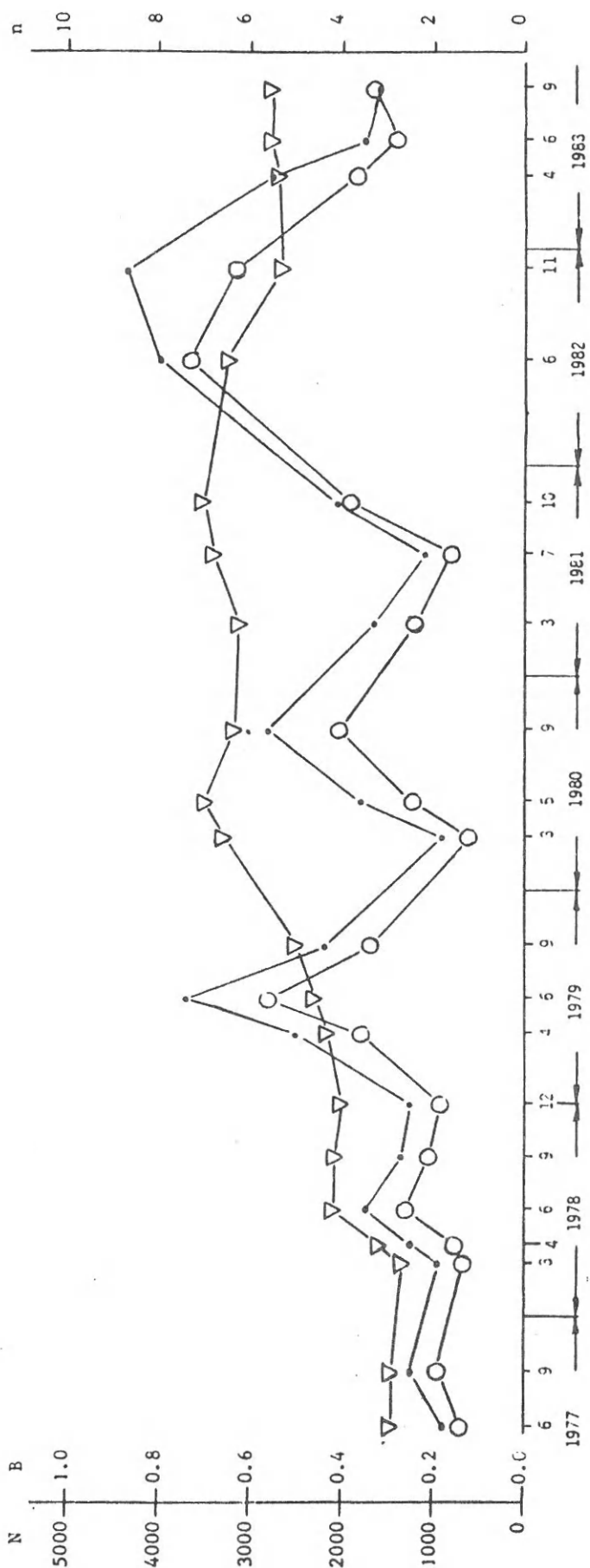
Fig. 2. Long term fluctuation of the number of taxa n (∇——∇), total meiofauna density (N.10 cm^{-2}) (o——o) and biomass B of the Nematoda (in mg dwt.10 cm^{-2})(.——.) in the Belgian coastal zone for the period 1977 – 1983.

Again this study demonstrates that the zone east of Ostend is markedly poorer than the Western part of the coastal zone: the number of taxa is half that found in the more sandy area west of Ostend.

Density.

The overall mean meiofauna density for the 80-81 period is $1.2 \cdot 10^6$ ind.m-2. The mean number per taxon for the three station groups for each sampling date is listed in table 2. The mean density both for the sand and muddy sand stations is $1.6 \cdot 10^6$ ind. m-2. In the mud stations a rather low mean density of $0.44 \cdot 10^6$ ind. m-2 was found. Maximum values are $8.8 \cdot 10^6$ ind. m-2 (10080, Sept. '80) and $5.8 \cdot 10^6$ ind. m-2 (12510, Sept. '80). Minimum densities are noted in the pure mud station 11880 : $0.02 \cdot 10^6$ ind. m-2 ..(Sept.'80) and $0.03 \cdot 10^6$ ind. m-2 (March '80).

The most dominant taxon are the Nematoda, with resp. 88.4 % dominance in the sand stations, 95.1 % in the muddy sand and 98.5 % in the mud stations.

From the other meiofaunal taxa, only harpacticoid copepods, turbellarians and gastrotrichs may be of some importance. An exceptionally high harpacticoid density was recorded at the mud station 11315 in July '81 ($0.47 \cdot 10^6$ ind. m-2 or 52 %). In the muddy sand and mud stations the second dominant taxon are the Turbellaria. Although extraction of preserved samples yields the best results for the majority of turbellarians (MARTENS, 1984), densities are to be considered as minimal estimates.

Except for Gastrotricha the importance of other meiofaunal taxa is generally negligible (less than 0.5 %).

Biomass.

Because nematodes generally are superdominant within the meiofauna, biomass fluctuations are very well correlated with density changes in this taxon (fig. 2). The average individual biomass, estimated for the May '80 and the October '81 samples, is 0.38 µg dwt. ind-1. The mean individual biomass for the sand, sandy mud and mud stations is resp. 0.54 µg dwt, 0.51 µg dwt and 0.19 µg dwt per nematode.

Due to the rather low nematode density found, especially in the mud stations, the total mean biomass is slightly lower than in other years (see fig. 2). This figure clearly shows the yearly periodicity both for density and biomass over the 1977 - 1983 period, with an absolute minimum for the March 1980 samples.

Harpacticoida of the coastal area.

The mean density of the harpacticoid copepods for all sampling data per sediment type is given in tables 3,4 and 5. The mean density in sandy sediments is 97 ind. 10 cm2, which is comparable to other studies in similar sediments, such as SCHEIBEL (1973,1976), ANKAR & ELMGREN (1978), ELMGREN et al. (1984) and COULL (1985), in which density ranges between 84 and 118 ind. per 10 cm2.

In the muddy sand and mainly in the mud samples density drops to very low numbers. The mean density is resp. 8 and 2 individuals per cm2. In most studies, such as BODIN (1984), AFLT (1975,1977), ANKAR & ELMGREN (1978) and MOORE (1979), the mean values are generally much higher (range : 11 - 175 ind. 10 cm2).

In this study 55 species belonging to 14 families were found. They are listed in table 6; twenty species occur only once. The most frequent species are *Paraleptastacus espinulatus* (f= 16), *Microarthridion littorale* (f= 11), *Halectinosoma sarsi* and *Leptastacus laticaudatus* which are found at 10 of the 33 sampling dates.

The families Cylindropsyllidae, Paramesochridae, Ectinosomatidae, Ameiridae and Diossacidae are qualitatively best represented by resp. 9,9,8,7 and 6 species. Quantitatively, the most important families are Tachidiidae (29 %), Ectinosomatidae (20,9 %), Cylindropsyllidae (20,6 %), Ameiridae (8,7 %) and Paramesochridae (8,3 %).

In sandy sediments, 43 species belonging to 11 families are found, while in muddy sand only 25 species occur. In mud stations the 11 species found belong to 7 families, of which 7 typically phytal forms occur only once.

Other community parameters, such as diversity and evenness, reflect the impoverishment of the harpacticoid fauna found in most of the mud and muddy sand stations. A mean number of 4 species per sample is found for the total coastal area (7 spp. West, 1 sp. East of Ostend). this results in a low diversity both for the Shannon-Wiener index H' and for the Brillouin index H :

	West	East
H'	1.97 bits/ind	0.36 bits/ind
H	1.56 bits/ind	0.34 bits/ind

Maximal diversity values are found in the sand station 11150 with H = 2.52. The minimum is H = 0 in both mud stations 11880 and 11315 where often only one or no harpacticoid copepod occurs. The mean H = 0.88 which is slightly lower than the mean 82-83 value of H = 0.96. The rather low mean Simpson's index SI = 0.27 means that in most samples often more than one species is dominant.

Table 3. Species composition and dominance of the Harpacticoida in
the sand stations 10080 and 11150 per sampling date.

ZANDSTATIONS Harpacticoida 1980-1981

Species	10080 3.80	10080 5.80	10080 9.80	10080 3.81	10080 7.81	10080 10.81	11150 3.80	11150 5.80	11150 9.80	11150 3.81	11150 7.81	11150 10.91	n	%D
Metacyclopina brevisetosa	-	-	-	-	-	-	-	-	0.5	-	-	-	0.0	0.04
Longipedia minor	-	-	-	-	1.0	0.5	-	-	-	-	-	-	0.1	0.13
Canuella perplexa	0.5	0.5	-	-	-	5.5	-	-	0.5	0.5	-	-	0.6	0.65
Arenosetella germ. germanica	-	-	-	-	-	-	-	4.0	1.5	-	11.5	7.5	2.0	2.11
Palectinosoma gothiceps	-	-	-	-	-	0.5	-	-	-	-	-	-	0.0	0.04
Palectinosoma herdmani	-	0.5	4.0	0.5	8.0	-	-	-	0.5	-	-	-	1.1	1.16
Palectinosoma propinquum	-	0.5	-	-	6.0	-	-	-	-	-	-	-	0.5	0.56
Palectinosoma sarsi	0.5	-	2.0	-	1.0	2.0	-	-	1.0	-	2.0	-	0.7	0.73
Hastigerella leptoderma	-	-	-	-	-	-	-	-	1.0	1.0	-	-	0.2	0.17
Pseudobradya beduina	-	0.5	10.0	-	-	1.5	-	-	2.0	-	0.5	-	1.2	1.25
Microarthridion littorale	-	-	-	-	11.0	-	0.5	-	-	-	0.5	-	1.0	1.03
Tisbe furcata	-	-	-	-	-	0.5	-	-	-	-	-	-	0.0	0.04
Dactylopodia vulgaris	-	-	-	-	1.0	-	-	-	-	-	-	-	0.1	0.09
Amniascoides debilis	-	-	-	-	1.0	-	0.5	-	-	-	-	-	0.1	0.13
Amphiascus varians	-	-	-	-	-	-	-	-	-	-	-	0.5	0.0	0.04
Pararamphiascopsis longirostris	-	-	-	-	-	-	-	-	0.5	-	-	-	0.0	0.04
Psammotopa phyllosetosa	-	-	-	-	-	-	-	3.0	20.5	-	9.5	6.5	3.3	3.40
Ameira brevipes	-	-	-	-	-	-	-	-	-	-	5.0	6.5	1.0	0.99
Ameira hyalina	-	-	-	-	-	-	-	-	33.0	0.5	-	5.0	3.2	3.31
Ameira parvula	-	-	2.0	-	1.0	29.5	-	-	-	-	-	-	2.7	2.80
Interleptomesochra eulitoralis	-	-	-	-	-	-	-	0.5	2.0	0.5	0.5	-	0.3	0.30
Sicameira leptoderma	-	-	-	-	-	-	-	1.5	-	-	-	-	0.1	0.13
Ameiridae sp.	-	0.5	-	-	-	-	-	-	-	-	-	-	0.0	0.04
Apodopsyllus sp.A	0.5	-	-	-	-	0.5	-	1.5	-	-	-	-	0.2	0.22
Diarthrodella secunda s.s.	-	-	-	-	-	-	0.5	-	-	-	-	-	0.0	0.04
Kliopsyllus constrictus s.s.	-	-	-	-	-	-	-	-	-	5.5	-	-	0.5	0.47
Kliopsyllus holsaticus s.s.	-	-	-	-	-	-	2.5	82.0	53.5	8.5	128.5	16.5	24.3	25.09
Kliopsyllus paraholsaticus	0.5	-	-	-	-	-	-	-	5.5	0.5	4.0	0.5	0.9	0.95
Leptopsyllus sp.A	-	-	-	-	2.0	-	-	-	-	-	-	-	0.2	0.17
Paramesochra sp.(Mielke)	-	-	-	-	-	-	-	7.0	1.5	2.5	1.0	-	1.0	1.03
Scottopsyllus Scottops. minor	-	-	-	-	-	-	-	0.5	-	1.5	-	-	0.2	0.17
Scottopsyllus Scottops. sp.C	-	-	-	-	-	-	-	2.5	-	-	-	-	0.2	0.22
Arenocaris bifida	-	-	-	-	-	0.5	-	-	-	-	-	-	0.0	0.04
Arenopontia sp.A	1.5	-	-	-	-	1.0	-	-	-	-	-	0.5	0.3	0.26
Cylindropsyllus remanei	-	-	-	-	-	-	-	0.5	-	-	-	-	0.0	0.04
Evansula pygmaea	-	-	-	0.5	-	-	-	17.0	4.5	2.5	51.0	6.5	6.8	7.06
Leptastacus laticaudatus intermedius	1.5	-	-	-	1.0	-	0.5	19.5	123.0	59.0	152.5	19.5	31.4	32.40
Leptopontia curvicauda	-	-	-	-	-	-	-	1.5	1.0	-	1.0	1.5	0.4	0.43
Paraleptastacus espinulatus	4.5	2.0	-	1.0	2.0	2.5	1.0	28.5	2.5	13.0	73.5	3.0	11.1	11.49
Paraleptastacus holsaticus	-	-	-	-	-	-	-	4.5	-	-	0.5	-	0.4	0.43
Paraleptastacus spinicauda	-	-	-	-	-	-	-	0.5	-	1.5	-	-	0.2	0.17
Enhydrosoma propinquum	-	-	1.0	-	-	-	-	-	-	-	-	-	0.1	0.09
Cletodidae sp.A	-	-	-	-	-	-	-	0.5	-	-	-	-	0.0	0.04
Aantal individuen :	10	5	19	2	33	47	6	175	255	97	442	74	97	
Aantal species :	7	6	5	3	10	12	6	17	18	13	15	12	43	

Table 4. Species composition and dominance of the Harpacticoida in the muddy sand stations 10500 and 12510 per sampling date.

ZAND-SLIB STATIONS Harpacticoida 1980-81

Species	10500 3.80	10500 5.80	10500 3.81	10500 7.81	10500 10.81	12510 5.80	12510 9.80	12510 3.81	12510 7.81	12510 10.81	n	%D
Canuella perplexa	3.0	–	0.5	1.0	0.5	–	–	–	–	–	0.5	6.02
Arenosetella germ. germanica	–	–	–	–	–	–	–	–	0.5	–	0.1	0.60
Halectinosoma gothiceps	1.0	–	–	–	–	–	–	–	–	–	0.1	1.20
Halectinosoma herdmani	1.5	–	0.5	2.5	–	–	–	0.5	–	–	0.5	6.02
Halectinosoma propinquum	–	–	–	–	–	–	3.0	–	2.0	–	0.5	6.02
Halectinosoma sarsi	3.0	–	1.5	1.5	–	0.5	–	–	–	–	0.7	7.83
Pseudobradya beduina	–	–	–	1.0	1.0	–	–	–	3.5	1.0	0.7	7.83
Pseudobradya minor	–	–	–	–	–	–	–	–	–	0.5	0.1	0.60
Puterrina acutifrons	–	–	–	–	–	–	–	–	–	0.5	0.1	0.60
Microarthridion littorale	–	2.5	–	12.5	–	–	–	–	3.5	–	1.9	22.29
Harpacticus littoralis	–	–	–	–	–	–	–	0.5	–	–	0.1	0.60
Dactylopodia tisboides	–	0.5	–	–	0.5	–	–	–	–	–	0.1	1.20
Dactylopodia vulgaris	–	–	1.0	–	–	–	–	–	–	–	0.1	1.20
Amphiascoides debilis	–	–	1.0	–	0.5	–	–	–	–	–	0.2	1.81
Amphiascus varians	1.0	–	1.0	–	–	–	–	1.5	–	–	0.4	4.22
Bulbamphiascus imus	–	–	3.5	–	0.5	–	–	–	–	–	0.4	4.82
Ameira hyalina	–	3.5	–	–	–	–	–	–	–	–	0.4	4.22
Ameira parvula	3.5	1.0	0.5	–	0.5	–	–	0.5	–	–	0.6	7.23
Proameira sp.A	0.5	3.0	–	–	0.5	–	–	–	–	–	0.4	4.82
Arodonsyllus sp.A	0.5	0.5	0.5	0.5	0.5	–	–	–	–	–	0.3	3.01
Scottopsyllus Scottops. minor	–	0.5	–	–	–	–	–	–	–	–	0.1	0.60
Mesochra pygmaea	–	–	0.5	–	–	–	–	–	–	–	0.1	0.60
Leptastacus laticaudatus intermedius	1.0	1.0	–	–	–	–	–	–	–	–	0.2	2.41
Paraleptastacus espinulatus	0.5	0.5	1.0	–	–	0.5	–	–	–	–	0.3	3.01
Esola bulligera	–	–	1.0	–	–	–	–	–	–	–	0.1	1.20
Aantal individuer :	16	13	13	19	5	1	3	3	10	2	8	
Aantal species :	10	9	12	6	8	2	1	4	4	3	25	

Table 5. Species composition and dominance of the Harpacticoida in the mud stations 11315 and 11880 per sampling date.

SLIBSTATIONS Harpactioda 1980-1981

Species	11315 9.80	11315 3.81	11315 7.81	11315 10.81	11880 3.80	11880 5.80	11880 9.80	11880 7.81	n	%D
Puterrina acutifrons	1.0	–	–	0.5	–	–	–	–	0.2	0.31
Microarthridion littorale	–	0.5	468.0	–	–	2.0	0.5	5.0	59.5	98.14
Dactylopodia tisboides	–	–	–	–	2.0	–	–	–	0.3	0.41
Diarthrodes pygmaeus	–	–	–	–	1.0	–	–	–	0.1	0.21
Amphiascoides debilis	–	–	–	–	0.5	–	–	–	0.1	0.10
Stenhelia (D.) norm. normani	–	–	–	–	0.5	–	–	–	0.1	0.10
Ameira parvula	–	–	–	–	1.0	–	–	–	0.1	0.21
Mesochra pygmaea	–	–	–	–	0.5	–	–	–	0.1	0.10
Paraleptastacus espinulatus	–	–	–	–	–	0.5	–	–	0.1	0.10
Laophonte cornuta	–	–	–	–	1.0	–	–	–	0.1	0.21
Paralaophonte congenera congenera	–	–	–	–	0.5	–	–	–	0.1	0.10
Aantal individuen :	1	1	468	1	7	3	1	5	61	
Aantal species :	1	1	1	1	8	2	1	1	11	

Table 6. Dominance and frequency (sampling data) of the Harpacticoida per species in the Belgian coastal waters for the 1980-81 period (with abbreviations used in fig. 5 for the more frequent species).

Species	Abr.	f	% D
Canuella perplexa	Cpe	9	2.29
Arenosetella germ. germanica	Age	5	0.70
Halectinosoma herdmani	Hhe	9	4.17
H. propinquum	Hpr	4	5.01
H. sarsi	Hsa	10	3.77
Pseudobradya beduina	Pbe	9	6.07
Euterpina acutifrons	Eac	3	7.50
Microarthridion littorale	Mli	11	21.48
Dactylopodia tisboides	Dti	3	1.45
Amphiascoides debilis	Ade	5	1.28
Amphiascus varians	Ava	4	2.17
Psammotopa phyllosetosa	Pph	4	0.69
Ameira hyalina	Ahy	4	1.57
A. parvula	Apa	9	5.11
Interleptomesochra eulitoralis	Ieu	4	0.06
Proameira sp. A	Pro	3	1.25
Apodopsyllus sp. A	Apo	8	1.07
Kliopsyllus holsaticus s.s.	Kho	6	5.78
K. paraholsaticus	Kpa	5	0.32
Paramesochra mielki	PaM	4	0.25
Scottopsyllus Scottop. minor	SSm	3	0.19
Arenopontia sp. A	Are	3	0.62
Evansula pygmaea	Epy	6	1.98
Leptastacus laticaudatus	Lli	10	7.44
Leptopontia curvicauda	Lcu	4	0.12
Paraleptastacus espinulatus	Pes	16	10.26

Species	f	% D
Metacyclopina brevisetosa	1	0.01
Longipedia minor	2	0.14
Halectinosoma gothiceps	2	0.25
Hastigerella leptoderma	2	0.05
Pseudobradya minor	1	0.33
Harpacticus littoralis	1	0.55
Tisbe furcata	1	0.04
Dactylopodia vulgaris	2	0.37
Diarthrodes pygmaeus	1	0.48
Bulbamphiascus imus	2	1.30
Paramphiascopsis longirostris	1	0.01
Stenhelia (D.) norm. normani	1	0.24
Ameira brevipes	2	0.33
Sicameira leptoderma	1	0.03
Ameiridae sp.	1	0.37
Diarthrodella secunda s.s.	1	0.30
Kliopsyllus constrictus s.s.	1	0.19
Leptopsyllus sp. A	1	0.14
Scottopsyllus Scottopsyllus sp. C	1	0.19
Mesochra pygmaea	2	0.87
Arenocaris bifida	1	0.04
Cylindropsyllus remanei	1	0.01
Paraleptastacus holsaticus	2	0.09
P. spinicauda	2	0.06
Enhydrosoma propinquum	1	0.18
Cletodidae sp. A	1	0.01
Esola bulligera	1	0.27
Laophonte cornuta	1	0.48
Paralaophonte congenera congenera	1	0.24

The fluctuations of the Pielou-, Heip- and Alatalo evenness indices follow an identical pattern, with the Pielou-index J always higher (fig. 3).
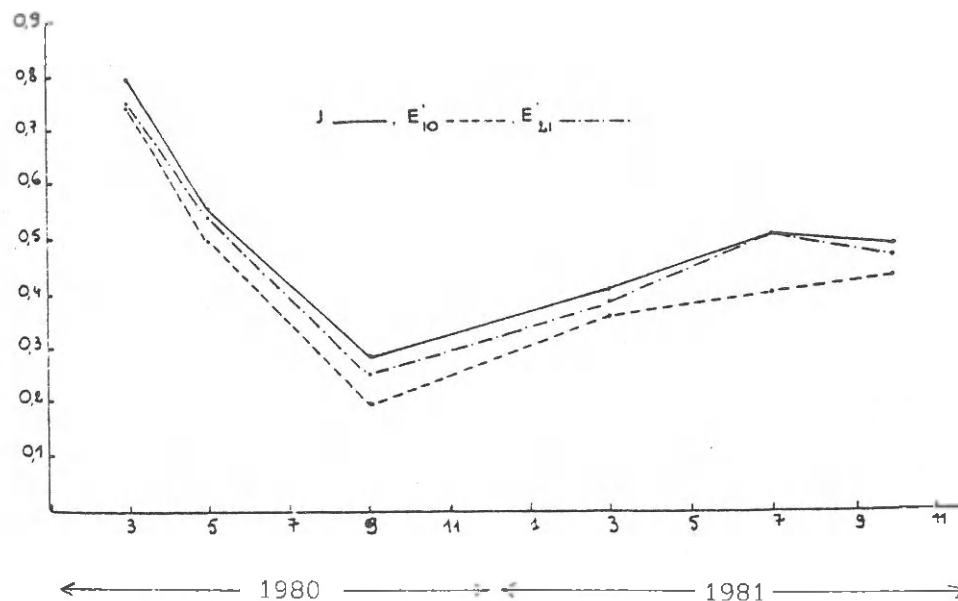
Fig. 3. Evolution of three evennes indexes for all stations over the 1980-81 period : Pielou index J, Heip index E'_{10} and Alatalo index E'_{21}.

The highest values are noted in spring 1980, then evenness drops due to the appearance of mesopsammic species to reach minimum values in Sept.'80, when *Leptastacus laticaudatus* and *Kliopsyllus holsaticus* become very dominant. In 1981 the equitability of the individuals among the species becomes better.

That the composition of the harpacticoid communities is well correlatred with the sediment type is demonstrated in the cluster diagrams (fig. 4). Cluster analysis is done with two similarity indices : the Sorensen-index - a binary index - on presence or absence of species between the stations and the Bray-Curtis-index which takes the relative densities into account. This was done once for all the 55 species and once for 26 species after elimination of all species with a frequency lower than 3.
The clustering of the stations over the 55 species is identical to that over 26 species for both indices, with an evidently higher degree of similarity for the 26 species.
With the Sorensen similarity index one can see a net separation of the sand stations from the other ones, although this is better illustrated by the Bray-Curtis plots. These graphs shows a net separation of the sand station 11150 both for the 26 and for the 55 species cluster. Also one can see that most samples of the other two stations west of Ostend (10500 and 10080) are grouped.
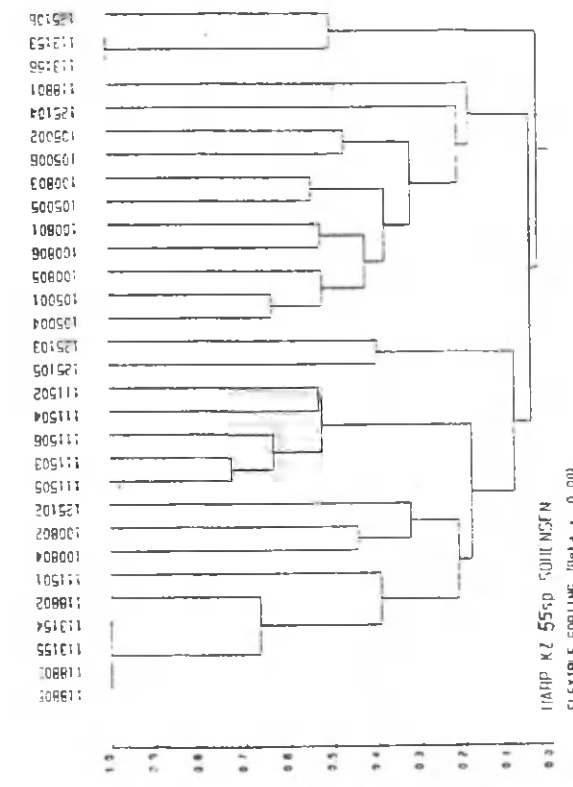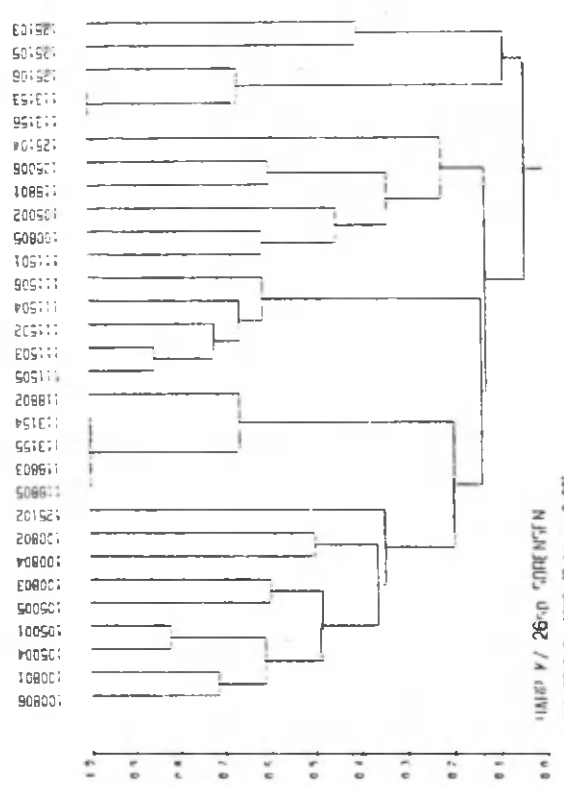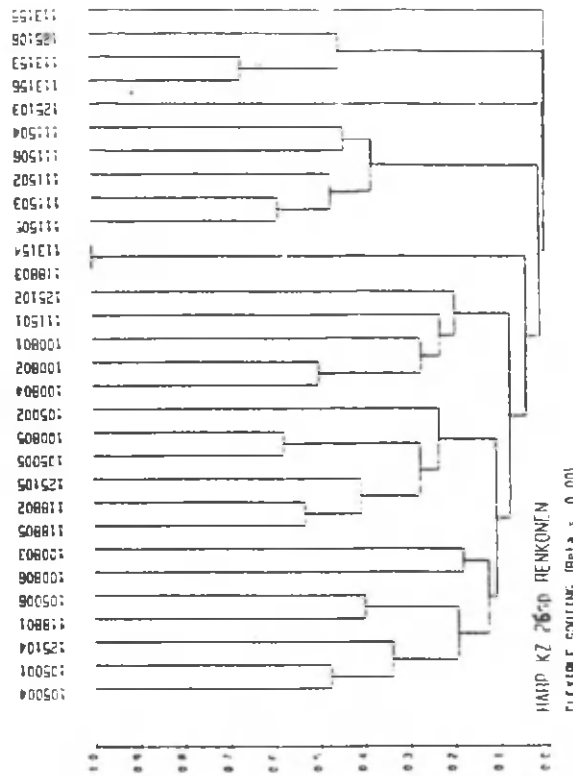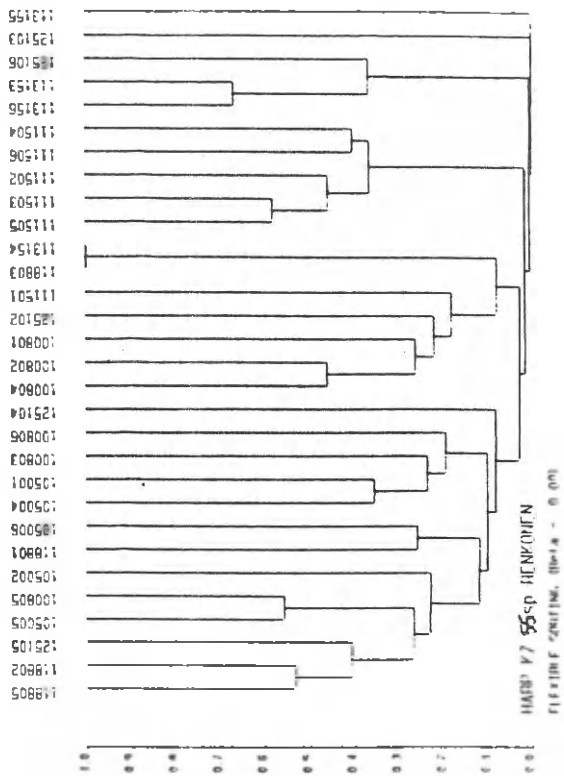
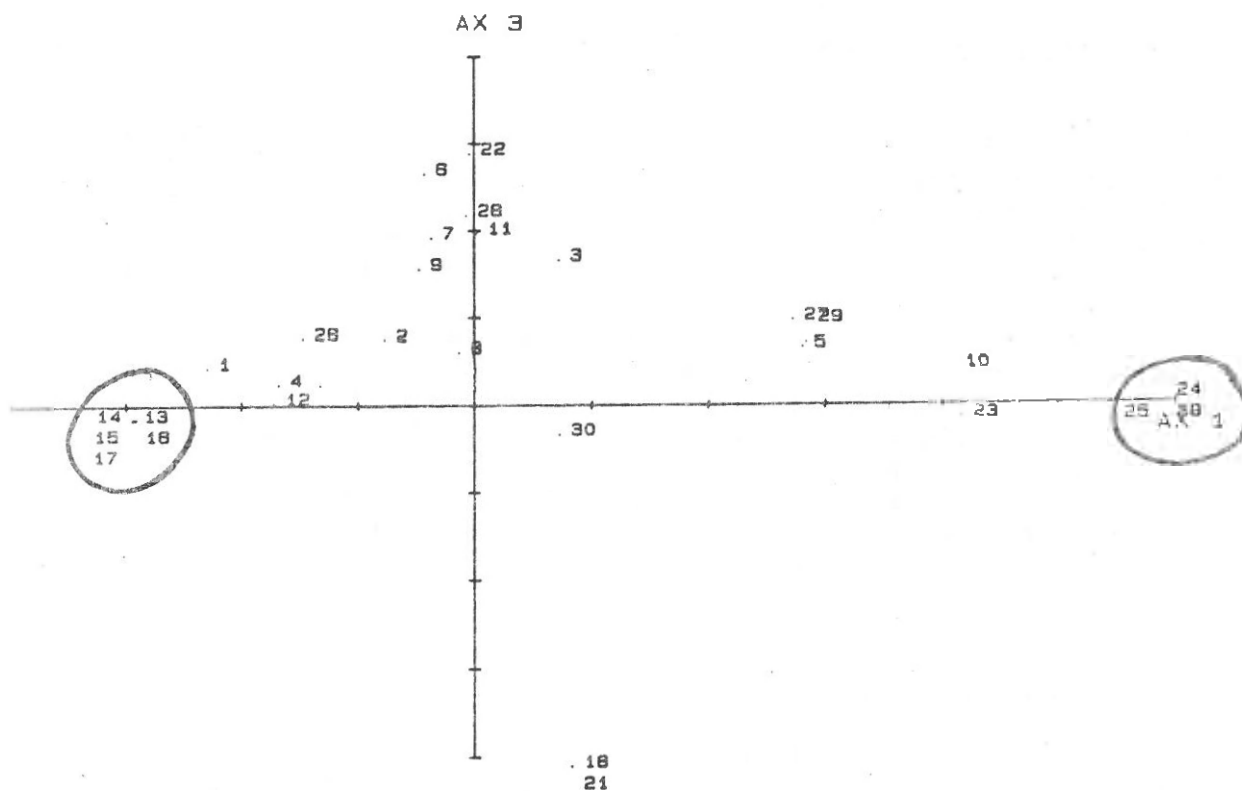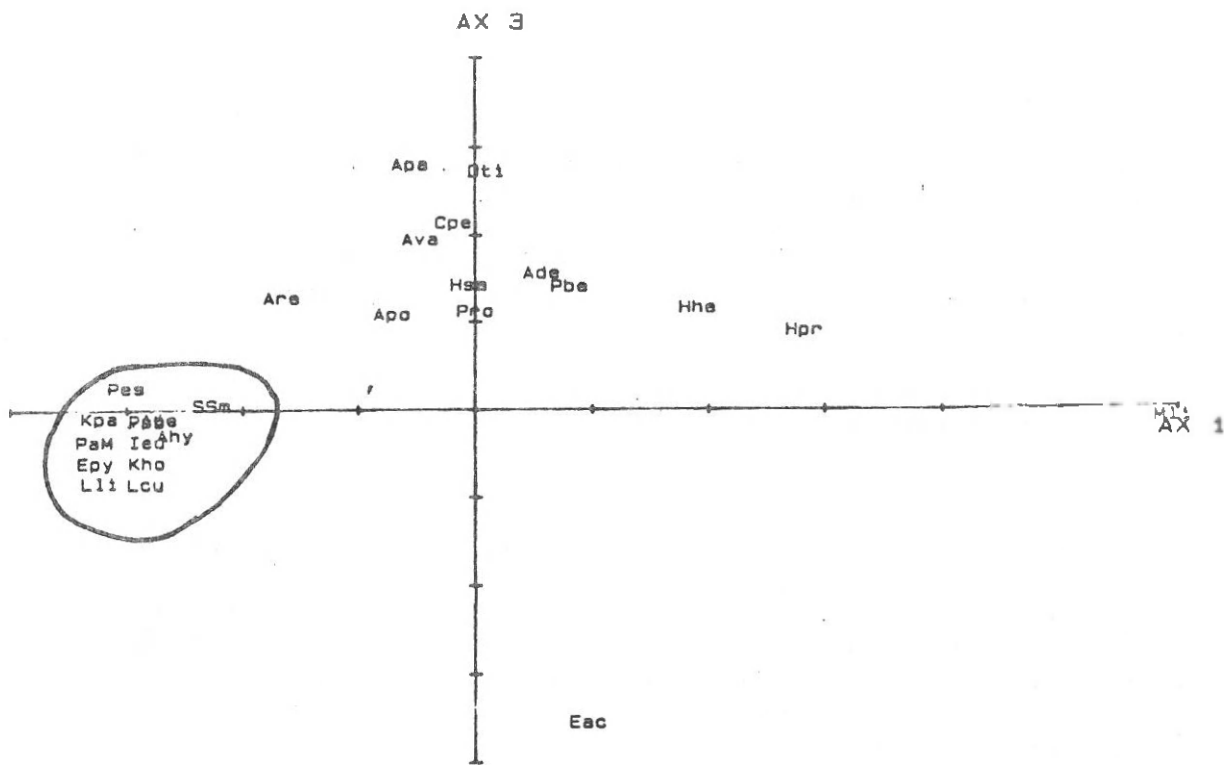Fig. 4. Cluster diagrams over 55 and 26 harpacticoid species (see text).

Fig. 5. Correspondence analysis over 26 harpacticoid species (top)
and stations (bottom) in six stations off the Belgian coast
for the '80-81 data.(For station numbers see table 2).

Because there was no marked difference between cluster analyses over the 55 and 26 species, correspondence analysis or reciprocal averaging was done for the smallest species group on the 80-81 data (fig. 5).

The abbreviations for the species used in this graph are listed in table 6. Two principal axes are plotted both for species and for stations. It is rather difficult to explain what all axes mean in ecological terms and allocate them to one of the numerous parameters acting within an ecosystem. In this study one can give a reasonable explanation for the two axes in the analysis of this harpacticoid communities.

Ax 1 is strongly correlated with sediment. In the top graph (fig. 5) one can distinguish on the left a group of representatives of the pure sand fauna. On the right of the graph the mud-dweller *Microarthridion littorale* (Mli) is netly separated from an intermediate group scattered around the Ax 3 which corresponds with the diverse muddy sand community. The same remarks can be made for the stations (bottom graph). Most samples of the sand station 11150 are concentrated in the left of this graph, while on the extreme right we find the mud samples with only *M. littorale* ; the bulk of the stations form a very heterogeneous intermediate group.

The parameter 'time' seems not to be of direct importance because the stations remain in the same group for sediment type and species composition.

CONCLUDING REMARKS

Considering the evolution of the total meiofauna from 1977 till 1983 one sees that peak values for all parameters studied are noted in summer '82. In the first study period (1977-79) there was an increasing trend both for meiobenthic diversity and for density and biomass. Density and biomass decrease slightly in the 80-81 period and reach their maxima in '82.

The diversity of the harpacticoid fauna from 1980 onwards is considerably higher than in the previous years. However, compared to other similar environments (e.g. MOORE, 1979; WILLEMS et al.,1982; BODIN, 1984) density and diversity are always low.

This study demonstrates again that the meiofauna in the Belgian coastal zone is 'poorer' compared to other localities of the North Sea.

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A re-evaluation of marine nematode productivity

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ABSTRACT

Nematodes are the most abundant multicellular animals in marine sedi-
ments but their role in the benthos has not been properly quantified yet.
In nearly all energy-flow budgets of marine systems their annual produc-
tion P is given as about nine times their mean biomass B and their part
in the total energy-flow is consequently estimated as anywhere between 3
and 30 % of the total (carbon) input in the benthic system. Our labora-
tory experiments demonstrate that nematode productivity is much higher
than P/B ~9 per year and may reach values of over 60 for bacterial grazers.
To obtain more reliable estimates for field populations we propose a re-
gression equation relating egg-to-egg development time T_{min} to temperature
(t) and adult female weight (W in μg wet weight) : $\log T_{min} = 2.202-0.0461t$
$+ 0.627 \log W$. When multiplied by the constant biomass turnover per gen-
eration $(P/B)_{gen} = 3$, development rate $1/T_{min}$ is a good predictor of daily
P/B. This method was applied to two series of field data. A rather stable
community from a sublittoral mud in the North Sea had an annual P/B = 20.
A less stable Aufwuchs community from *Sargassum* in Japan had an annual
P/B = 58.

Density of marine nematodes is in the order of 10^5-10^6 ind. per m^2, their biomass usually ranges between 0.1 and 1 g dry weight per m^2 (Heip *et al.*, 1985). There is experimental evidence that they stimulate mineralisation of organic matter (Findlay & Tenore, 1982) and nutrient regeneration (Tietjen, 1980) by grazing on bacteria. They cycle an important proportion of the sediment pool of some heavy metals (e.g. Cd) (Frithsen, 1984). They are eaten by small crustaceans (Gerlach & Schrage, 1969; Feller, 1984) and fish (Lasserre *et al.*, 1976), thus forming a link between bacterial production and higher trophic levels. The rates of these processes are largely unknown. These rates may be estimated from energy flow through the populations (Crisp, 1971). An important part of the energy intake is channeled into biomass production. Production estimates of nematode populations in the sea do not exist : many species reproduce continuously throughout the year and the logistics of sampling subtidal sediments also prohibit the use of the classical methods in production studies (analysis of growth or mortality of cohorts in the field). Nearly the whole literature on benthic productivity uses an annual P/B around nine (McIntyre, 1969; Gerlach, 1971; Warwick & Price, 1979) as representing the annual biomass turnover of marine nematodes and even meiofauna in general. Gerlach's estimate P/B = 9 is based on a study of one brackish-water species in the laboratory with a biomass turnover of three per generation. Three generations per year is an average for the few long-lived meiofauna species for which the life-cycle was known at the time. Warwick & Price (1979) calculated P/B = 8.7 from respiration measurements and the relationship between respiration and production proposed by McNeil & Lawton (1970).

A production in each generation of three times the average biomass is a valid figure for the several copepods, ostracods and nematodes where it has been verified (Heip *et al.*, 1982; Herman *et al.*, 1984). Field data for nematodes do not exist. Since the birth rate of a population in the stable age distribution is equal to its daily P/B (Zaika, 1973) we constructed life tables for four species of nematodes cultured in our laboratory (Vranken, 1985). The average value obtained from these experiments was P/B = 2.98 $\pm$ 0.13 (n=7) per T_{min}. From these experiments it also became clear that fecundity of nematodes is much higher than previously thought. In a recent review (Zaika & Makarova, 1979) an average fecundity of twenty eggs per female was proposed. However, a single female of *Monhystera disjuncta* in agnotobiotic conditions (Dougherty, 1960) produces over 200 eggs during the 70 days that her productive adult life lasts,

which represents more than fifteen times her own body weight (Vranken, 1985). When fed in monoxenic cultures on an optimal diet the figure rises to over 500 eggs. These eggs develop into adults within two weeks. The rhabditid *Pellioditis marina* has an even higher reproductive potential, producing over 600 eggs per female which mature in less than five days (Vranken & Heip, 1983).

For most marine nematodes studied the average duration of egg-to-egg development is in the order of two to three weeks at the annual mean temperature in the habitat. This indicates maximum annual P/B ratios in the order of 50 to 70. For the best studied species, *Monhystera disjuncta*, the yearly P/B was estimated as 69 from three times the number of generations produced in the field calculated from development time and temperature (Vranken & Heip, 1985) and as 66 from the daily birth rate, which is a linear function of temperature in all species studied (Vranken & Heip, 1985). These figures are almost an order of magnitude higher than assumed in the literature.

In order to better assess the productivity of marine nematodes in the sea we calculated a multiple linear regression between duration of egg-to-egg development T_{min} and temperature t (°C) and adult female body weight W (in µg wet weight) for all species from temperate areas (maximum temperature lower than 22° C) for which reliable data exist (Table 1). The resulting equation (1) has a temperature coefficient corresponding to a Q_{10} = 2.95 and a very steep dependence on body weight, indicating that the spectrum of biomass in a nematode community will strongly influence its production :

$$\log T_{min} = 2.202 - 0.0461 \; t + 0.627 \log W \qquad (1)$$

$$(R^2 = 0.88; \quad F(2,46) = 173; \quad n = 49)$$

As an example eq. (1) was used to determine the annual production of a subtidal community from a muddy sediment (median grain size 45 µm) off the Belgian coast in the North Sea (Vincx & Heip, 1984) and from an Aufwuchs community on *Sargassum confusum* in Japan (Kito, 1982). The North Sea station is polluted and characterized by a low diversity community dominated by *Sabatieria punctata* (av. 84.5 %) and *Daptonema tenuispiculum* (av. 8.4 %). The biomass structure (males, females and juveniles) was determined each month. The average biomass was 1.10 g ww per m^2. The P/B was calculated for each month as $1/T_{min}$ x D x 3, with D the number of days in the month. Total production so calculated amounted to 22.2 g ww per m^2 per year and the annual P/B of this community is P/B = 20. The Aufwuchs

community from *Sargassum* showed a marked seasonality with maximum numbers in Spring and Summer and virtually disappeared in Winter. *Monhystera refringens, Chromadora nudicapitata, Chromadora heterostomata, Araeolaimus elegans* and *Theristus acer* were the five dominant species. The average biomass of this community, again determined from monthly samples, was 157 mg ww per m^2, its annual production 9144 mg ww per m^2. The annual P/B = 58. The calculation proposed here still has speculative aspects. These include two extrapolations : 1) laboratory rates are used to estimate development rates in the field; and 2) data based on a limited number of species are extrapolated to all species in a community. Our equation is based on all the reliable data available in the literature and includes 15 populations belonging to 12 species. For *Oncholaimus oxyuris, Eudiplogaster pararmatus* and *Chromadora nudicapitata* we dispose of data on growth rates in the field and in laboratory conditions (Heip *et al.*, 1978; Smol *et al.*, 1980; Romeyn *et al.*, 1983; Vranken, 1985). These show a good agreement between development rates realized in the field, and those predicted from laboratory experiments. Although very limited, this data set suggests that our first extrapolation may be valid.

The second extrapolation, from the limited set of cultured species to all species in a community, is the most far-reaching assumption in our method. Three species (*Oncholaimus oxyuris, Paracanthonchus caecus* and *Eudiplogaster pararmatus*), possess either low fecundity or slow development rates and may be considered relatively 'conservative' species. The majority of our data are from opportunistic species able to realize high population growth rates (Heip *et al.*, 1985). This of course, reflects a quite 'natural' selection of species by experimental nematologists. Nematode-communities in the field, especially of subtidal sediments, are often dominated by more conservative species. Due to the inclusion of many opportunistic species, our equation may overestimate the productivity of these communities. Unless more dominant species from marine communities are cultured, we cannot assess the importance of this factor. In any case, in our data set the more 'conservative' species did not deviate in a systematic way from the pattern shown by the other species. Body weight may well be a good predictor of the strategy of a particular species.

In a similar approach to the one adapted here, Vranken & Heip (1985) showed a relationship between egg weight and embryonic development time at 20° C. This relationship predicted the embryonic development time of *Sabatieria vulgaris* from the sluice dock of Ostend (Belgium), which was not included in the data-set, exactly (prediction : 9.87 d, experimental :

9.92 d). Unfortunately we were not able to maintain the cultures long enough to determine generation times.

Our regression equation is a new tool to estimate nematode production indirectly, requiring only knowledge of the biomass spectrum of the nematode community in the field and of the annual temperature regime of the habitat. Other methods to determine productivity of field populations, indirectly have been reviewed by Heip *et al.*, (1985).

In our opinion, the use of a single P/B value for nematodes, and a fortiori for the meiofauna as a whole is invalid. Nematode productivity, especially that of members of 'Aufwuchs' communities may be much higher than previously thought. Nematodes are a significant component in the energy flow in shallow-water marine ecosystems.

ACKNOWLEDGEMENTS

Table 1. List of species used to calculate the relationship of T_{min} versus temperature and body weight; data coded 1 are compiled by Heip *et al.* (1985); others, labeled 2 are from Vranken & Heip (in press).

Species	Reference
Monhystera denticulata	1
Monhystera parva	2
Monhystera disjuncta	1,2
Diplolaimella spec. 1	2
Diplolaimelloides bruciei	1
Theristus pertenuis	1
Chromadora nudicapitata	1,2
Neochromadora poecilosomoides	Vranken, 1985
Paracanthonchus caecus	2
Chromadorita tenuis	Jensen, 1983
Eudiplogaster pararmatus	1
Oncholaimus oxyuris	1

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THIS PAPER NOT TO BE CITED WITHOUT PRIOR REFERENCE TO THE
AUTHORS.

MEIOFAUNAL GRAZING ON BACTERIA: A PRELIMINARY REPORT. 3136

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ABSTRACT.

In situ measurements of meiofauna grazing rates on
bacteria were made. The bacteria were labeled at the start
of the experiment, and the label in bacteria, nematodes,
copepods, and polychaetes after different incubation times
was determined. Uptake dynamics of the radiolabel in the
bacteria was highly non-linear. After approximately one
hour the bacteria had reached maximum labeling.
Accumulation in the meiofauna was linear in time. Uptake
rates were estimated from the slope of the accumulation
line.
Even assuming that the accumulation of label in the
meiofauna corresponds to the somatic production only, and
that grazing is 12.5 times higher than this value, the
nematodes and copepods take only a minor fraction of the
bacterial standing stock per day (0.5 - 3 %). Grazing by
polychaetes is much more important. It is concluded that
meiofauna is not able to control the bacterial populations
in the sediments.

INTRODUCTION.

It is well known that meiofauna populations feed to a
large extent on bacteria in the sediments. Several
nematode species can be succesfully cultivated on bacteria
(review in Heip et al. 1985), and the same is true for the
other dominant group in the meiofauna, the harpacticoids
(Rieper, 1978). This grazing activity may have important
effects on the bacterial dynamics. Pamatmat & Findlay
(1983) showed that nematodes prolong the lag phase of
bacterial growth, and have a stimulatory effect on energy
flow when the bacteria reach steady state. Stimulation may
follow from bioturbation (Tietjen, 1980), addition of
nutrients and mucus production (Riemann & Schrage, 1978).
Only in a few studies has the grazing rate of
meiofauna been estimated. In the laboratory, ingestion
rates have been measured for some nematodes (Tietjen, 1980;
Admiraal et al. 1983) and copepods (Rieper, 1978). In
situ estimates were made by Montagna (1984).
Montagna (1984) used the model of Daro (1978) to
estimate in situ ingestion rates. Radiolabel is added at
the start of the expriment, and the model assumes linear
label accumulation in the bacteria, and hyperbolic
accumulation in the meiofauna. However, as is partially

shown by Montagna's (1984) results, the applicability of this model is questionable, especially with respect to the linearity of the radiolabel accumulation in the bacteria.

One of the aims of the present work was to delineate the most relevant time scales and physiological parameters to allow an estimation of grazing rates. Our intention was to use the Daro (1978) model. Therefore we added unlabeled glucose to dilute the radiolabel, so that it would be accumulated in a linear way into the bacteria.

We also performed a 'concentration experiment': as we anticipated a too low abundance of bacterivorous meiofauna in sandy sediments, we tried to concentrate them in less sediment, so as to achieve higher radioactivity counts in the meiofauna.

MATERIAL AND METHODS.

Replicate meiofauna cores (10 cm inner surface plexi tubes) were taken from one Reineck box core (0.5 x 0.5 m) in four stations during the 'Boulogne - Den Helder' cruise of the RV **Belgica** between 18/6/85 and 28/6/85. The stations are:

702: $51^{\circ} 22.63'$ N, $3^{\circ} 18.68'$ E: a muddy sediment station off the Belgian coast; upper centimeter of sediment is fluid; the station is very eutrophicated.

730: $51^{\circ} 17.47'$ N, $2^{\circ} 31.45'$ E: medium sand station off the Belgian coast (western side).

A04: 52 2.00 N, 3 35.00 E: fine sand station off the Dutch coast.

B15: $51^{\circ} 58.00'$ N, $3^{\circ} 55.00'$ E: a station off the Dutch coast, similar to A04.

701: $51^{\circ} 22.63'$ N, $3^{\circ} 9.25'$ E: a station near station 702, and similar to it; here only one additional experiment was conducted.

At stations A04, B15 and 702, we performed our experiments in the following way. The upper two centimeters of sediment from 5 replicate cores were transferred into separate 60 ml plastic vials. To these vials approximately 10 µCi 14C-glucose (270 mCi/mmol) and 50 µl 'cold' glucose (1g/l) were added. After incubation at sea water temperature (14 C) during 0 h (at point 702: 0.5 h), 1 h, 2 h, 3 h and 4 h one replicate was fixed with formaline (final concentration: 4 %). Two 1 ml subsamples were taken immediately upon fixation: one for the enumeration of the bacteria, and one for radioactivity counts in the bacteria. The latter subsample was filtered on a 0.1 µm millipore filter and washed with sterile sea water. The filter was preserved for determination of the radioactivity. (The methods and results of the work with the bacteria will not be detailed here).

At station 730 the upper 10 cm of sediment were transferred into a 500 ml beaker, and decanted 4 times with filtered seawater. A 7 % MgCl2 solution in sea water was then added to the sediment, the animals were anaesthesized during 5 min, and decanted 4 times afterwards with filtered

seawater.   The animals on the 38 µm sieve were transferred
into a 60 ml plastic vial, 5 ml of sediment was added, and
the experiments were treated in the same way as described
above.

      Upon   return to the laboratory, the meiofauna was
extracted from the sediment. Sand sediments were decanted
10 times with filtered sea water on a 38 µm sieve.   Muddy
sediments were centrifuged with LUDOX (Heip et al., 1985).
All animals were then picked out under the dissecting
microscope, washed two times in filtered sea water, and
transferred into a droplet of distilled water in a
scintillation vial. The material was digested overnight in
1 ml of LUMASOLVE (Lumac); as scintillation liquid 10 ml of
LIPOLUMA (Lumac) was added. Scintillation was counted in a
Beckman liquid scintillation counter.

      A laboratory experiment was conducted with the
nematode Monhystera disjuncta. This nematode was cultured
according to the methods described by Vranken et al.
(1985).   Bacteria of the strain ISC2 (belonging to the
Alteromonas   haloplanktis   group)   were   labeled   with
3H-thymidine (this experiment was primarily set up to
invasigate the possibility of simultaneously measuring
bacterial productivity and meiofauna grazing).

      A cohort of nematodes was grown (see Vranken et al.,
1985).   For each experiment, 100 nematodes of age 8 d were
transferred to a petri-dish containing labeled bacteria: 50
ul of the bacterial suspension had been added in a
ring-shaped excavation in the 0.5 % bacto-agar (Difco).

      Bacteria were grown for 8 h in heart infusion broth,
harvested by centrifugation and resuspended in 25 ml
sterile sea water containing 0.125 g glucose and 50 uCi
3H-thymidine (uCi/mmol).   After incubation in a shaker
for 24 h they were harvested, rinsed with sterile sea
water, and resuspended in 1 ml sterile seawater.   This
yielded final bacterial concentrations of about 5 10^{10} ml^{-1}.
The exact bacterial concentration in each experiment was
determined with a Petroff-Hauser counting chamber.

      The experiments were incubated at 17°C for 0.5 h (2
replicates), 1 h (2 replicates), 2 h, 3 h and 4h (1
replicate each).   Upon termination of the incubation time
the animals were fixed with formaline 4 %, and treated as
in the field experiments.

RESULTS.

      The results concerning the bacteria will not be
detailed here.   However, it is important to note that,
despite the dilution of the radioactive glucose with cold
glucose, the label accumulation in the bacteria is clearly
non-linear over the time scale of our experiments.   A
typical example is shown in Fig. 1a for station 702, where
additional incubation experiments of 6 h and 10 h were
added for the bacterial incorporation. After approximately
1 h a plateau level of accumulation is reached.   The
variations afterwards clearly fall within the range of
measurement error.   Note that we had no value at t = 0 h

for this experiment; we took the mean of the other three values, which were 6700, 6700 and 4800 cpm ml^{-1}). Variations in the label accumulation after 1 h or more of incubation were not correlated with bacterial numbers in the experiments indicating that these variations were due to experimental error, rather than to spatial variations in bacterial densities. Therefore the labeling of the bacteria was calculated as the mean incorporation in the bacteria (cpm ml^{-1}) for all experiments of 1 h or more incubation time, divided by the mean number of bacteria ml. (mean of all the experiments).

An additional experiment at station 701 shows that what we measured is indeed bacterial uptake. Incubations were made of 4 sections of a core: 0-2 cm, 2-4 cm, 4-6 cm and 6-8 cm depth. The incorporation into the bacteria after 4 h as a function of depth, is shown in Fig. 1b. Clearly the bacterial activity declines rapidly with depth in this muddy station.

It is clear that with such non-linear accumulation in the bacteria, one cannot expect the accumulation in the meiofauna to be hyperbolic, as is predicted by Daro's (1978) model. In fact, as is shown by Figs. 2, 3 and 4, accumulation in nematodes, copepods and small polychaetes is more or less linear from 1 h incubation time onwards. The 'concentration experiment' at station 730 yielded high counts per individual, but a very irregular pattern in time for the nematodes. The results were somewhat better, but still difficult to interpret for the copepods. At station B15 significant numbers of interstitial harpacticoids were present (10-75 per experiment). We accomodated for their much smaller body size by counting them as 1/10 of the other copepods.

In Figs. 2, 3 and 4 a straight line is fitted by linear least squares through the points (except for t = 0h) where a line provides a reasonable approximation. It is apparent that these lines intersect the Y-axis at positive values. For the nematodes both slope and intercept are very similar for the two sandy stations B15 and A04. At station 702 the slope is higher, and the intercept lower than at the other stations. For the copepods the slopes are generally similar, but the intercept is puzzelingly high in station B15, where the fit of the linear regression is also poor.

This accumulation pattern is similar to that found in the laboratory experiments with 3H-thymidine labeled bacteria. Accumulation was linear from 0.5 h incubation time onwards, with an intercept well above zero (Fig. 5). Most probably these dynamics result from the uptake of bacteria in the nematode's gut, resulting in a sudden increase in labeling of the nematodes. After the gut has been filled, the rise in labeling corresponds to the uptake of organic material from the food into the body tissue.

4. DISCUSSION.

The results of the experiments are expressed as turnover rates of the bacteria in Table 1. In the first half of this table the actual uptake rates (calculated from the slopes of the label accumulation lines) are expressed. These values are not equal to the grazing rates, as they do not take into account the assimilation efficiencies, nor the respiration. Laboratory experiments with 14C-labeled glucose currently being executed indicate that the slope of the accumulation line is about equal to the rate of stable incorporation, and thus of somatic production.

Table 1.

Station	702	B15	A04
A. Accumulation rates			
Nematodes	$0.30 \ 10^{-3}$	$0.57 \ 10^{-3}$	$0.66 \ 10^{-3}$
Copepods	$2.70 \ 10^{-3}$	$0.33 \ 10^{-3}$	-
Polychaetes	$1.35 \ 10^{-3}$	$2.94 \ 10^{-2}$	$9.89 \ 10^{-2}$
B. 'Potential grazing'			
Nematodes	$0.38 \ 10^{-2}$	$0.71 \ 10^{-2}$	$0.82 \ 10^{-2}$
Copepods	$3.38 \ 10^{-2}$	$0.41 \ 10^{-2}$	-
Polychaetes	$1.69 \ 10^{-2}$	$36.8 \ 10^{-2}$	$124 \quad 10^{0}$

Table 1. Uptake of bacteria by meiofauna and polychaetes, expressed as turnover rates of the bacteria (d^{-1}). A. Uptake as calculated from the slopes of the lines in Figs. 2, 3 and 4. B. Potential grazing rates, i.e. maximum rates assuming that the label accumulation rates are 8 % of the actual number of bacteria ingested (see text for explanation).

With the 3H-tymidine labeled bacteria, the slope of the regression line even underestimates the growth of the nematodes. The slope of the regression in Fig. 5 corresponds to an uptake of $2.7 \ 10^{2}$ bacteria nematode^{-1} h^{-1} or 0.02 µg dwt nematode^{-1} d^{-1}. (the dry weight of a bacterium was determined by weighing as $0.32 \ 10^{-12}$ g). At an age of 8 d, *Monhystera disjuncta* has a dry weight of approximately 0.1 µg. In these monoxenic cultures the nematodes achieve an exponential growth, with growth rate around 0.5 d^{-1} . Thus the measured incroporation rate is only about half of what would be expected for growth alone. This is apparently not the case with the 14C-labeled bacteria. Most probably the thymidine is incorporated into the bacterial DNA, and selectively taken up at a lower rate than other organic substances by the near-adult nematodes, where cell division is very limited.

If we assume that the label incorporation rate equals the production rate, then the grazing rate must be at least an order of magnitude higher. With a production efficiency P/(P + R) of 40 % (Herman et al., 1984), and an Assimilation/Consumption ratio of 20 %, the factor would be 2.5 x 5 = 12.5. Speculative "turnover rates" due to grazing are added in Table 1, using this conversion factor. These values probably represent maximum estimates. They were only calculated for comparison in the case of the small polychaetes. In these animals the dynamics of the label uptake are probably different. As the animals are much larger than the meiofauna, their gut residence time is probably much longer too. In that case the measured accumulation is probably a good approximation of the realized grazing.

It is apparent from this table that the 'strict' meiofauna, without polychaetes, consumes only a minor fraction of the bacterial biomass per day. Polychaetes are about 2 orders of magnitude more important as food consumers. These results are comparable to those obtained by Montagna (1984), who found bacterial turnover rates of $5.76 \; 10^{-3} \; d^{-1}$ due to nematodes, $6.0 \; 10^{-3} \; d^{-1}$ due to copepods, and $0.77 \; d^{-1}$ due to polychaetes (means of his summer and winter values). If the conclusions from these studies can be generalized, meiofauna may be of minor importance as regulators of bacterial activity, at least in a quantitative way. Even qualitative influence, in the sense that meiofauna may influence the composition of the bacterial community, is not very likely. The bacteria outgrow the influence of the meiofauna all too easily. It is not plausible that the majority of the bacteria in our experiments were in a latent phase. The glucose added amounted to 100 ug per experiment. This was incorporated in about 1 h. Estimating the bacterial volume as $0.3 \; \mu m^3$ (Montagna, 1984), the bacterial biomass in one experiment is about $1 - 8$ mg C. The bacteria thus incorporated 0.5 to 4 % of their biomass h^{-1}, i.e. doubling times of 1 d to 8 d. (These estimates are not very accurate, and influenced by the - unnatural - addition of glucose. However, the very rapid reaction of the bacteria to the addition of substratum also indicates that they were in an active state).

Although the meiofauna apparently does not influence the bacterial dynamics to any significant extent, the reverse may still be true. It is known from culture experiments that nematodes require quite high bacterial densities for optimal growth (Schiemer, 1982; Vranken, pers. communication). The limiting factor seems to be the density of the food, not the absolute amount offered, indicating that it requires too much energy for the meiofauna to sample the bacteria when they are scarce. Therefore an important part of the bacterial standing stock in sediments may not be exploitable for the meiofauna.

Bacteria are not homogeneously distributed in the sediments. We hypothesize that only the dense aggregations of bacteria, e.g. on detritus particles, are useful as food to the meiofauna. This would imply that it is well possible that these animals are food limited, while living

amidst an amount of food one order of magnitude higher than their own biomass. Compare it to the fact that Belgium has to import cadmium ores, while the sediments in the North Sea contain (far too) large amounts of it.

Further experiments will be needed to confirm these conclusions. We are currently investigating in the laboratory the uptake dynamics of labeled food by the nematode *Monhystera disjuncta*. As many data on the population dynamics, growth and respiration of this species are available (Vranken et al., in prep.), the label accumulation will be readily comparable to other terms in the energy budget. Field experiments similar in design to these reported here will be performed soon. Special care will be devoted to proper replication in each station. An experiment with a larger sediment volume will be performed to estimate the polychaetes' feeding rate. The values for polychaetes given here are based on 2 - 24 animals, and should be viewed with caution for that reason. However, the counts for the polychaetes are high enough to allow a 10-fold increase in sediment volume with the same amount of label added.

ACKNOWLEDGEMENTS.

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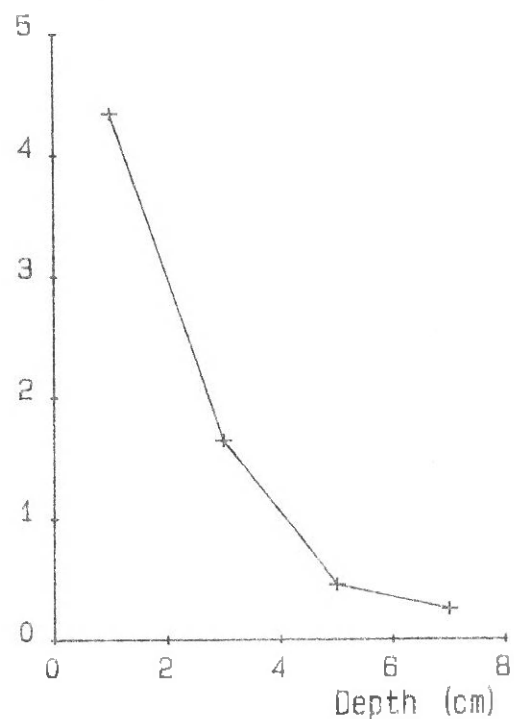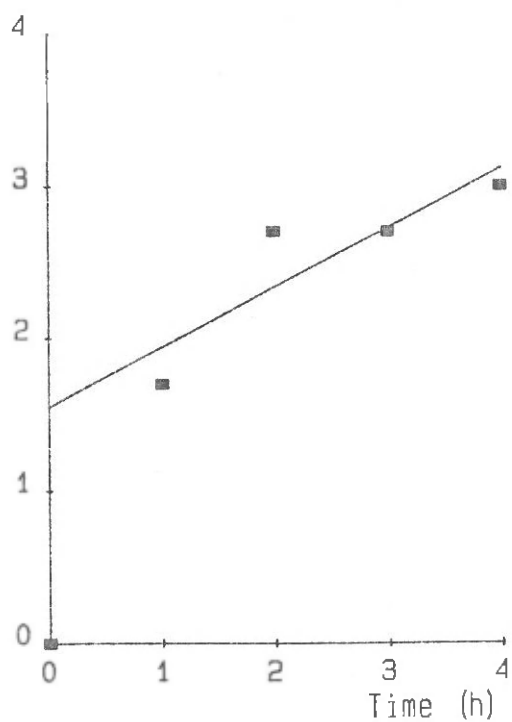   Pellioditis marina). Dis. Aquat. Org. 1: 69-78.

702

cpm/ml (*10^5)
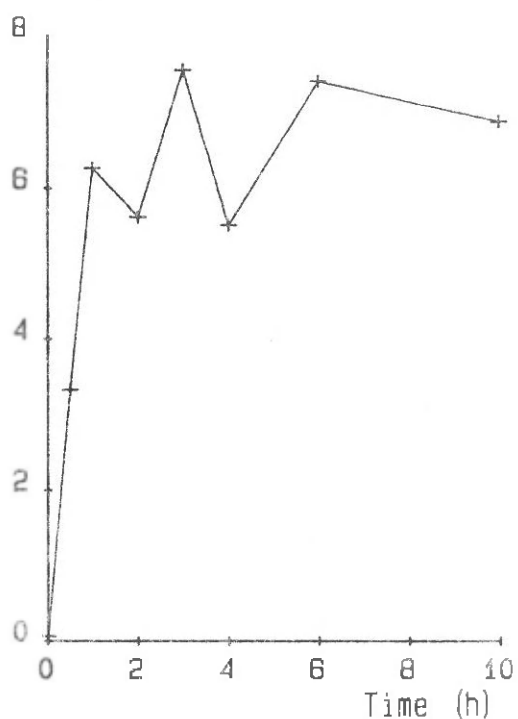
701

cpm/ml (*10^5)

Fig. 1. Accumulation of radiolabel in the sediment bacteria. A. Time course of accumulation in the bacteria at station 702. B. Bacterial accumulation after 4 h incubation as a function of depth in the sediment at station 701.

A04

Bact/nem (*10^4)

702

Bact/nem (*10^4)
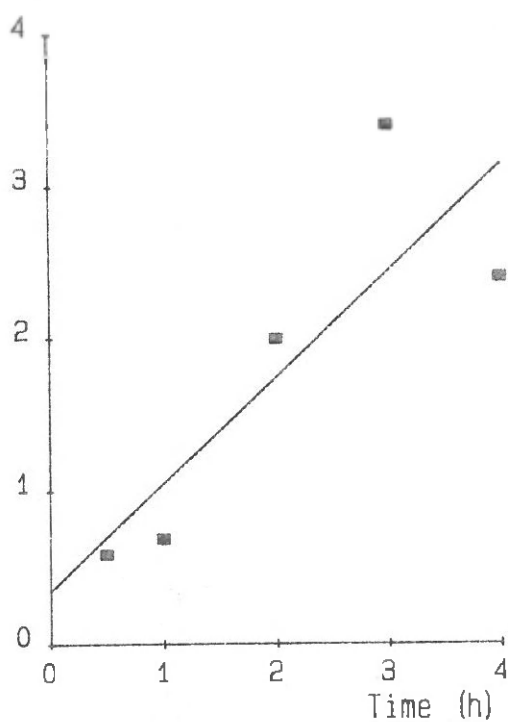
B15

Bact/nem (*10^4)

730

Bact/nem (*10^4)

Time (h)
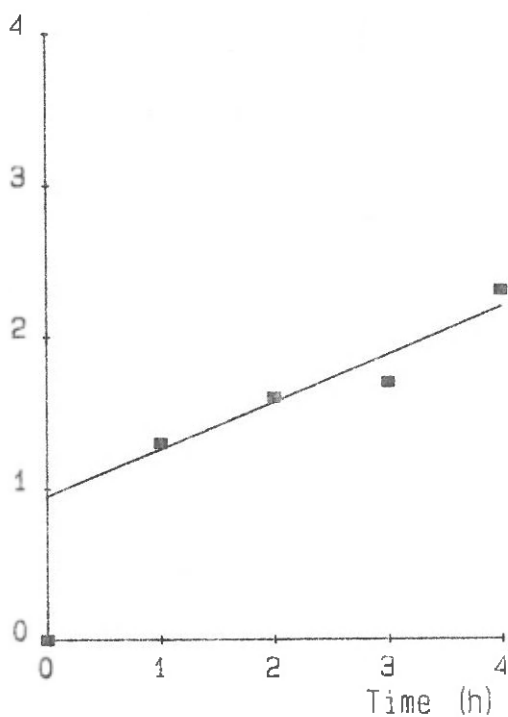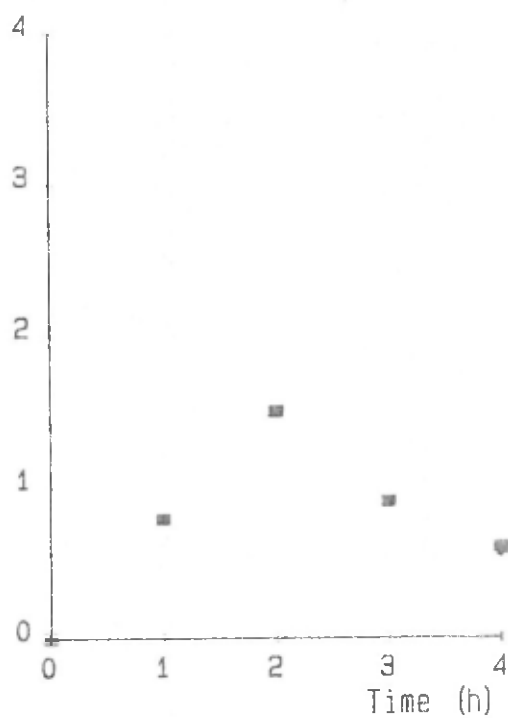
Fig. 2. Accumulation of radiolabel in the nematodes at four
experimental stations. Accumulation is expressed as
no. of bacteria per nematode: cpm per nematode was
divided by cpm per bacterium.

702

Bact/cop (*10^4)
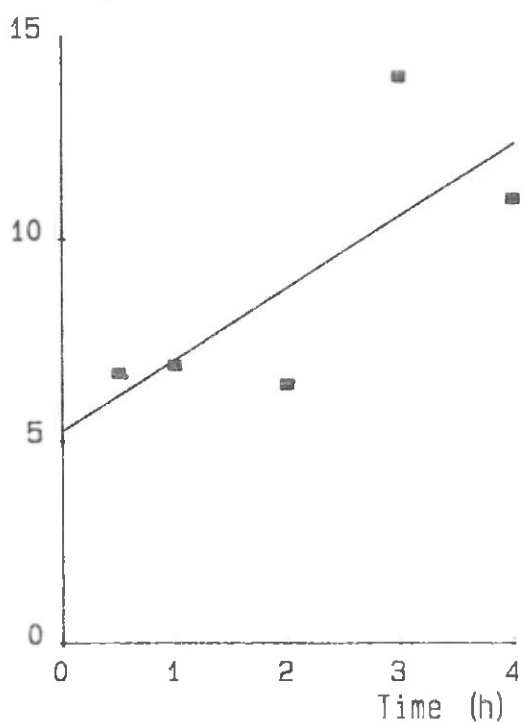
730

Bact/cop (*10^4)
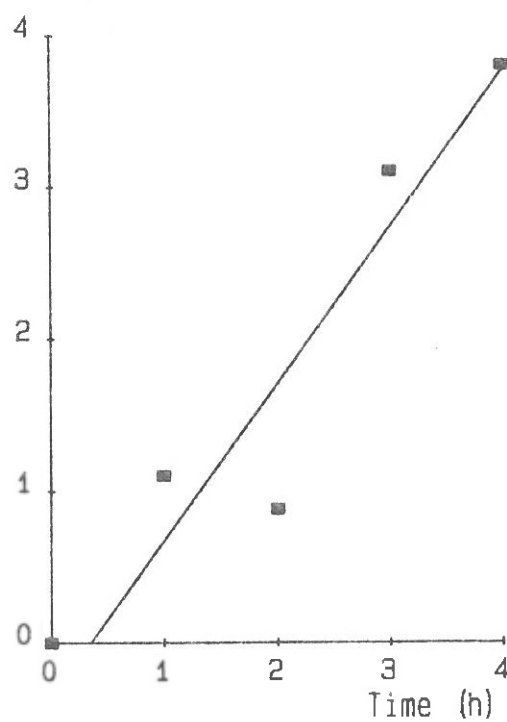
B15

Bact/cop (*10^4)

Fig. 3. Accumulation of radiolabel in the copepods at four
experimental stations. Accumulation is expressed as no.
of bacteria per copepod.

Fig. 4. Accumulation of radiolabel in the polychaetes at four experimental stations. Accumulation is expressed as no. of bacteria per polychaete.

Bact/nem (*10^5)

Fig. 5. Accumulation of radiolabel in the laboratory experiment with 3H-thymidine labeled bacteria. Accumulation is expressed as no. of bacteria per nematode.
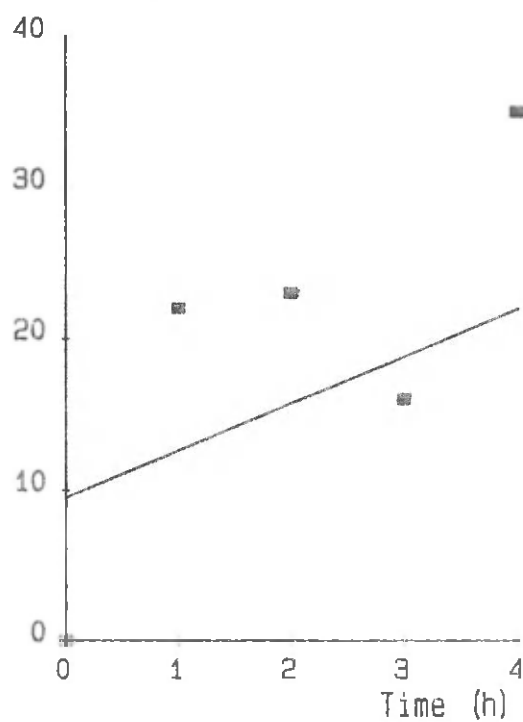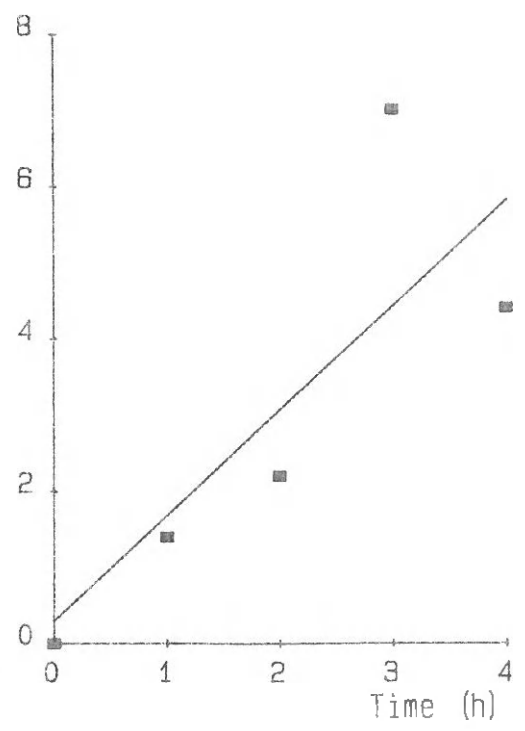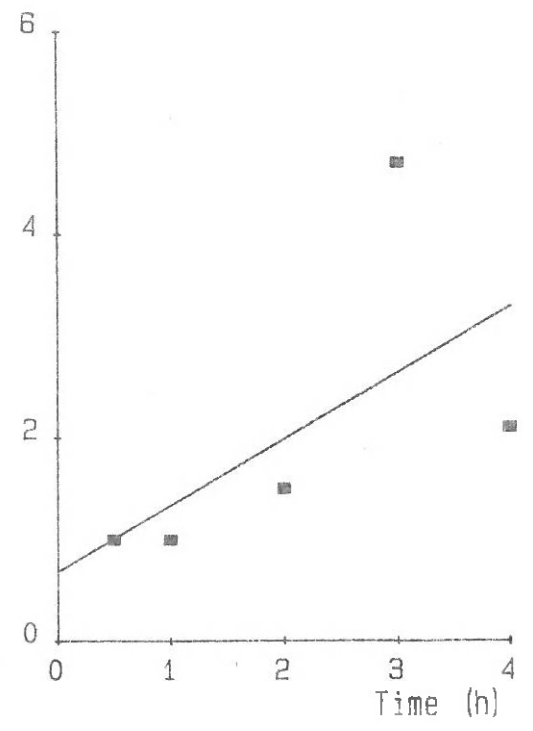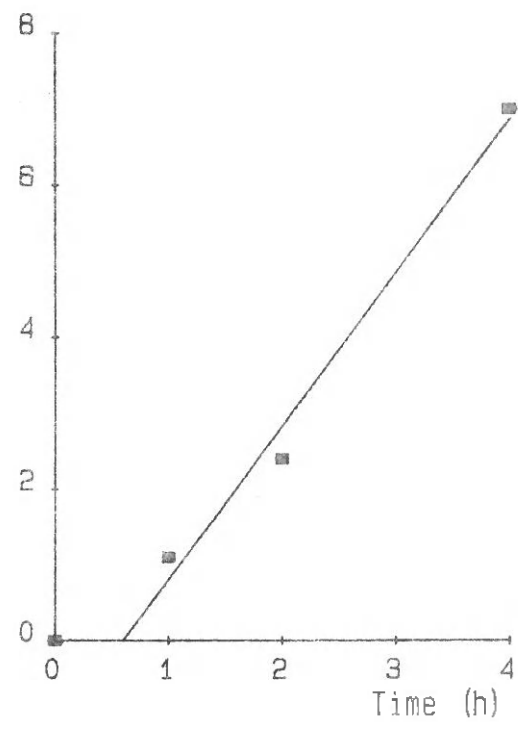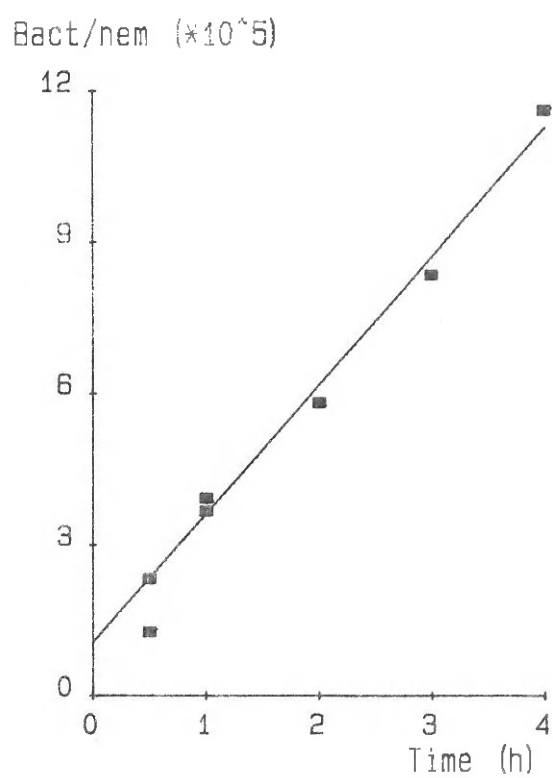
Life history, food consumption and food resource
partitioning in two sympatric gobies *Pomatoschistus minutus*
and *P.lozanoi* in the Belgian coastal waters.

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1. INTRODUCTION

The search for interspecific competition is a
fashionable pursuit for ecologists (Schoener 1983). The
number of studies claiming to demonstrate competition is
influenced by scientific editors, referees and the
behaviour of scientists in general (Connell 1983).
Competition as the main factor that determines the
distribution, abundance and resource use of species in
natural communities is directly related to the Darwinian
theory of evolution (Darwin, 1859).
The impossibility of indefinite coexistence of two or
more species limited by the same resource was first modeled
mathematically by Volterra (1928). The competitive
exclusion principle (Hardin, 1960) states that n species
cannot coexist on fewer than n resources or in fewer than n
niches. The principle is in a sense tautological: if it is
valid we will be unable to demonstrate competition in
coexisting species (Slobodkin 1961), so ecologists may well
be hunting the ghost of competition past (Connell 1980).
Armstrong & McGehee (1980) have shown that systems can
be constructed where n species coexist on $k<n$ resources or
limiting factors. Important aspects of their model are
non-linearity of the functional response and relaxation of
fixed density assumptions. The species can then coexist
because of internally generated cyclic behaviour. This
makes competitive exclusion less probable in ecosystems
with important seasonal cycles. Wiens (1977) presents some
evidence from a variable environment where competition
seems to be temporally sporadic and impotent.
Pomatoschistus species are the most abundant fish in
the Belgian coastal waters and are an important food source
for several commercial demersal fish species, notably *Gadus
morhua* and *Merlangius merlangus* (Redant 1977).
Data on life history, density, biomass and food
consumption of two sympatric gobies of the genus
Pomatoschistus collected in the shallow coastal waters of
the Southern Bight of the North Sea are presented here.
The data are analysed with special emphasis on feeding
ecology and food niche segregation. Field evidence for
present-day competition is examined.

2.   MATERIALS AND METHODS

Approximately monthly samples were obtained from the bycatch of a commercial shrimp trawler "062" operating in the Westdiep-Trapegeer area off Nieuwpoort (Fig 1) from May 1984 through December 1984. During the exceptionally cold months of early 1985 no samples were taken because most fish and shrimp moved to deeper areas out of reach of the small trawlers. In April 1985 fish were obtained from the Spring Survey of the Fisheries Research Institute Ostend with the vessel "Broodwinner". The sampling stations 16, 17, 19, 23, 24 and 91 (Fig 1) are situated in the shrimp fisheries area normally visited by the 062. Gobies from these sampling stations were pooled to yield a sample similar to the 062 samples. In June and August 1985 samples were again available from the 062. In September 1985 fish were obtained from the same sampling stations in another survey with the Fisheries Research Institute (Table 1). Both vessels are beam trawlers. The 062 has two 7 meter beams, the Broodwinner has two 6 meter beams. Both are equiped with standard commercial shrimp nets with an 18 mm stretched mesh in the cod end. Trawl speeds are 2 to 3 knots.

On board all fish are immediately anaesthesized in a Benzocaine (Ethylamino-4-benzoate) solution in sea water to prevent regurgitation of stomach contents. Within 15 minutes after capture the fish are preserved in neutralised formaldehyde 7% final concentration.

At least three months after capture, to allow for shrinkage to stabilize, all gobies are identified and measured to the nearest mm. All lengths are standard lengths, measured from the tip of the mouth to the base of the tail fin. A linear regression analysis was performed on a December subsample of *Pomatoschistus lozanoi* to determine the relationship standard length-total length.

If a sufficient number of fish of the same 5 mm size class of both species was present in the same trawl from nightly samples from the Trapegeer area (51° 07'40'' NB, 02° 30'40''OL to 51° 08'40"NB, 02°34'20"OL), thirty fish from each species were selected at random from that size class for stomach analysis.

On May 17, 1984 fishing was only done at daytime and numbers of *Pomatoschistus minutus* were so low that *P. minutus* and *P. lozanoi* from all trawls were pooled. On July 20, 1984 samples were obtained during the day and in the following night. Because no overlapping size classes were found, *Pomatoschistus minutus* were again pooled from all trawls and *Pomatoschistus lozanoi* of size classes comparable to the August and September samples were taken from a nightly trawl in the Trapegeer area. All other 062 samples are night samples, all Broodwinner samples are daytime samples.

Absolute densities calculated by the Fisheries Research Institute are not yet available, but from relative densities in the different samples, taking hours of trawling into account, and average monthly densities per 1000 m2 from Redant (1978), approximate densities for 1984 can be estimated (Table 1). Efficiency of the net was assumed to be 25% for all size classes. Average biomass per m2 for 1984 was calculated with the Total Ash Free Dry Weight (TW) of the median of every 5 mm size class from length to TW regressions for *Pomatoschistus minutus* and *P.lozanoi* in Table 2. It is assumed that the yearly average does not differ substantially from the average based on May to December monthly averages.

As gobies do not have a functional sphincter at the gastrointestinal junction the food items in the entire gastrointestinal tract, excluding the rectum, are examined under a dissecting microscope. Every food item in the gastrointestinal tract (hereafter refered to as "stomach") is identified, if possible and reasonably practical to species level. Hydroidea, phytal material and detritus are not regarded as prey. All food items, except calanoids and harpacticoids are measured to the nearest .1 mm using a drawing mirror and curvimeter. If prey are incomplete , loose parts that have a linear relationship with total length e.g. a carapax or a telson are measured, so that conversion to Ash Free Dry Weights (AFDW) is possible. If a prey item is partly in the rectum a subjective estimate is made of what proportion remains in the "stomach", and only that proportion is counted for percentage AFDW calculations. It is counted as a full prey for numerical percentage calculations.

Length to AFDW relationships of prey are derived from various sources (Table 2). For *Pomatoschistus minutus*, *P.lozanoi* and bivalve siphons relationships are determined from our own samples (Figs 2,3,4 and Table 2). Undigested bivalve siphons are obtained from the esophagus of fish. A subjective technique is used to estimate the AFDW of the tentacle crowns of *Lanice conchilega*. If these are present their volume is compared "de visu" to a volume of undigested tentacles from the esophagus of a fish of the same size class and sampling date. Comparison of the AFDW of some esophagus samples with their estimated volume shows the "de visu" method to be reasonably accurate if crude designations like double, half, a third or a fourth are used. Luckily the epidermis of Lanice seems to resist digestion rather well, therefore bulk of the tentacles (and body size for entire animals) in the stomach may resemble original bulk quite closely. Errant polychaetes are not always eaten whole so using body width as a measure of body length is dubious. We try to make an educated guess of the original size of the fragment by estimating the number of segments, measuring the AFDW of the fragment as present in the stomach, measuring jaws or other undigested parts, etc... Still, polychaete importance is probably underestimated by this technique.

Other soft bodied animals like oligochaeta and nemertineans, though not rare in the ecosystem, were never found in the stomachs. This may be an artifact. Nematode consumption is certainly underestimated because of rapid digestion (Hofsten et al. 1983). Over 90 % of the nematodes encountered in the stomachs are *Mesacanthion spec.* a 2-3 mm long Enoplid. This does not reflect its predominance in the ecosystem, but the fact that its cuticula has three layers probably slows digestion (M.Vincx, pers.comm.).

For some food categories we assigned an AFDW independent of length (Table 2). Most of these food items, except the chaetognaths, are very small and the influence of even a very wrong AFDW will be marginal. For the chaetognath *Sagitta* a length to Dry Weight regression is stated in Feigenbaum (1979). Unfortunately he does not state the relationship between the size of the undigestable grasping spines and the total body length. We chose an intermediate value from his regression and, although we measured different sizes of grasping spines, allocated .500 mg AFDW to every chaetognath. This corresponds to a *Sagitta* of about 15 mm.

Another problem is the possibility that food items in the stomachs of prey species are mistaken for food items taken by the fish. In crustaceans the carapax is usually so well preserved that food remains stay contained. In chaetognaths on the contrary the body wall is rapidly digested and prey items are scattered in between those of the fish. No attempt is made to distinguish between e.g. calanoids consumed by the fish and those primarily consumed by the chaetognaths. Conceivably a correction for this bias is possible if the average number of calanoids per chaetognath is known.

Stomach analysis data should always give a measure of the relative importance of different prey items and a measure of the bulk of the food present in the stomach. Results must allow comparison with other areas, other species or other seasons, therefore they must be objective and expressed in convertible units (Berg 1979). The Points method and Frequency of Occurrence method, still widely used, must be considered obsolete. Percentage AFDW and Numerical Percentage are calculated in this study. Bulk of food present in the stomach was measured by drying the examined stomach contents at $120°C$ for 2 hours. It is expressed as Fullness Index (FI = Dry Weight of stomach content * 100 / Total Ash Free Dry Weight).

Individual Total Weight (TW) is the sum of Somatic Weight (SW) and Gonad Weight (subsequently GW). Somatic Weight is determined by drying individual fish for 5 days at $60°C$ and subsequently incinerating the dried remains at $550°C$ for 2 hours, the difference between Dry Weight and Ash Weight is the Ash Free Dry Weight. Ash Free Dry Weights can be converted to caloric content. The complete digestive tube is added to the fish after stomach analysis and before drying so that it is included in SW and TW measurements.

To determine the reproductive state of the fish ovaria and testes are examined and staged according to an adaptation of the classification devised by Miller (1961) for *Gobius paganellus* . From all stage II and riper ovaries a few eggs are isolated at the anterior margin at about half length of the ovary for measurement of egg diameter. Gonad Weights are determined in a similar way as SW but gonads are dried at 120°C for 2 hours and then incinerated. Of stage I gonads only Dry Weight is determined. Because of their extremely low weight there will only be the slightest overestimate of GW, using DW instead of AFDW. Gonad maturation is expressed as Gonadosomatic Index (GSI): GW * 100 / TW. Data on gonad maturation are not reported extensively here because the selection of certain size classes for stomach analysis was given priority. For calculating average GSI of a population fish should be selected at random, not stratified for size.

All sizes are in mm, all weights in mg unless otherwise stated.

Niche overlap is measured by calculating Renkonen's index (Renkonen 1938).

3. RESULTS

3.1. Identification.

Of 12725 gobies of the *Pomatoschistus minutus* complex
(Webb 1980) examined, 9398 (74%) are *Pomatoschistus
minutus*, 3327 (26%) are *Pomatoschistus lozanoi*. Other
Pomatoschistus species encountered are *Pomatoschistus
pictus* and *Pomatoschistus microps*. These are very rare
(less than 20 each) and will not be dealt with here.
    With some experience the identification of gobies of
the *P.minutus* complex is not difficult. Webb (1980) cites
three important differences: the number of vertical c-rows
of papillae on the jaw (higher in Pomatoschistus minutus,
but with an overlap in number), the fact that the 2nd and
4th c-rows descend beyond the horizontal d-line in
Pomatoschistus lozanoi and the difference in vertebral
number (though also with an overlap). We have found many
gobies with the 4th c-row continuing under the d that were
clearly *Pomatoschistus minutus* according to a variety of
other criteria: size, pigmentation, number of vertical rows
etc.... With the same criteria we found many
Pomatoschistus lozanoi that do not have a 4th c-row
continuing below d.

3.2. Life history.

    Length-frequency distribution of the 1984 and 1985
samples are shown in Fig 5 for *Pomatoschistus minutus* and
in Fig 6 for *P.lozanoi*. It must be stressed that sample
sizes depicted are not directly related to densities:
different numbers of trawls of different duration are
pooled per sampling date. To reduce this bias
length-frequency is converted to length-percentage
frequency distribution (Figs 7 and 8). Thirty fish is
taken to be the minimal size for a length-percentage
frequency distribution to be meaningful.
    In spring *Pomatoschistus minutus* 1+ adults are ready
to spawn as demonstrated by nuptial colours and high GSI.
By June they become extremely rare. Most *P.minutus* have
probably spawned by then and are dead or dying. In July
the 0+ juveniles recruit into the net, having attained a
body length of about 30 mm. Growth is extremely rapid as
by the end of September a sizeable proportion of the
population attains adult length, GSI is still very low.
Recruitment continues through October as seen by the slight
decrease of average length in an increasing population of
rapid growers (Table 3). In 1985 there is a similar
pattern, with the recruits of the fall of 1984 spawning and
dying in spring and early summer.
    Pomatoschistus lozanoi is much more abundant in late
spring than *P.minutus*. The population consists of 1+
adults with little or no nuptial colouring though GSI is
already quite high. There seems to be virtually no somatic
growth (constant mean length with no change in density) but
gonads are developing. Adult size averages less than in
Pomatoschistus minutus. By July nuptial colouring is very
pronounced and GSI peaks.

In August the first juveniles recruit into the net, a sizeable population of 1+ adults with high GSI is still present. By the end of September virtually all 1+ have disappeared and population buildup begins. A large proportion of the *Pomatoschistus lozanoi* population seems to pass the winter at subadult length. The 1985 data confirm this general pattern.

The relationship found for conversion of standard length to total length is:

Total length = 1.089 + 1.157 * Standard length.

(n=96, r2=.99) (Fig 9).

3.3. Density, biomass and food consumption.

Estimated yearly average density is 80 *Pomatoschistus minutus* per 1000 m2 and 40 *P.lozanoi* per 1000 m2 (Table 1). This represents 33.19 mg and 8.45 mg AFDW per meter square, for *Pomatoschistus minutus* and *P.lozanoi* respectively. With an 8.6% of body weight daily consumption, calculated by Andersen (1984) for *Pomatoschistus microps*, this amounts to 969 mg AFDW /m2/yr for *Pomatoschistus minutus* and 247 mg AFDW /m2/yr for *P.lozanoi*.

3.4. The food of *Pomatoschistus minutus*.

Data from the stomach analyses of 359 *P.minutus* are summarized in Table 4. A survey of food categories that provide at least 10% of AFDW for a certain size class at any one time may give some insight into the feeding of *Pomatoschistus minutus*. Siphons of bivalves are responsible for the bulk of energy derived from molluscs, small spat is eaten infrequently and individual biomass is low. Errant polychaetes are only important if a large *Nephtys* or *Sthenelais boa* is eaten, this is rather infrequent. Sedentary polychaetes, mostly *Lanice*, and some *Pectinaria* are very important, except in September. This is probably not a seasonal effect of some sort but an artifact of the trawl just missing the fields of *Lanice*: in a later trawl of the same night only a few meters deeper down on the slope of the Trapegeer most *P.minutus* did eat *Lanice* (Hamerlynck, unpublished data). Calanoid copepods are important in autumn, especially for the smaller size classes. There is a huge peak in the abundance of *Temora longicornis* at that time (Polk et al. 1975). Caridean decapods are also very important. In spring nearly 50% of caridean bulk is provided by *Pontophilus trispinosus*, later in the year nearly 100% by *Crangon crangon*. It is not clear why gobies predate on the first recruits of *Pontophilus* that appear in spring (Labat 1984) and not on the second batch that arrives at the end of the summer. Perhaps it is not a seasonal effect at all: the May sample includes fish from different areas than the later samples. Mysids are frequently consumed and have high individual AFDW, most are *Schistomysis spec.*.

In gammaridean amphipods *Bathyporeia* species are only
found in May, the same remark may apply as in *Pontophilus*.
Pariambus typicus is very important in July and August,
this seems to be an effect of the presence of larger
animals: mean AFDW of consumed *Pariambus* is .031 mg in
August (n=277) and .015 mg in September (n=1584). No fish
other than *Pomatoschistus* species were found in goby
stomachs. Partial digestion makes identification very
difficult. However, the life history data suggest that
predation is on juvenile *P.lozanoi* in September, and that
cannibalism (in May) is less important. Table 6 lists all
food items found in *Pomatoschistus minutus* and *P.lozanoi*.

3.5. The food of *Pomatoschistus lozanoi*.

     Data from the stomach analyses of 255 *Pomatoschistus
lozanoi* are summarized in Table 5 . When a similar survey
of the data as in *P.minutus* is done the importance of
Lanice in August, and its near absence in the other months
is striking. The importance of calanoids is rather similar
to that in *P.minutus* in September, but *P.lozanoi* is also
predating strongly on the spring peak. Mysids are almost
always the most important food, except in May when
gammaridean amphipods are more prominent.As in *P.minutus*
predation on the juveniles of the other species is more
important than cannibalism.

3.6. Changes in the predation with fish size.

     There are obvious qualitative and quantitative changes
in the importance of different food categories with
increasing fish length for *Pomatoschistus minutus* (Fig 10).
The range of lengths of *P.lozanoi* investigated does not
allow definite conclusions to be drawn, but data suggest
that changes in the predation with increased length are
less pronounced than in *P.minutus* (Fig 11).
     Average AFDW of ingested prey does not differ
substantially in *Pomatoschistus minutus* from the 35-39 mm
to the 50-55 mm size class (Fig 12), despite a 3.2 fold
increase in body weight of the median fish between those
size classes (Fig 13). In the larger size classes 55-59
and 60-64 mm there is a strong increase of average prey
size, but still not proportional to the weight increase of
the predator. When we look into the different prey
categories we see that the decrease in number of calanoids
per stomach (Fig 14) with increasing fish length is
probably an important factor in the increase of average
prey size. In harpacticoids (Fig 14) the pattern is rather
different with a first substantial reduction to around 1
per stomach at 45-49 mm and a second drop to about one in
every 5 stomachs at 55-59 mm. Increase in mean bivalve
siphon size is marginal (Fig 15) so the very substantial
increase (Fig 10) of the importance of siphons to the
energy budget can be attributed almost exclusively to an
increase in numbers per stomach. No consistent pattern can
be seen in the average size of Crangon crangon and
Schistomysis spec. (Fig 15).

In gammaridean amphipods there is an increase in average prey size (Fig 15). There is virtually no change in average weight of Microprotopus maculatus, a small ampipod consumed in very large numbers by all Pomatoschistus size classes examined (Fig 16). Thus the increase in average gammaridean AFDW is largely due to increased capture of a few Gammarus and Atylus gammarideans with a relatively high AFDW (Fig 16). Only 4 *Pomatoschistus lozanoi* were eaten by *P.minutus*, the one eaten by a fish from the 60-64 mm has a very high AFDW and thus contributes significantly to the average increase of prey AFDW. The same can be said of the single *Liocarcinus* and *Nephtys* that are responsible for nearly all brachyuran and polychaete AFDW (Fig 10).

3.7. Food niche segregation.

Niche overlap is measured by: $Cxy = 1 - 1/2 \left(\sum^{i} |pxi - pyi| \right)$ (Renkonen 1938).

Its calculation for similar size classes of both fish per sampling date shows quite strong overlap (.44 to .72) in the food niche of *Pomatoschistus minutus* and *Pomatoschistus lozanoi* (Table 6). It is most pronounced in the smaller size classes (35-39 mm) and is stronger in October than in September. The consumption of some 17% (AFDW) by *P.minutus* of *Lanice conchilega* in October does not compensate for the large overlap of *Schistomysis spiritus* and *Temora longicornis* consumption.

Niche breadth indices were not calculated, because standardization is not without problems (Colwell & Futuyma 1971). A simple count of food categories found exclusively in *Pomatoschistus minutus* stomachs (n=44), food categories common to both species (n=42) and those found exclusively in *P.lozanoi* stomachs (n=6) does indicate that *P.minutus* is more of a generalist, and that *P.lozanoi* is more of a specialist (Table 6).

To demonstrate the nature of the difference in food niche all prey items were lumped into three categories: benthic, epibenthic and pelagic (Table 8). Calculations of cumulated percentage AFDW of these categories per sampling date are shown in Table 9. It is clear that the *Pomatoschistus lozanoi* population extracts a more substantial proportion of its energy from the pelagic system in comparison to the *P.minutus* population in all but one month. The result of August seems completely aberrant.

4. DISCUSSION

4.1. Identification.

In the laboratory viable larvae have been produced by hybridisation of Pomatoschistus minutus with P.lozanoi (Fonds 1973) and individuals have been found in nature with morphological and biochemical characteristics intermediate between those of Pomatoschistus minutus and P.lozanoi (Swedmark 1968, Fonds 1973, Wallis & Beardmore 1980, Webb 1980). Individuals with intermediate morphological characteristics are extremely rare in our study. This may be explained in part by our criteria for deciding in favour of one species or the other. Our rule is: if the 2nd vertical c-row continues under d the fish is a *Pomatoschistus lozanoi*, if it does not, it is a *P.minutus*.

4.2. Life history.

Our life history data are similar to the results of other studies that distinguish Pomatoschistus minutus from P.lozanoi (Fonds 1973, Wallis & Beardmore 1984, Claridge et al. 1985). The precise mechanisms of reproductive isolation and of temporal segregation of reproduction are still unknown. There may be direct competition for nest sites, a resource that can be monopolisized. We have still very few data on gonad ripening but it seems that in early spring *Pomatoschistus minutus* is ready to spawn, while an important part of the *P.lozanoi* population has to channel some of its energy into somatic growth before ripening of the gonads starts.

4.2. Density, biomass and food consumption.

Admittedly our estimated densities may be wrong. If they are reasonably accurate the consumption of nearly 1 g AFDW per m2 per year for *Pomatoschistus minutus* and of .25 g AFDW per m2 per year for *P.lozanoi* is very high for animals of such low biomass. Pihl (1985) calculated consumption of mobile epifauna in a shallow bay in western Sweden. For *Pomatoschistus minutus* he found a consumption of 1 g AFDW per m2 per year for a production of .24 g per m2 per year. The finding of exactly the same consumption figure does not mean it is correct. Their study area is rather different from ours: it is a shallow bay of less than one meter depth. It is only visited by *P.minutus* juveniles in summer and autumn (Pihl & Rosenberg 1982). Evans (1983, 1984) working in a similar bay estimates consumption by *P.minutus* to be .4 g AFDW per m2 per year. He uses ingestion of 3% of body weight per day from Healey (1972) for his calculations. This just shows the uncertainty of a figure like 1 g AFDW per m2 per year, it can easily be 50 % higher or lower, depending on the parameters chosen for calculation.

The impact of this predation on the fauna is also difficult to assess. Experiments with inclusion (Berge & Hesthagen 1981) and exclusion (Berge & Valderhaug 1983) of *P.microps* suggest that predation impact is small: only in the exclusion experiments a slight increase in ostracods and amhipods was seen. In most other experimental studies epifauna is claimed to regulate infauna (reviewed by Peterson 1979).

Most authors agree that the Atlantic-Mediterranean gobies of the genus *Pomatoschistus* are not food limited but predator controlled (Healey 1971, Evans 1983, Miller 1984).

4.3. Foraging strategy.

An ontogenetic shift in prey selection is one of the mechanisms through which a reduction of intraspecific competition may occur: if small fish of the same species eat other prey than large fish they occupy different niches (Grossman et al. 1980). An alternative hypothesis is that as fish size increases larger prey become available and fish shift their effort towards more profitable (increased energy gain per unit handling time) prey (reviewed in Pyke et al. 1977). Many authors have tried to correlate mouth size or gape to prey size (reviewed by Gibson 1982).

Villiers (1980) shows in the Meditteranean goby *Deltentosteus quadrimaculatus* that with increasing fish size the relative importance of different food types changes. After settlement on the bottom the 20-25 mm gobies eat a mixed diet of meiofauna and small macrofauna. Over 25 mm they become exclusive macrofauna feeders.

Our data suggest that mouth size is not an important factor in these changes: 35-39 mm gobies do swallow prey items almost as large as the largest ones found in the 60-64 mm size class. In most studies on mouth size effects no attempt is made to distinguish effects of increasing mouth size from other length-increase related effects. The logic of an upper limit to prey size passing through a certain mouth size may be irrelevant in nature.

No significant trend is found in most mean prey sizes within a given food category, so *Pomatoschistus minutus* is a "switcher" type predator. The same phenomenon is observed by Grossman (1980) in *Lepidogobius lepidus*. What seems to increase most with increasing size is the chance of capturing a large food item. What may cause this difference?

Most authors claim that gobies are visual predators that lie motionless on the bottom until a prey item is spotted and caught after a short swimming bout (reviewed by Gibson 1982). Thus we may assume encounter rate between predator and prey to be independent of predator size. When the length of the chase increases the profitability of a prey of a certain energy content decreases (MacArthur & Pianka 1966). Length of chase (equivalent to handling time) depends on the relative swimming speeds of predator and prey, and swimming speeds are strongly correlated with size. The decreasing importance of small items like harpacticoids and calanoids in larger gobies may be related to their decreasing profitability to large predators. Only if they are very close to the predator the energy gain expected by their capture exceeds the investment required as was demonstrated for *Spinachia spinachia* (Kislalioglu & Gibson 1976). This is the energy maximizing strategy (Schoener 1971).

An alternative hypothesis is time minimizer strategy. Gobies rely to a large extent on camouflage to elude predators. When attacking a prey they are more conspicious to visually oriented predators. Long chases of large prey items by small gobies may thus be selected against. Minimizing time spent chasing prey may increase individual fitness to a larger extent than maximizing energy gain, as gobies are probably predator controlled rather than food limited.

Intraspecific competition may also contribute to the lower probability for capture of large prey by small gobies. As a big goby swims faster it will reach a food item sooner than a small goby if it is spotted from the same distance at the same time.

There are still a lot of flaws in our thinking about this subject. Light intensities at 7 meter depth in the very turbid coastal waters of the North Sea must be virtually zero. All observations on goby foraging are necessarily done in aquaria or pools with excellent visibility. Preliminary investigations into cyclic feeding activity suggest most foraging activity to be concentrated at night (Hamerlynck & Catrysse, unpublished data). Visual clues may therefore be less important in foraging than claimed by most authors. Feeding rythms may be related to prey activity and/or activity of predators on gobies.

Besides circadian rythms there may be tidal rythms. Healey (1971) suggests that in an estuarine environment juvenile *Pomatoschistus minutus* feed at high tide and adults at falling tide. There may be considerable variation from one study area to another (Gibson & Hesthagen 1975).

Another flaw is the importance of bivalve siphons and sedentary polychaetes in the diet. This is rather surprising for a feeding strategy of pouncing on moving prey.

4.4. Food niche segregation.

The result of niche overlap calculation seems in accordance with the strong food niche overlap (.80 to .86) found between *Pomatoschistus minutus* and *P.microps* in October and November in Gulmarsvik, a shallow bay on the Swedish west coast (Pihl 1985).

This does not mean that there is competition: during the autumn peak of the zooplancton food may not be a limiting resource. Renkonen's index is simple to calculate, but that does not reduce the difficulties of interpretation. On the contrary its use is strongly critisized because it ignores variation in resource state abundance (Hurlbert 1978). It is also inappropriate in that it is not expressed in units that are relevant for a discussion of competition, resource relationships and the like. It is just an index and certainly no proof of competition as mistakingly asserted by Thorman (1982). The calculation of a more sophisticated index that takes frequency of interspecific encounter and directionality into account is not feasible yet because we lack data on prey abundances.

The suggestion made by Hamerlynck et al. (1985) that the *Pomatoschistus lozanoi* and *P.minutus* occupy more or less separate food niches in the coastal waters of the Southern Bight is confirmed by this study. Fonds (1973) did not find a difference in frequency of occurrence of different prey items between *P.minutus* and *P.lozanoi* from the North Sea, however he did find that in the Waddenzee stomachs of *P.minutus* contained more often harpacticoid copepods and polychaetes, and that stomachs of *P.lozanoi* contained more often mysids. Frequency of occurrence is a very crude measure of prey importance because it is not related to a measure that can be converted into energy content. Thus one harpacticoid in every stomach is equivalent to 1000 harpacticoids in every stomach. A more sophisticated analysis might have given the same results for the North Sea as found in our study. Claridge et al. (1985) find no differences in percentage weight composition between *Pomatoschistus minutus* and *P.lozanoi* from the inner Severn. The diet of both species consisted almost entirely of *Gammarus salinus* and *Neomysis integer* in all seasons. Possibly prey diversity so high up the estuary is very low and little or no bivalves and polychaetes are available. The presence of only 20% *P.minutus* suggests that the area is less favourable for this species than the coastal waters of the North Sea. Another bias may be introduced by their sampling method: fish are collected from the intake screens of power stations. Perhaps bottom dwelling fish are only rarely caught and only those *P.minutus* foraging in the water column are sampled.

Support to the hypothesis (Hamerlynck et al. 1985) that differences in the pattern of the sensory papillae are of adaptive significance to the observed feeding patterns is given by Gibson & Ezzi (1981): *Pomatoschistus norvegicus* which has a pattern similar to *P.lozanoi* is also feeding primarily on mysids and calanoids.

4.5. Spatial segregation.

Food niche segregation of the kind described here implies vertical spatial segregation between the species with *Pomatoschistus minutus* confined to the bottom and *P.lozanoi* cruising at some distance from the bottom in the water column.

Though not analysed in detail here there is a strong indication of some horizontal spatial segregation between the species. In spite of large numbers of fish collected and considerable size overlap of both populations (Figs 5 and 6) only one overlapping size class is found in October and none are found in December in any single trawl. The fact that this segregation seems stronger when population size increases may be an effect of competition. An alternative hypothesis is that horizontal segregation is directed by preferred prey abundance.

4.6. Competition.

Interspecific competition may be defined as follows: with two species sharing at least one common resource the presence of species A has a negative influence on fitness in species B. Proof of this effect requires field experiments: species have to be transplanted and/or enclosed without ill effects, they must be stocked at different densities when alone (to assess intraspecific competition) and when together in close to ambient conditions (Conell 1983). Competition is often inferred from indirect evidence like retarded growth because fitness is too difficult to measure directly (e.g. Werner & Hall 1976).

In a recent review of field experiments Schoener (1983) emphasises that little or no studies were done on food competition in marine vertebrate carnivores. The coastal zone of the North Sea with its frequent storms, low transparency, high current speeds and large tidal amplitude is not very amenable to experimental studies. Thus another approach is warranted.

Hutchinson (1958) distinguishes between fundamental and realized niche. The fundamental or "pre-competitive" niche is larger than the actual or realized niche of a population in a certain environment. Actual niche is expanded in the absence of competitors to fundamental niche and vice versa. Thus observations of niche shifts in the presence and absence of competitors are seen as strong indicators of competition.

The temporal segregation of spawning combined with the annuality of *Pomatoschistus species* creates a situation where adults of one species are present and adults of the other species are lacking because the 1+ have died and the 0+ are still too small to compete. This is precisely what we observed in the beginning of July 1984 (compare Figs 5 and 6).

Stomach analysis data for adult *Pomatoschistus lozanoi* in July do not reveal a major niche shift in the absence of its supposed competitor. This suggests absence of active present-day competition. Of course temporal effects of fluctuation in prey abundance may make niche widening impossible. This is improbable because the very small *P.minutus* present eat *Pariambus typicus* of sizes apparently still profitable to 60-64 mm adults (data of September). Alternatively *Pomatoschistus minutus* may still be present but catchability is reduced by spawning activities (Miller 1984). The territorial bottom dwelling spawners may be even more agressive than usual, keeping *P.lozanoi* confined to the water column.

The strong niche shift towards benthic feeding by adult *Pomatoschistus lozanoi* in August in the presence of 0+ *P.minutus* of the same length class may be a belated response to the disappearence of 1+ *P.minutus*, if gobies need a lot of time to adapt to a new situation. This is highly improbable in annual fishes adapted to a very dynamic ecosystem. Moreover data from laboratory experiments (Edlund & Magnhagen 1981, Magnhagen & Wiederholm 1982) show an immediate niche shift response in *Pomatoschistus minutus* and *P.microps*. When alone both species eat similar amounts of *Corophium* and chironomids. When together *P.microps* switches to *Corophium* and its feeding rate is reduced.

Another hypothesis is that *Pomatoschistus lozanoi* is confined to the bottom in August because of spawning activities at that time, e.g. it can not go feeding in the water column without ruining its chances for successful reproduction.

5. CONCLUSIONS

Pomatoschistus species are versatile microcarnivores that consume large amounts of food. They derive their energy from secondary consumers in the water column, from the meiobenthos and from secondary and tertiary consumers in the benthos and epibenthos. The data suggest that the small mobile epifauna plays an important role in the food web of the shallow coastal waters inshore of the Flemish Banks (Southern Bight of the North Sea) .

The lack of niche shift in *Pomatoschistus lozanoi* in July suggests present-day competition to be unimportant in explaining food niche segregation between *P.minutus* and *P.lozanoi*, but alternative explanations can be found. The mechanisms directing the complex pattern of temporal, spatial and food niche segregation in the two species of Pomatoschistus can only be elucidated by field and laboratory experiments with adequate controls for prey abundance variability and for effects of spawning behaviour.

The function and adaptive significance of the species diagnostic papillary pattern in the feeding ecology of gobies requires further investigation. The morphology of the 2nd vertical c-row of papillae is a reliable criterion for the separation of *Pomatoschistus lozanoi* from *P.minutus.*

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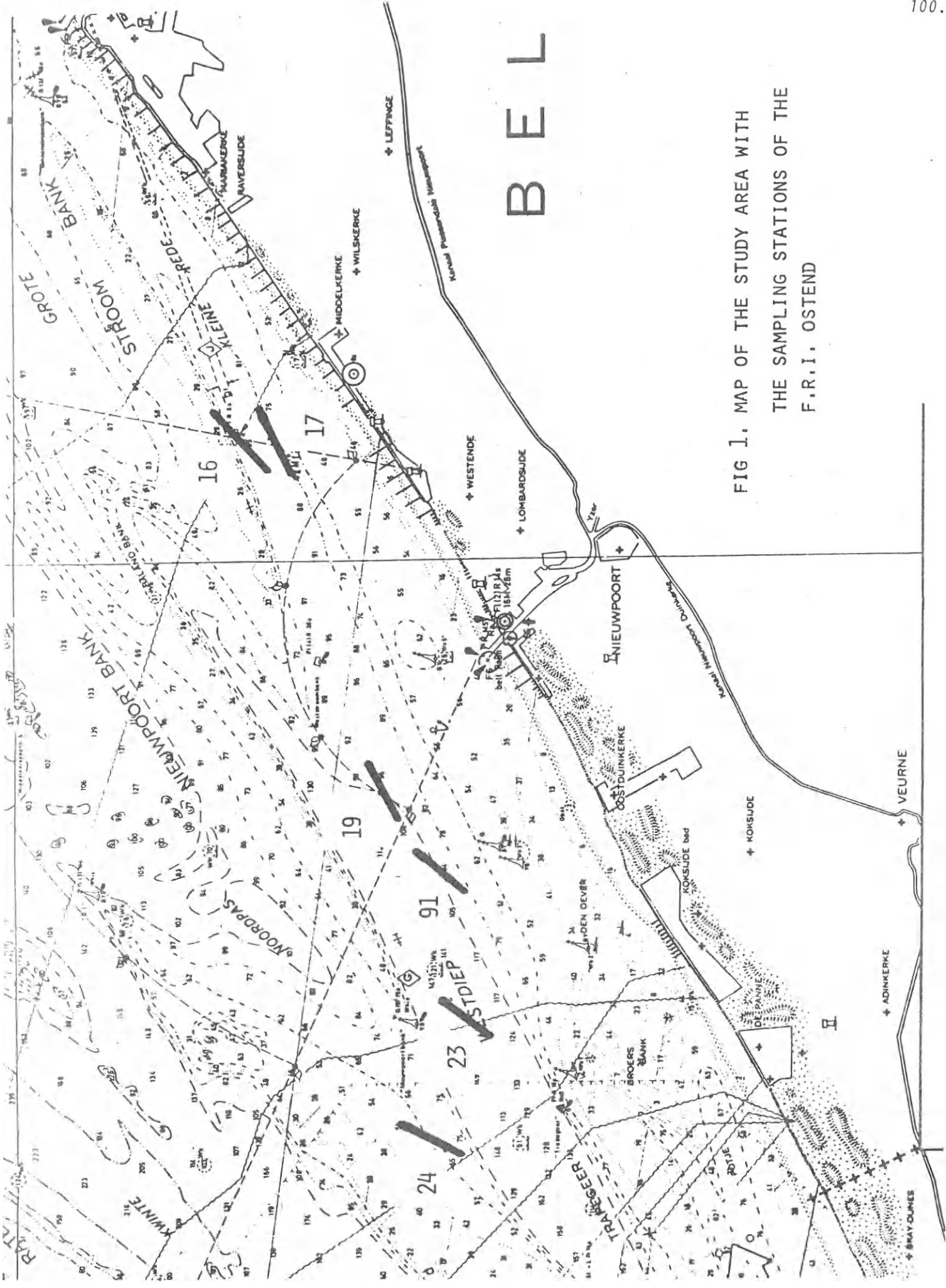
FIG 1. MAP OF THE STUDY AREA WITH
THE SAMPLING STATIONS OF THE
F.R.I. OSTEND

AFDW (mg) P.lozanoi   n=111
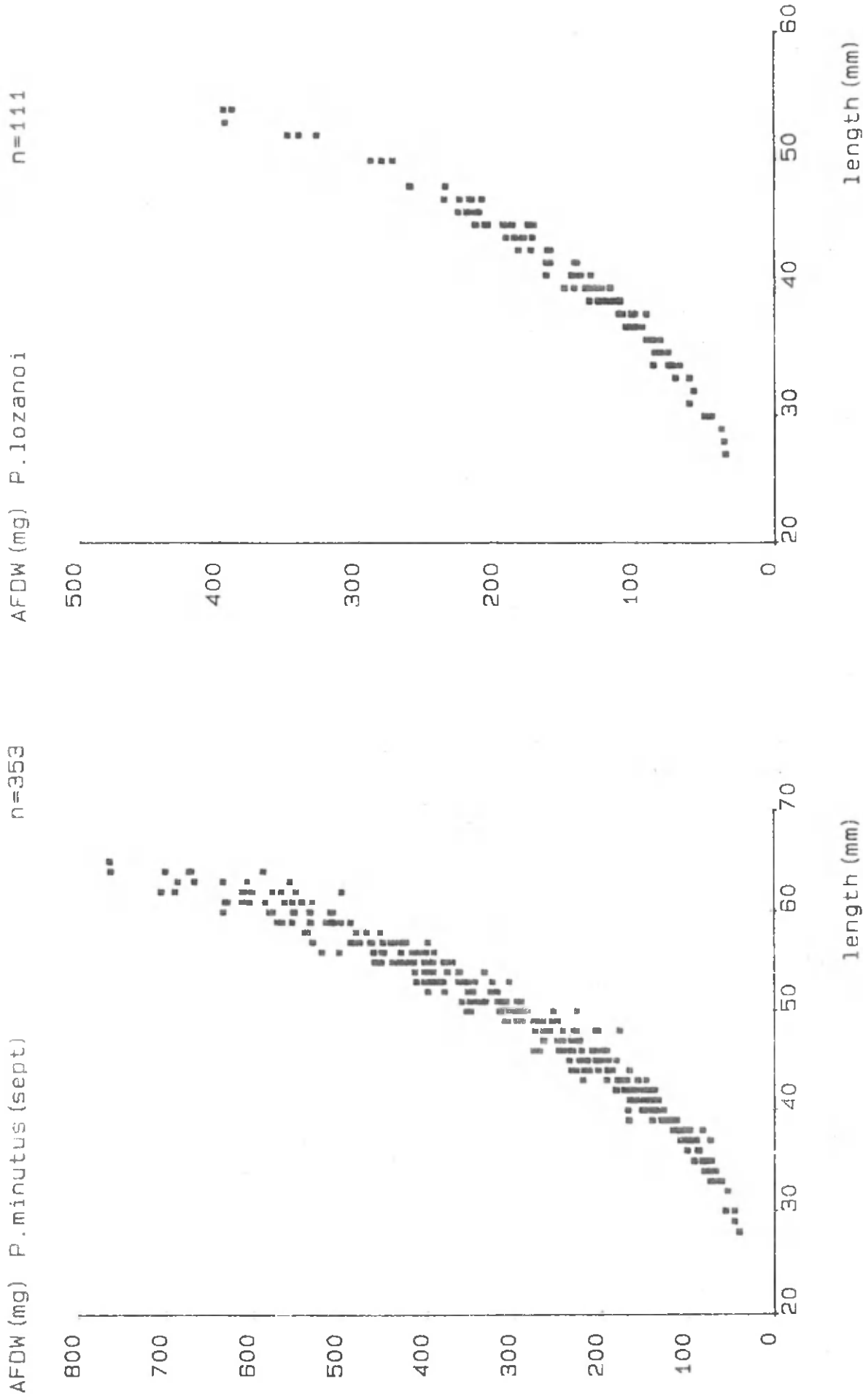
length (mm)

FIG 3. RELATIONSHIP STANDARD LENGTH TO AFDW

POMATOSCHISTUS LOZANOI

AFDW (mg) P.minutus (sept)   n=353

length (mm)

FIG 2. RELATIONSHIP STANDARD LENGTH TO AFDW

POMATOSCHISTUS MINUTUS
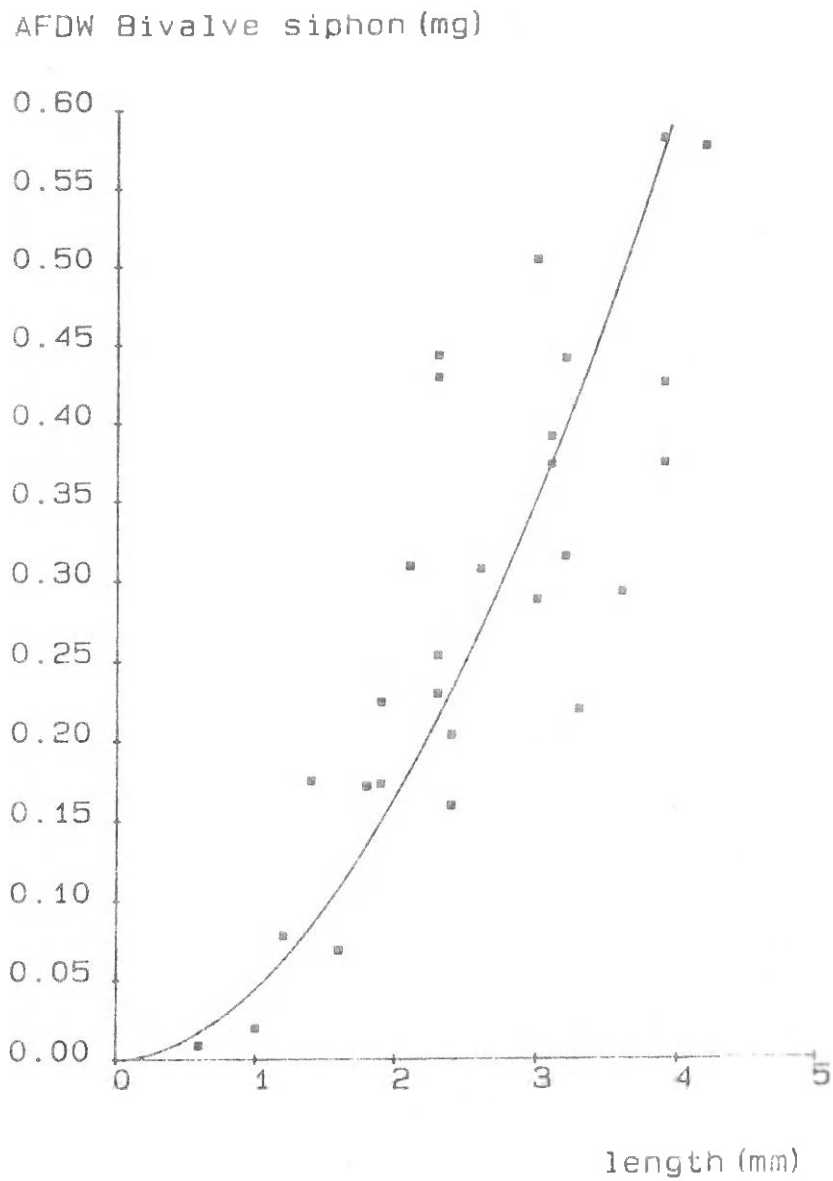
FIG 4. RELATIONSHIP BIVALVE SIPHON LENGTH TO
BIVALVE SIPHON AFDW
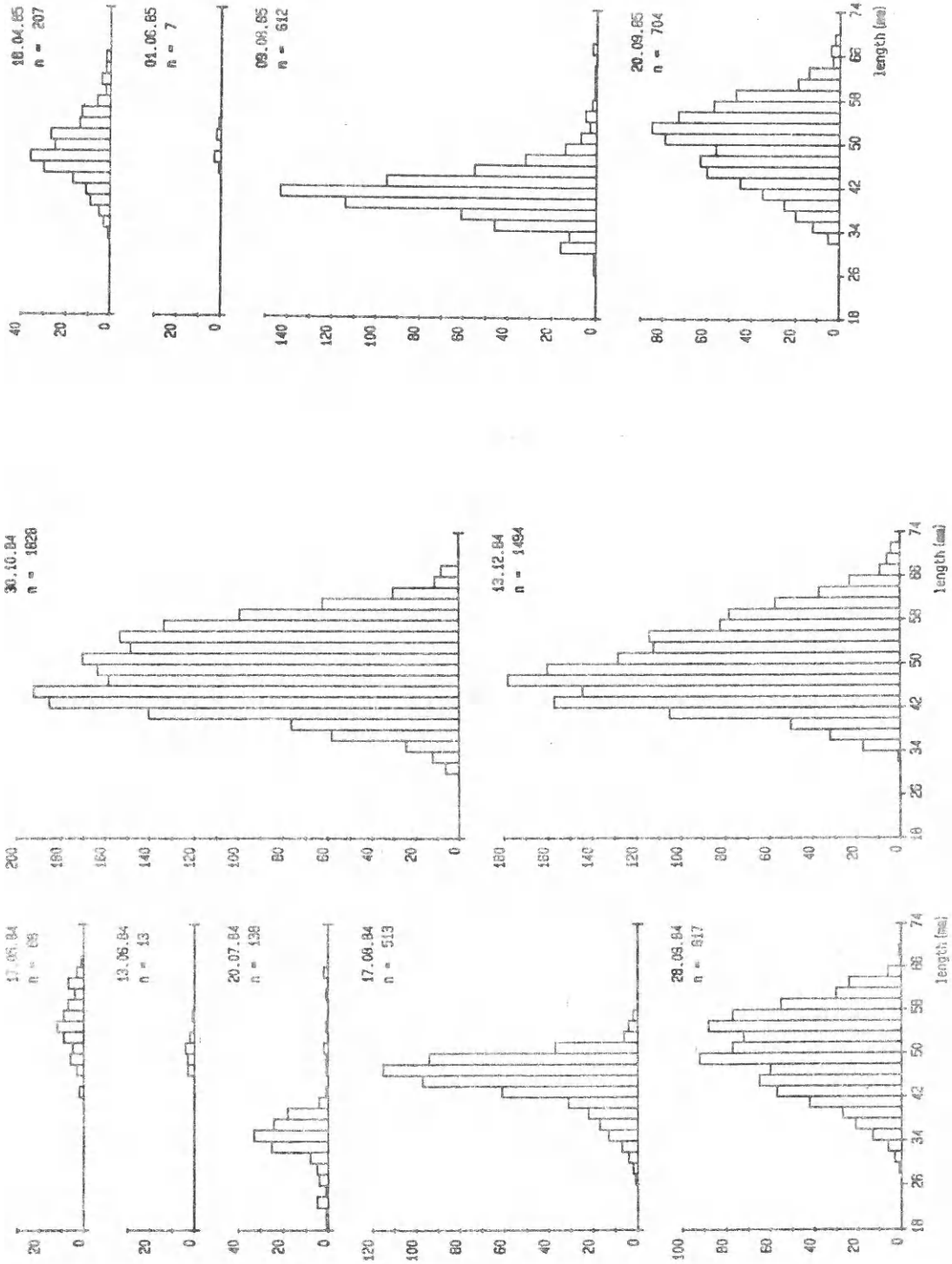
FIG 5. LENGTH-FREQUENCY DISTRIBUTIONS POMATOSCHISTUS MINUTUS

Pomatoschistus lozanoi
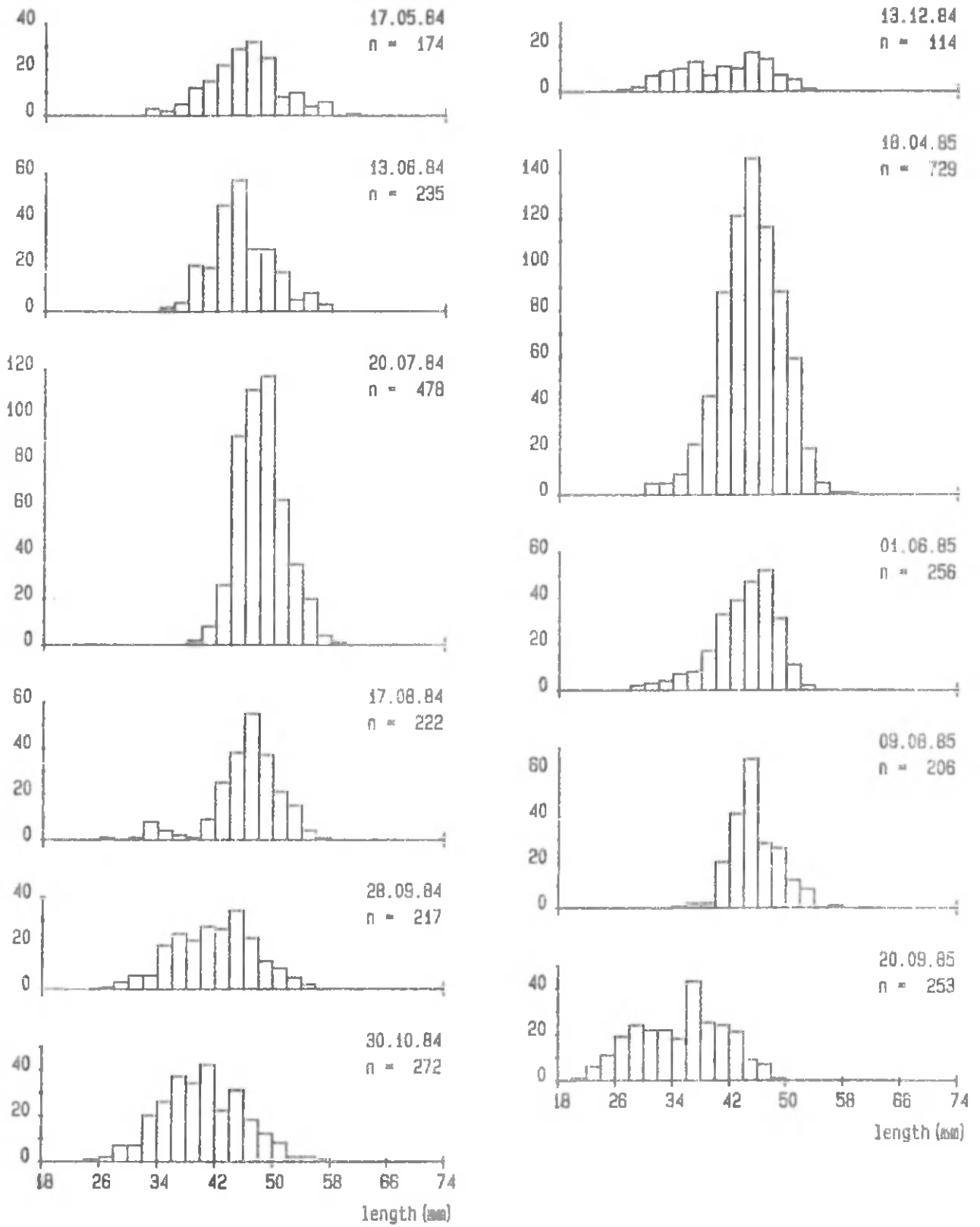
FIG 6. LENGTH-FREQUENCY DISTRIBUTIONS POMATOSCHISTUS LOZANOI

Pomatoschistus minutus (%)
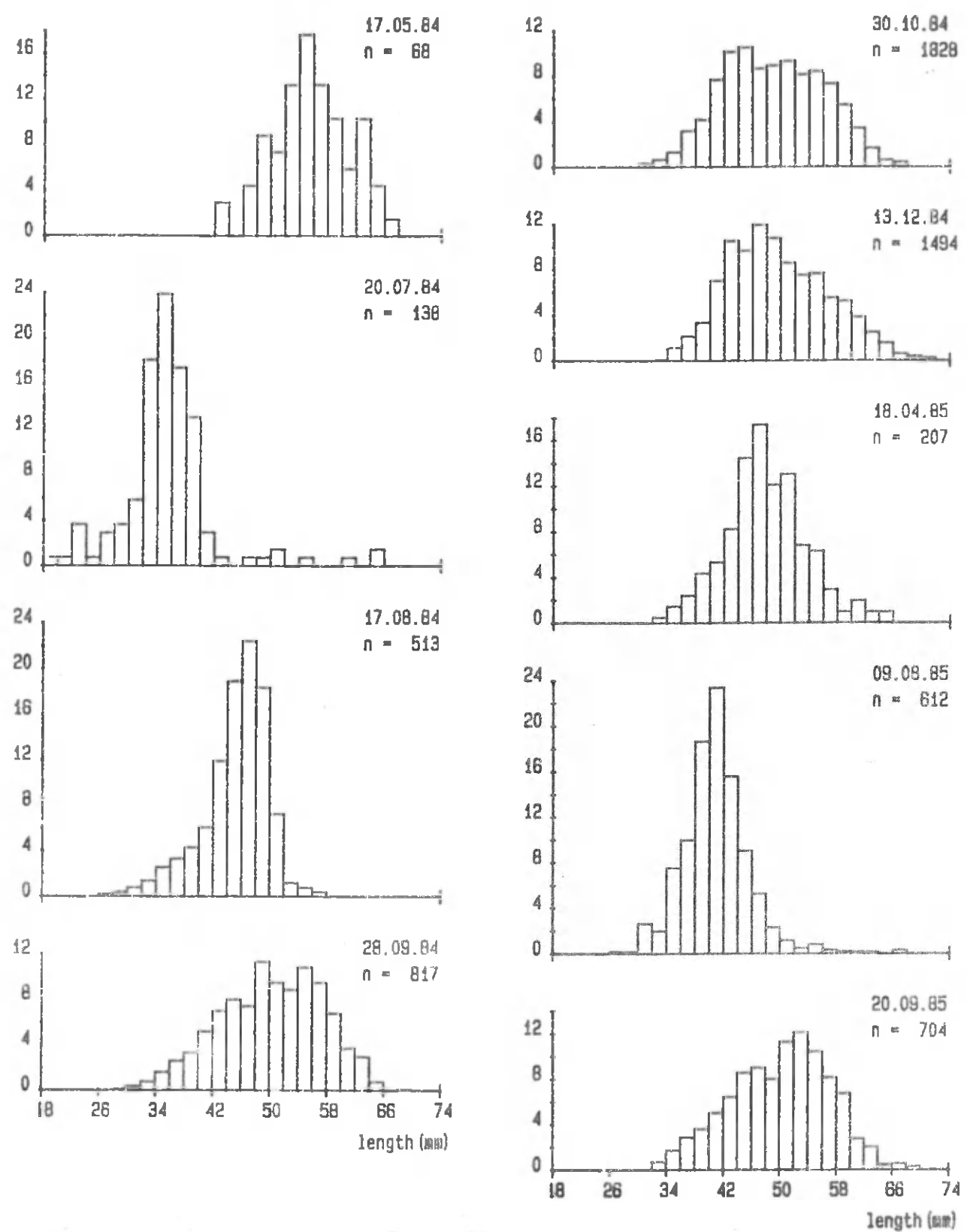
FIG 7. LENGTH-PERCENTAGE FREQUENCIES POMATOSCHISTUS MINUTUS

Pomatoschistus lozanoi (%)
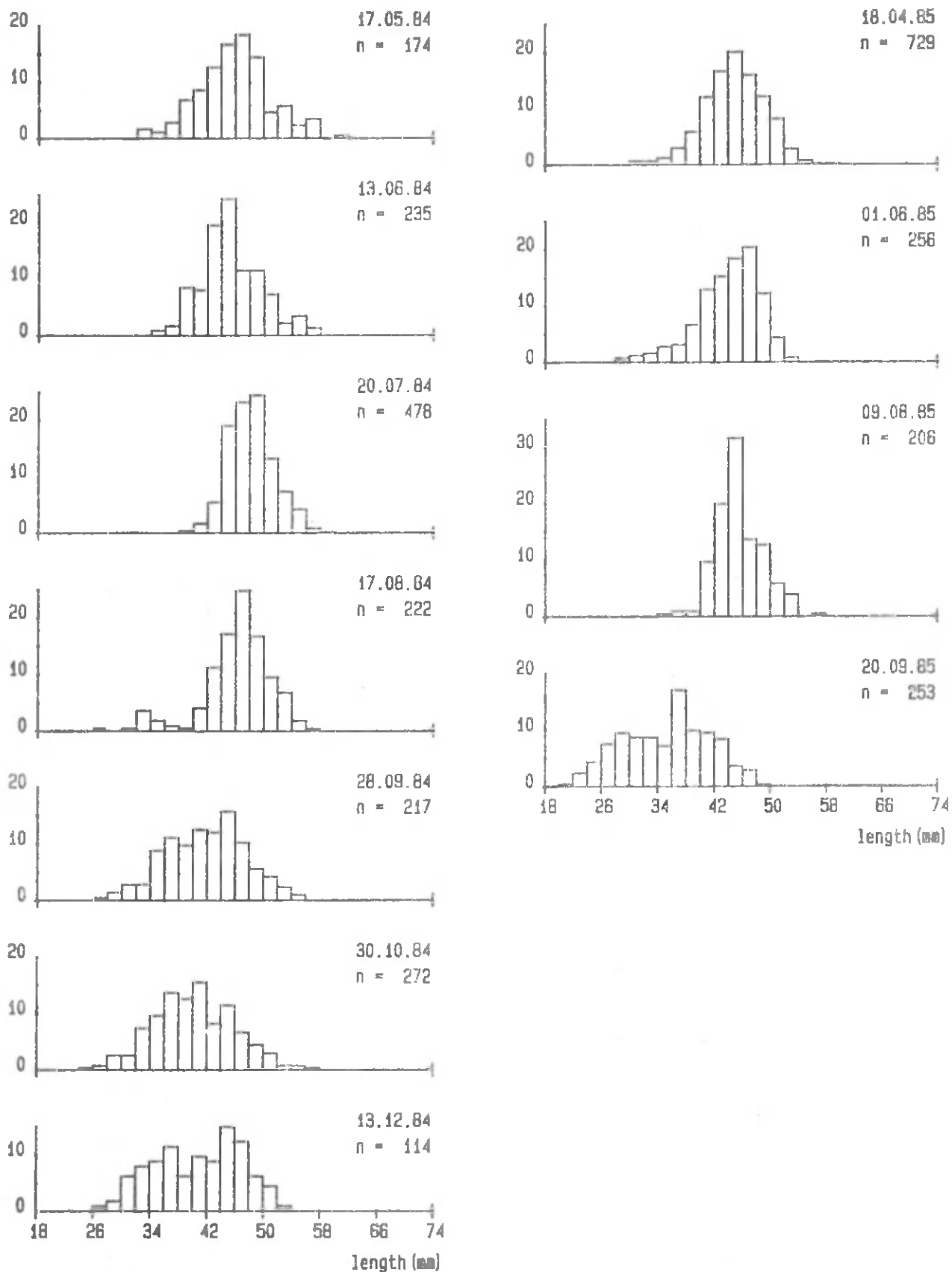
FIG 8. LENGTH-PERCENTAGE FREQUENCIES POMATOSCHISTUS LOZANOI
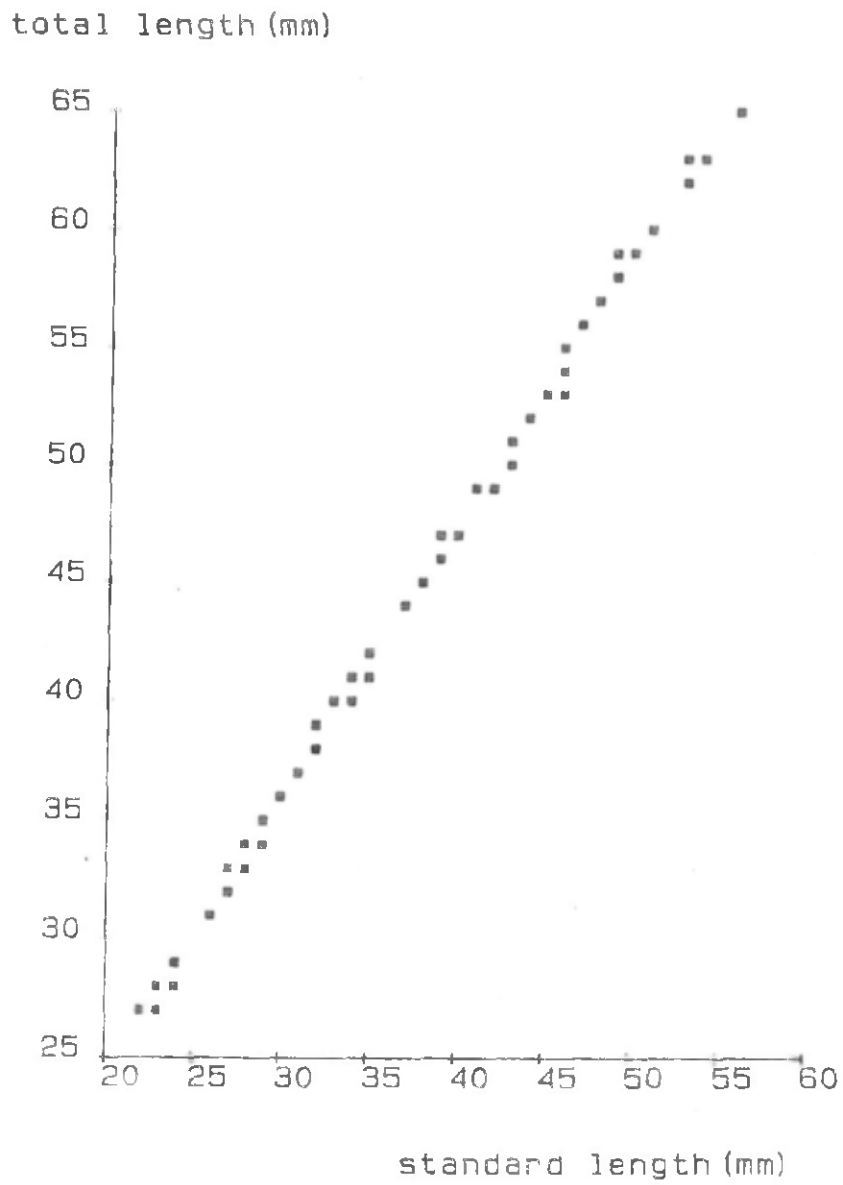
FIG 9. RELATIONSHIP STANDARD LENGTH TO TOTAL LENGTH
POMATOSCHISTUS LOZANOI

Pomatoschistus minutus (Sept)   n=178

% AFDW

length (mm)
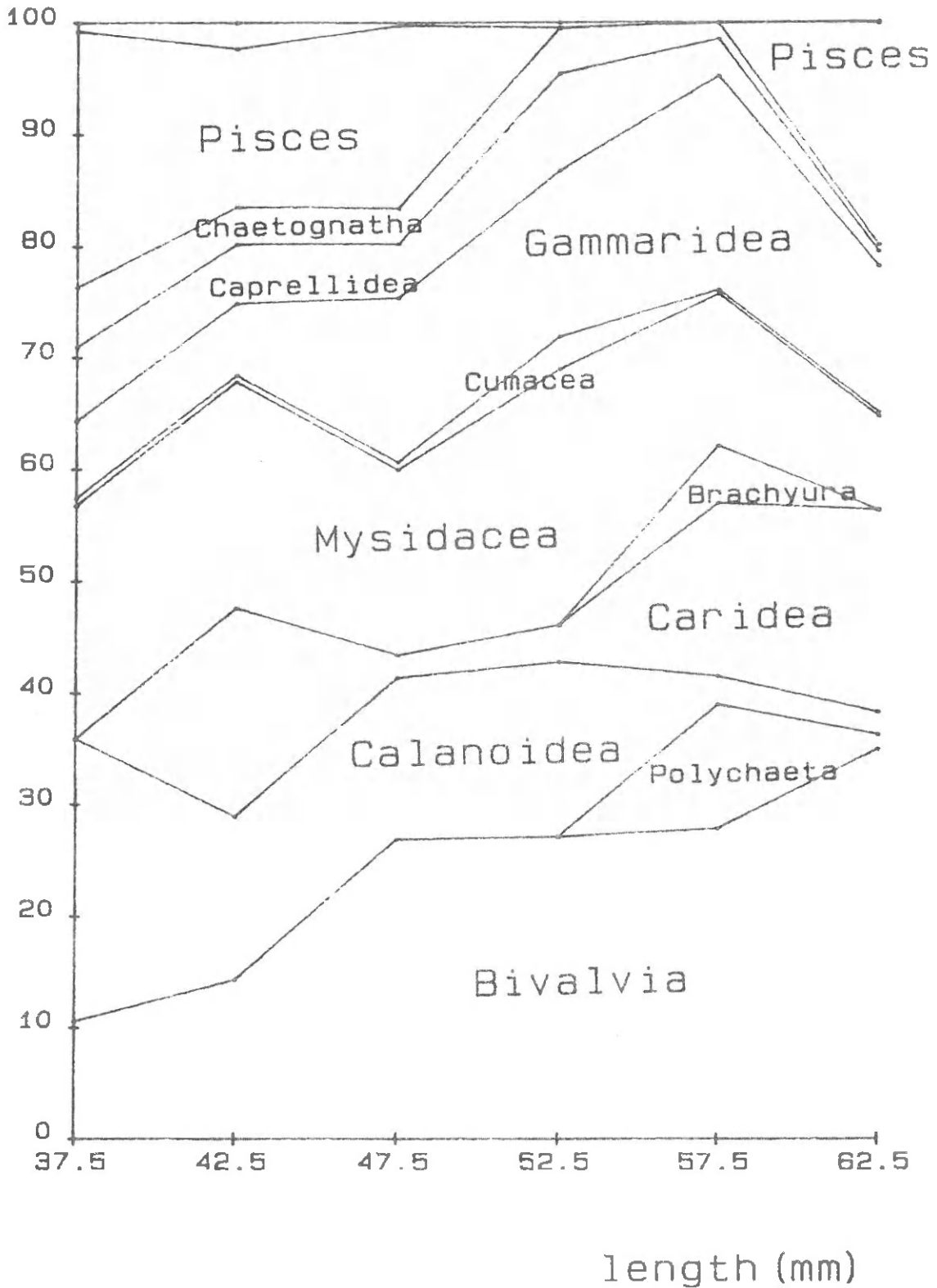
FIG 10. FOOD COMPOSITION IN PERCENTAGE AFDW FOR DIFFERENT
SIZE CLASSES OF POMATOSCHISTUS MINUTUS
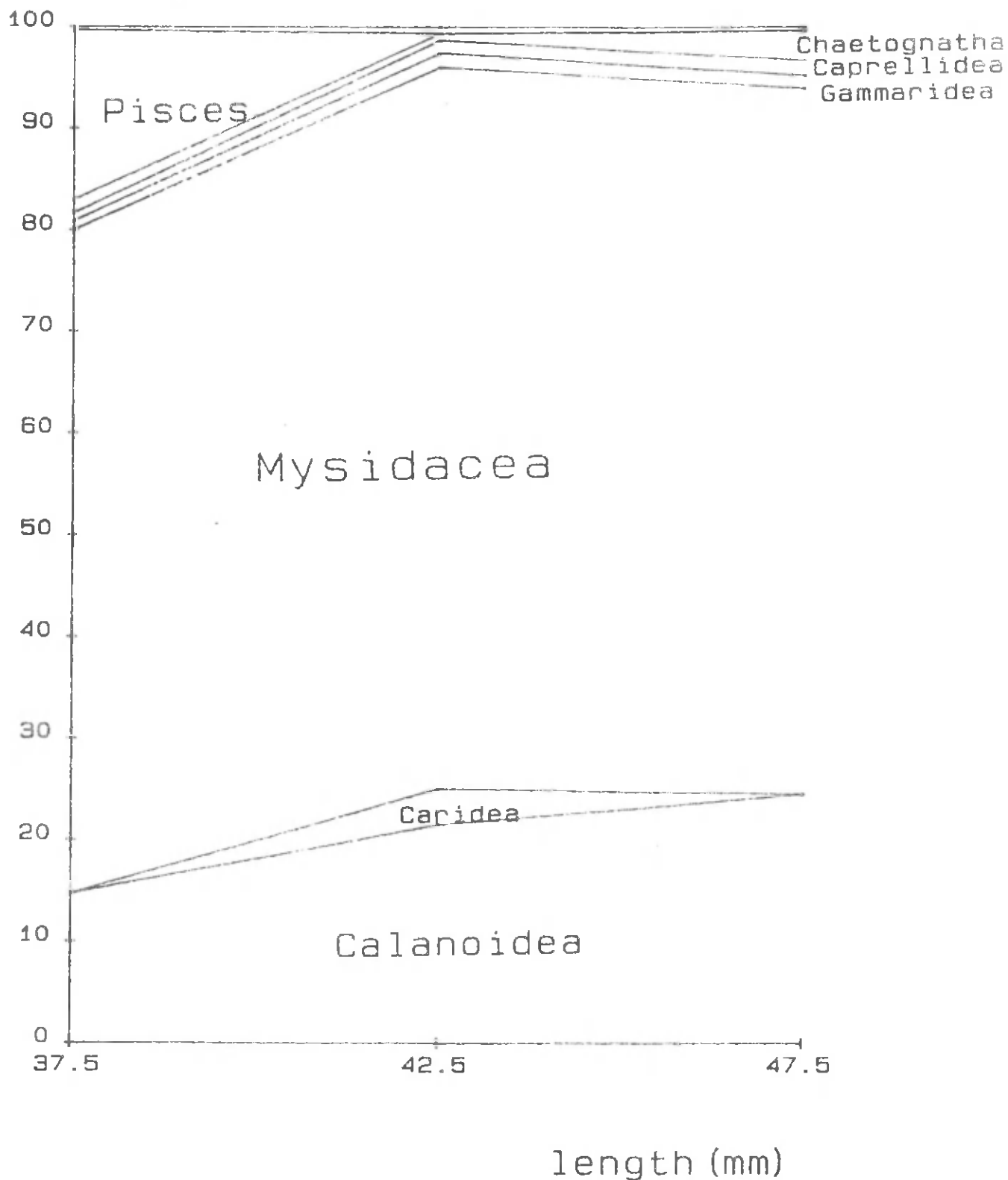
FIG 11. FOOD COMPOSITION IN PERCENTAGE AFDW FOR DIFFERENT
SIZE CLASSES OF POMATOSCHISTUS LOZANOI

Average prey AFDW P.min. (Sept)

AFDW (mg)

length (mm)
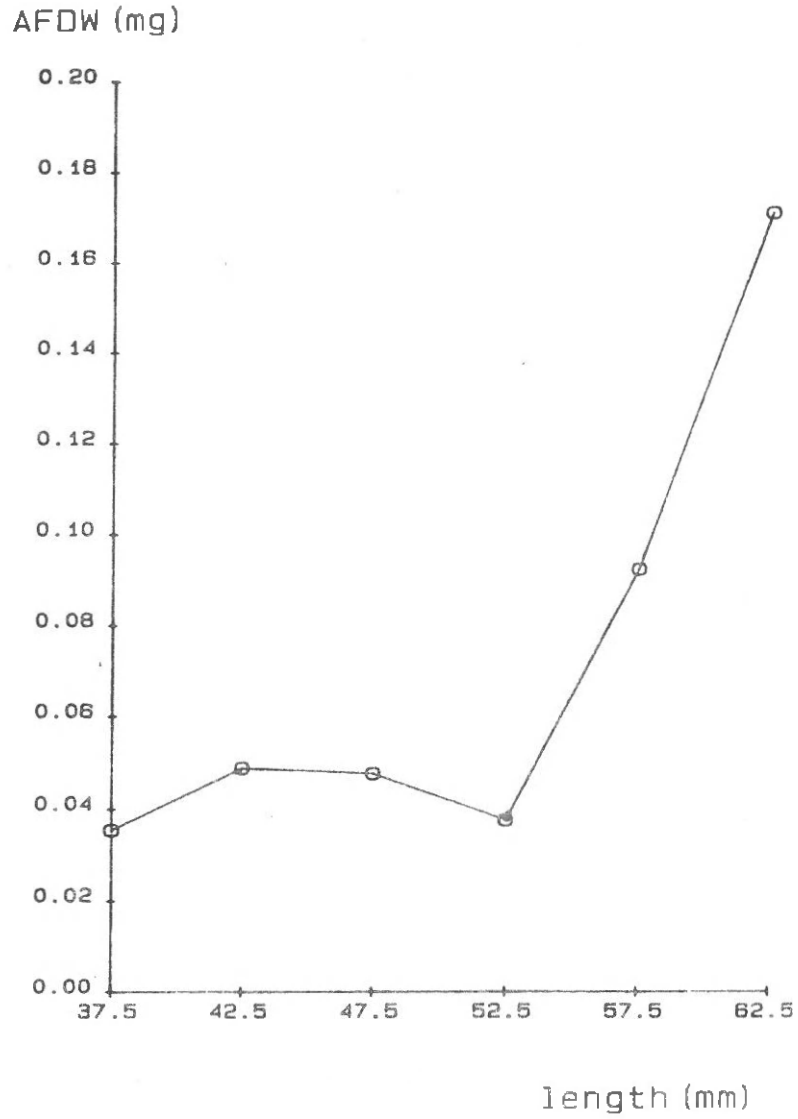
FIG 12. AVERAGE AFDW OF INGESTED PREY FOR
DIFFERENT SIZE CLASSES OF POMATOSCHISTUS MINUTUS

AFDW Predator/Average AFDW Prey

length (mm (
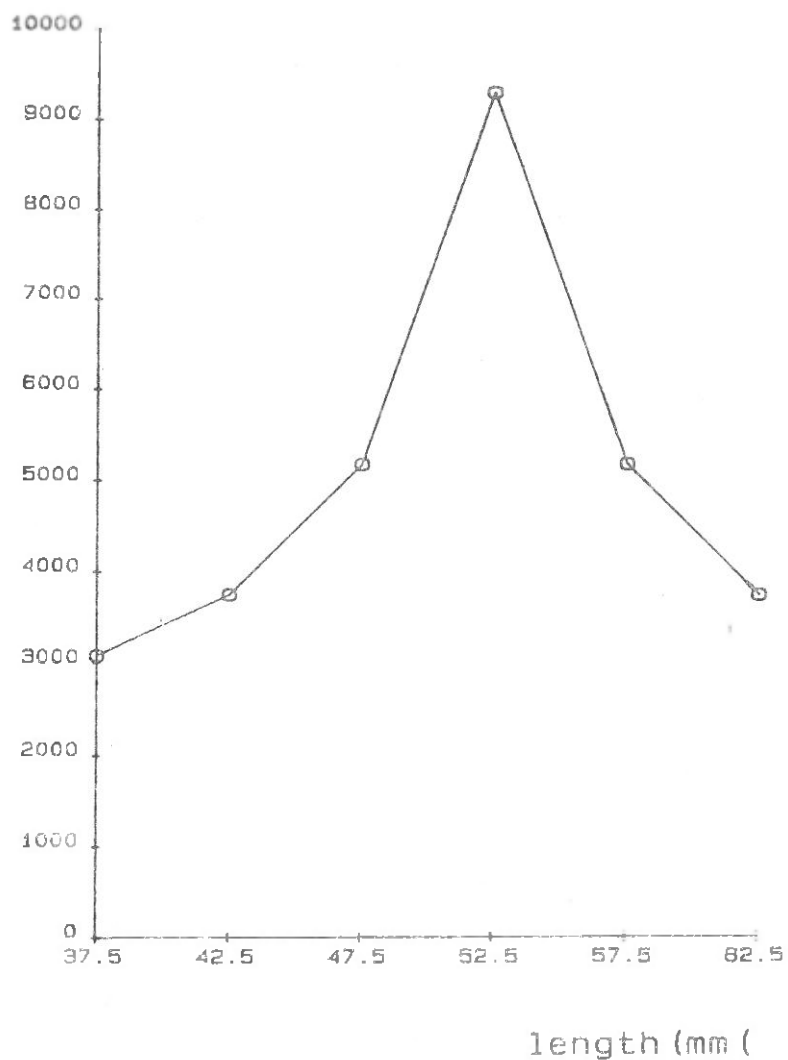
FIG. 13. RELATIVE BIOMASS (IN AFDW) PREDATOR-PREY

FOR DIFFERENT SIZE CLASSES OF POMATOSCHISTUS MINUTUS

Average number of Calanoids per stomach
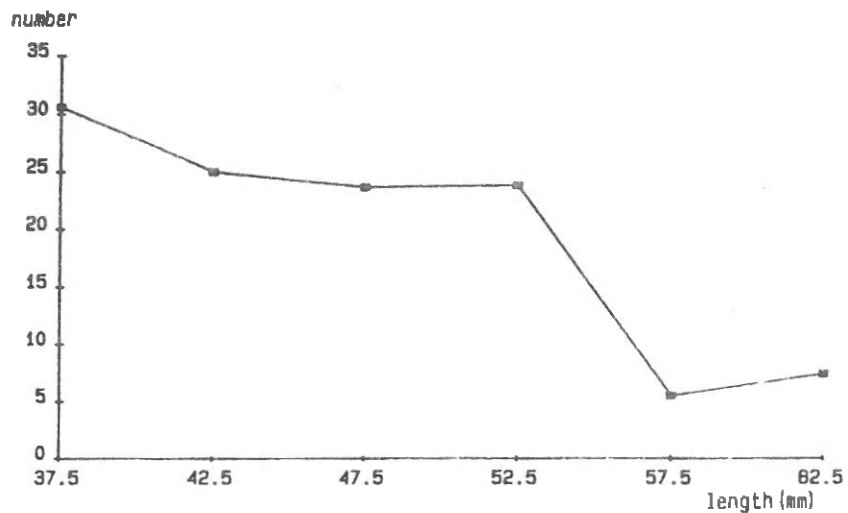
number

Average number of Harpacticoids per stomach
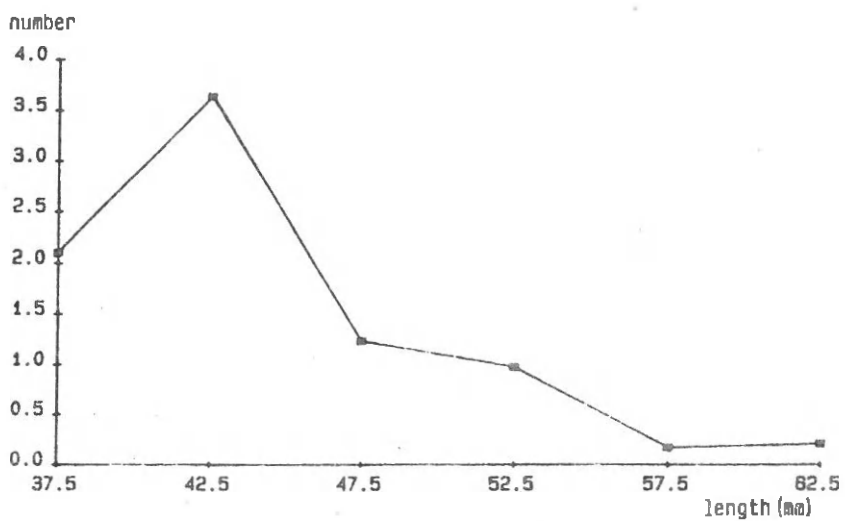
number

FIG 14, AVERAGE NUMBER OF CALANOIDS AND HARPACTICOIDS
        FOR DIFFERENT SIZE CLASSES OF POMATOSCHISTUS MINUTUS
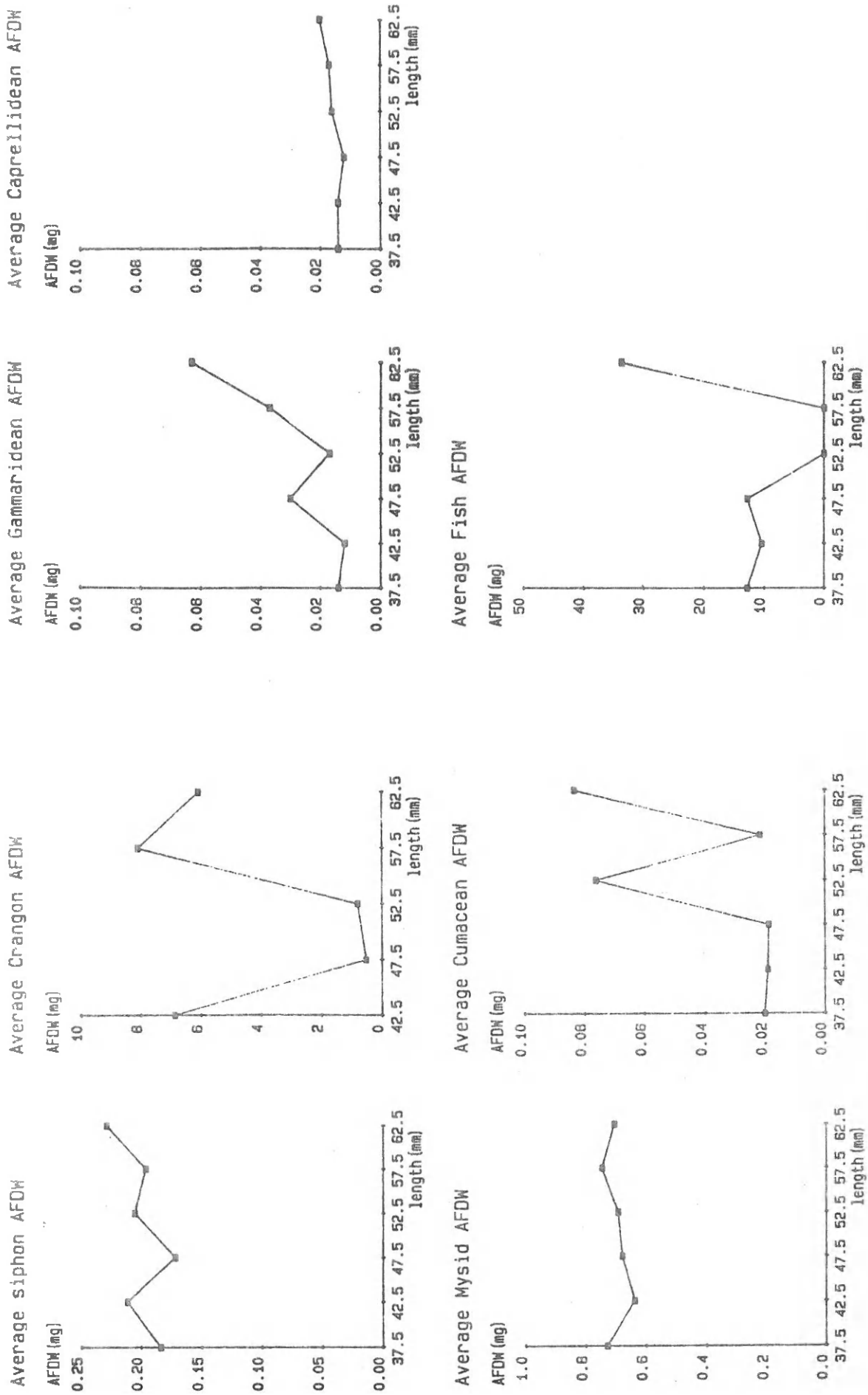
FIG 15. AVERAGE AFDW'S OF DIFFERENT FOOD CATEGORIES FOR DIFFERENT SIZE CLASSES OF POMATOSCHISTUS MINUTUS

Average AFDW Microprotopus maculatus

Average AFDW other gammarideans
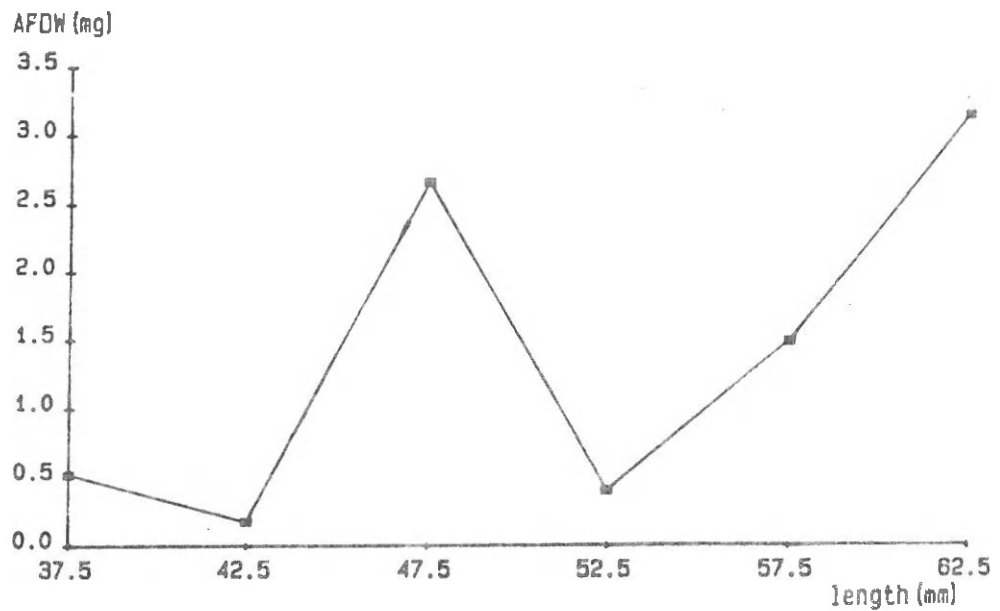
FIG. 16. AVERAGE AFDW'S OF MICROPOTOPUS MACULATUS
AND OTHER GAMMARIDEAN AMPHIPODS FOR DIFFERENT
SIZE CLASSES OF POMATOSCHISTUS MINUTUS

Ship	Date	PM:n=	dens	biom	*	PL:n=	dens	biom
062	17.05.84	68	6	2,6	*	174	40	7,3
062	13.06.84	13	3	1,3	*	235	40	8,2
062	20.07.84	138	15	1,6	*	479	40	15,1
062	17.08.84	513	100	21,7	*	222	50	11,3
062	28.09.84	817	175	57,0	*	217	25	4,2
062	30.10.84	1828	250	75,8	*	432	75	7,0
062	13.12.84	1494	250	72,4	*	114	30	6,2
BRW	18.04.85	202			*	730		
062	01.06.85	7			*	256		
062	09.08.85	612			*	206		
BRW	20.09.85	706			*	262		

TABLE 1. SAMPLING DATES, NUMBERS PER SPECIES, DENSITY AND

BIOMASS

PM= Pomatoschistus minutus
PL= Pomatoschistus lozanoi
Densities in number per 1000 m^2
Biomass in mg AFDW per m^2

ASH FREE DRY WEIGHTS OF PREY SPECIES

Nematods: width × length^2 /1600000 = fresh weight
AFDW = 15% of fresh weight
source: Andrassy 1956

Bivalvia: ln AFDW = -4.052 + 2.817 ln length
source: Govaere 1978
siphons: log AFDW = 1.876 + .043 log length
n = 29; r^2 = .80

Polychaeta:
Stenelais boa: ln AFDW = -4.389 + 1.785 ln length
Eteone spec.: ln AFDW = -5.717 + 1.530 ln length
Anaitides spec.: ln AFDW = -5.882 + 1.674 ln length
Nephtys spec.: ln AFDW = -7.139 + 2.489 ln length
Polychaeta errantia indet cfr. Anaitides
source: Govaere 1978
Nereis spec.: log body weight = 3.3 × log jaw
length + 1.57
source: Olive & Garwood 1981
Spionidae: ln AFDW = -6.030 + 1.831 ln length
Capitella capitata: ln AFDW = -6.354 + 2.051 ln length
Pectinaria koreni: ln AFDW = -6.918 + 2.689 ln length
Lanice conchilega: ln AFDW = -6.918 + 2.181 ln length
source: Govaere 1978
Sabellinae indet. cfr. Lanice

Ostracoda: .002 mg assigned value

Calanoidea: .016 mg assigned value

Harpacticoids:
Longipedia minor, Canuella perplexa, Halectinosoma
sarsi, Thompsonula hyaenae: .004 mg assigned value
all others: .002 mg assigned value
copepodites: 1/3 adult weight
source: R. Herman pers. comm.

Cirripedia: AFDW of an amphipod of the same length as the cirri, assigned value

Caridea: log AFDW = .00046 + 3.321 log length
source: van Lissa 1977
zoea: .01 mg assigned value

Brachyura: log wet weight = -3.961 + 3.160 log carapax
breadth; AFDW = 20% wet weight
source: Borremans 1982
Portunidae zoe: .01 mg assigned value
Portunidae megalopa: .015 mg assigned value

Mysidacea: ln AFDW = -4.422 + 1.924 ln length
source: Govaere 1978

Cumacea: ln AFDW = -6.078 + 2.525 ln length
source: Govaere 1978

Isopoda: cfr. amphipoda

Amphipoda:
Bathyporeia spec.: ln AFDW = -8.674 + 4.563 ln length
all others: ln AFDW = -6.958 + 3.225 ln length
source: Govaere 1978

Chaetognatha: .5 mg assigned value from Feigenbaum 1979

Pisces: total weights = somatic weight + gonad weight
Pomatoschistus minutus: log AFDW = -3.40976 + 3.460 log length; n = 191; r^2 = .98
Pomatoschistus lozanoi: log AFDW = -3.40566 + 3.448 log length; n = 113; r^2 = .97
Pomatoschistus spec. cfr P. minutus

TABLE 2. LENGTH TO AFDW RELATIONSHIPS OF PREY SPECIES

P.minutus	Mean	Stand.Dev.	n
May 1984	55,3	5,31	68
June 1984	50,2	2,85	13
July 1984			
Yearcl. 0+	33,8	4,27	130
Yearcl. 1+	55,1	6,96	8
Aug 1984	44,7	4,57	513
Sept 1984	49,7	7,14	817
Oct 1984	48,5	7,10	1828
Dec 1984	49,1	7,13	1494
Apr 1985	47,4	5,86	207
June 1985	48,0	2,65	7
Aug 1985	40,5	4,78	612
Sept 1985	49,6	6,97	704

P.lozanoi	Mean	Stand.Dev.	n
May 1984	45,3	5,10	174
June 1984	44,8	4,29	235
July 1984	47,4	3,25	478
Aug 1984			
Yearcl. 0+	33,3	2,59	17
Yearcl. 1+	46,8	3,28	205
Sept 1984	41,4	5,61	217
Oct 1984	39,7	5,66	272
Dec 1984	40,4	6,02	114
Apr 1985	44,4	4,30	729
June 1985	43,6	4,45	256
Aug 1985	45,1	3,38	206
Sept 1985	34,7	6,14	253

TABLE 3. MEAN LENGTHS OF POMATOSCHISTUS MINUTUS AND P.LOZANOI PER SAMPLING DATE

P.minutus	Nem	Biv	P.E.	P.S.	Ost	Cal	Harp	Cirr	Car	Bra	Mys	Cum	Iso	Gam	Cap	Chae	Pisc	AFDW(mg)
MAY 1984																		
l 50-54 n=19	0,0	6,0	9,1	28,4	3,0				25,5		0,2	0,2	27,7	0,0	0,5			111,1
l 55-59 n=18		11,0	0,9	36,2	6,0	0,0			10,7		4,9	1,3	19,4	1,2	2,3		5,4	66,7
JULY 1984																		
l 30-34 n=38	0,0	0,1	2,6	16,6	2,4	0,3			28,9	0,2	40,4	0,1		0,4			8,0	14,3
l 35-39 n=46		2,2	0,4	6,0	4,1	0,2				0,2	12,5	1,0		4,8			33,5	19,6
AUG. 1984																		
l 45-49 n=30	0,0	0,3	0,8	39,7	0,0						0,1	5,2		8,3			45,6	18,8
SEPT. 1984																		
l 35-39 n=29	0,0	10,5	0,4		0,0		25,3	0,4	20,9			0,7		6,9	6,4	5,4	22,9	55,9
l 40-44 n=30	0,0	15,3	0,0	0,1	0,0		15,8	0,5	18,2	0,0	20,2	0,6		4,5	5,3	3,3	14,2	75,7
l 45-49 n=30	0,0	26,9	0,1		0,0		14,5	0,2	2,0		16,5	0,7	0,0	14,7	4,8	3,2	16,4	78,1
l 50-54 n=30	0,0	27,1	0,0	0,3			15,7	0,1	3,3	0,0	22,9	2,9		14,8	8,7		4,1	72,8
l 55-59 n=30	27,8	11,0	0,2				2,5	0,0	15,5	5,1	13,6	0,4	0,0	19,1	3,3		1,4	104,4
l 60-64 n=29	35,0	1,2	0,1			0,0	2,0	0,0	18,1	0,0	8,3	0,4	0,1	13,0	1,3	0,6	19,9	169,1
OCT. 1984																		
l 35-39 n=30		0,1		16,6			14,3	0,0	63,3			0,3		2,2	2,1		1,2	43,1

TABLE 4. FOOD COMPOSITION IN % AFDW FOR POMATOSCHISTUS MINUTUS

Nem= nematodes; Biv= bivalves; P.E.= errant polychaetes; P.S.= sedentary polychaetes;
Ost= ostracods; Cal= calanoids; Harp= harpacticoids; Cirr= cirripedia; Car= caridean decapods
Bra= brachyurans; Mys= mysids; Cum= cumaceans; Iso= isopods; Gam= gammaridean amphipods
Cap= caprellids; Chae= chaetognaths; Pisc= Pisces

P.lozanoi	Nem.	Biv.	P.E.	P.S.	Ost.	Cal.	Harp.	Car.	Bra.	Mys.	Cum.	Iso.	Gam.	Cap.	Chae.	Pisc.	AFDW(mg)
MAY 1984																	
1 45-49 n=30	0,0		0,7	0,0	21,1	0,0			1,9	11,4	0,2		23,8	2,5	40,8		78,9
1 50-54 n=21		8,1	0,5		15,7	0,0			9,5	12,5	0,2		52,4	0,9			42,2
JULY 1984																	
1 40-44 n=30	0,0	2,7		0,3		0,0			2,9	69,4	0,1		0,5	0,2	0,6	13,7	83,7
1 45-49 n=30		0,7		0,3		0,0			7,0	49,0			0,2	0,1	42,7		93,7
AUG. 1984																	
1 45-49 n=30		95,8		0,1		0,0							3,6	0,6			60,3
SEPT. 1984																	
1 35-39 n=30					14,7	0,0			0,0	65,5	0,1	0,0	1,0	0,7	1,3	16,7	76,7
1 40-44 n=30	0,2	0,0			21,5	0,0			3,5	71,0	0,4	0,0	1,4	1,3	0,7		74,8
1 45-49 n=24	0,1				24,5	0,0			0,1	69,5	0,2	0,0	1,3	1,5	2,9		67,8
OCT. 1984																	
1 35-39 n=30		0,6			7,1	0,0			1,5	88,5	0,3		0,1	0,0	2,0		102,6

TABLE 5. FOOD COMPOSITION IN % AFDW FOR POMATOSCHISTUS LOZANOI

Nem= nematodes; Biv= bivalves; P.E.= errant polychaetes; P.S.= sedentary polychaetes

Ost= ostracods; Cal= calanoids; Harp= harpacticoids; Car= caridean decapods;

Bra= brachyurans; Mys= mysids; Cum= cumaceans; Iso= isopods; Gam= gammaridean amphipods

Cap= caprellids; Chae= chaetognaths; Pisc= Pisces.

TABLE 6.   SYSTEMATIC LIST OF FOOD ORGANISMS IN STOMACHS

	P.min	P.loz
Phylum NEMATODA		
Sabatieria hilarula (De Man, 1922)	+	−
Mesacanthion spec.	+	−
Enoplidae spec.	+	+
Leptolaimidae spec.	+	−
Nematoda spec.	+	−
Phylum MOLLUSCA		
Cl.Bivalvia		
Cerastoderma edule (Linnaeus, 1758)	+	−
Abra alba (Wood, 1802)	+	−
Tellina fabula (Gronovius, 1781)	+	−
Spisula spec.	+	−
Bivalvia indet.	+	+
Bivalvia siphons	+	+
Phylum ANNELIDA		
Cl.Polychaeta		
O.Errantia		
Sthenelais boa (Johnston, 1839)	+	−
Eteona spec.	−	+
Anaitides mucosa (Oersted, 1843)	+	−
Anaitides groenlandica (Oersted, 1842)	+	−
Anaitides spec.	+	−
Nereis spec.	+	−
Nephtys hombergii (Savigny, 1818)	+	+
Nephtys spec.	+	−
Polychaeta Errantia indet.	+	−
O.Sedentaria		
Spionidae spec.	+	−
Capitella capitata (Fabricius, 1780)	+	−
Pectinaria koreni (Malmgren, 1865)	+	−
Lanice conchilega (Pallas, 1766)	+	+
Lanice tentacle crowns	+	+
Sabellinae indet.	+	−
Phylum ARTHROPODA		
Subph.Crustacea		
Cl.Ostracoda		
Ostracoda indet.	+	+
Cl.Copepoda		
O.Calanoidea		
Temora longicornis (Muller, 1792)	+	+
Centropages hamatus (Lilljeborg, 1853)	+	+
Calanoidea indet.	+	+

	P.min	P.loz
O.Harpacticoidea		
Longipedia minor (T.& A. Scott, 1893)	+	-
Canuella perplexa (T.& A. Scott, 1893)	+	-
Halectinosoma propinquum (T.& A.Scott, 1894)	+	-
Halectinosoma sarsi (Boeck, 1872)	+	-
Pseudobradya beduina (Monard, 1935)	+	-
Euterpina acutifrons (Dana, 1884)	+	+
Microarthridion littorale (Poppe, 1881)	+	+
Thompsonula hyaenae (I.C. Thompson, 1889)	+	+
Harpacticus littoralis (Sars, 1910)	+	-
Tisbe furcata (Baird, 1837)	+	+
Tisbe spec.	+	+
Altheuta interrupta (Goodsir, 1845)	+	+
Dactylopodia tisboides (Claus, 1863)	+	-
Dactylopodia vulgaris (Sars, 1905)	+	+
Ameira parvula (Claus, 1866)	+	-
Cl.Cirripedia		
Cirripedia indet. cirri	+	-
Cl.Malacostraca		
O.Decapoda		
InfraO.Caridea		
Hippolyte varians (Leach, 1814)	+	+
Crangon crangon (Linnaeus, 1758)	+	+
Pontophilus trispinosus (Hailstone, 1838)	+	+
Caridea zoe	+	+
InfraO.Brachyura		
Carcinus maenas (Linnaeus, 1758)	+	-
Liocarcinus spec.	+	-
Portunidae zoe	+	+
Portunidae megalopa	+	+
O.Mysidacea		
Gastrosaccus spinifer (Goes, 1864)	+	+
Schistomysis spiritus (Norman, 1860)	+	+
Schistomysis spec.	+	+
Mesopodopsis slabberi (van Beneden, 1861)	+	-
O.Cumacea		
Cumopsis goodsiri (van Beneden, 1861)	+	-
Pseudocuma longicornis (Bate, 1858)	+	+
Diastylis rathkei (Kroyer, 1841)	+	-
Diastylis lucifera (Kroyer, 1841)	+	+
Diastylis spec.	+	+

	P.min	P.loz
O.Isopoda		
Eurydice pulchra (Sars, 1899)	+	+
Idotea linearis (Bate & Westwood, 1868)	+	−
O.Amphipoda		
SubO.Gammaroidea		
Orchomene nana (Kroyer, 1864)	−	+
Amphilochus neapolitanus (Della Valle, 1893)	+	−
Stenothoe marina (Bate, 1856)	+	−
Gammarus crinicornis (Stock, 1966)	+	+
Gammarus spec.	+	+
Maera grossimana (Montagu, 1808)	+	−
Melita obtusata (Montagu, 1813)	+	−
Bathyporeia elegans (Watkin, 1938)	+	+
Bathyporeia guilliamsoniana (Bate, 1856)	−	+
Bathyporeia spec.	+	+
Urothoe poseidonis (Reibisch 1905)	+	−
Perioculodes longimanus (Bate & Westwood, 1868)	−	+
Calliopius laeviusculus (Kroyer, 1838)	+	−
Pontocrates arenarius (Bate, 1858)	+	+
Atylus falcatus (Metzger, 1871)	+	−
Atylus swammerdami (Milne-Edwards, 1830)	+	+
Ampithoe rubricata (Montagu, 1808)	−	+
Aora typica (Kroyer, 1845)	+	−
Gammaropsis nitida (Stimpson, 1853)	−	+
Microprotopus maculatus (Norman, 1867)	+	+
Jassa falcata (Montagu, 1808)	+	−
SubO.Caprellidea		
Pariambus typicus (Stebbing, 1888)	+	+
Subph. Uniramia		
Cl.Insecta		
Diptera indet.	+	−
Phylum CHAETOGNATHA		
Sagitta spec.	+	+
Phylum CHORDATA		
Cl.Pisces		
Pomatoschistus minutus (Pallas, 1770)	+	+
Pomatoschistus lozanoi (De Buen, 1923)	+	+
Pomatoschistus spec.	+	+

	Cx y
Aug 40-44mm	0,44
Sept 35-39mm	0,56
Sept 40-44mm	0,44
Sept 45-49mm	0,38
Oct 35-39mm	0,72

TABLE 7. RENKONEN SIMILARITIES FOOD

POMATOSCHISTUS MINUTUS-LOZANOI

BENTHIC PREY	EPIBENTHIC PREY	PELAGIC PREY
NEMATODA	POLYCHAETA ERRANTIA	CALANOIDEA
BIVALVIA	EPIBENTHIC HARPACTICOIDA	PELAGIC HARPACTICOIDEA
POLYCHAETA SEDENTARIA	CARIDEA	MYSIDACEA
OSTRACODA	CUMACEA	PELAGIC ISOPODA
CIRRIPEDIA	GAMMAROIDEA	CHAETOGNATHA
BRACHYURA	EPIBENTHIC ISOPODA	PISCES
CAPRELLIDEA		

TABLE 8. PREY CATEGORIES CONVERSION TO FOOD NICHE CATEGORIES

P.minutus	BENTHIC	EPIBENTH	PELAGIC
May 50-59mm n= 37	40	51	9
July 30-39mm n= 84	54	18	28
Aug 45-49mm n= 30	86	9	5
Sept 35-49mm n= 89	24	18	58
Sept 35-64mm n= 178	32	28	40
Oct 35-39mm n=30	19	3	79

P.lozanoi	BENTHIC	EPIBENTH	PELAGIC
May 45-54 mm n= 51	1	41	57
July 40-49mm n=60	2	5	93
Aug 45-49mm n= 30	96	4	0
Sept 35-49mm n= 84	1	3	96
Oct 35-39mm n= 30	1	2	96

TABLE 9. PERCENTAGE ASH FREE DRY WEIGHTS OF PREY

IN FOOD NICHE CATEGORIES

A comparative study of the macrobenthos of three sandbanks in the Belgian coastal waters in 1980-1984

Has sand exploitation an influence on the macrobenthos?

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Abstract

Three sandbanks (Goote Bank, Buiten Ratel and Kwinte Bank)
were investigated over a period of five years (1980-1984)
in order to establish the impact of sand and gravel
extraction on the macrobenthos. Two stations on each
sandbank were sampled twice a year.

All six stations are characterized by clean sand ; the
median grain size of the sand fraction ranges between fine
sand (in the southern stations of the Kwinte Bank and the
Buiten Ratel) and coarse sand (in the northern stations of
the Kwinte Bank and Buiten Ratel) ; the stations on the
Goote Bank are characterized by fine to medium sand. The
macrobenthos is a typical sand fauna ; 118 species were
identified.

Density (range : 37-3337 ind./m.) and species number (range
: 1-34) can be correlated with sediment characteristics.
Both increase with increasing grain size. Diversity (H' ;
range : 0.00-4.15 bits/ind.), evenness (J ; range : 0.00-
0.76) and dominance (SI ; range : 0.09-1) do not follow the
same trend. In genera, there is no indication of changes
in any of these parameters due to sand and gravel
exploitation over the five years.

However, at one station of the Goote Bank a striking fall
in density, species number and diversity was recorded in
April 1982 ; this may be due to the sandextractions on the
Goote Bank . The fauna recovered within seven months.

Introduction

In the late seventies a research programme was set up by
the "Management Unit of the Mathematical Model North Sea"
(Ministry of Public Health, Belgium) in order to make a
study of the Flemish and Zeeland Banks, as they became of
interest to dredging companies. At that time the demand
for sand and gravel increased and moreover the exploitation
of terrestiral sand pits became expensive and gave rise to
environmental concern. Therefore concessions were given
for exploitation in the North Sea in two well delimited
areas. Zone I including the Thornton Bank and Goote Bank
and Zone II including the Oost Dyck, Buiten Ratel and
Kwinte Bank (figure 1).

This study is part of a research programme and investigates
the impact of sand and gravel extractions on the benthic
fauna, the macrobenthos particularly. Several papers and
reports have already appeared on the subject, concerning
both macro- and meiobenthos (Vanosmael et al., 1979 ;
1982a; 1982b ; 1984 and Willems et al., 1982a ; 1982b).
This report presents a summary of the results from a long
term survey (5 years) on three sandbanks : the Goote Bank
(zone I) is exploitated partially by the Ministry of Public
Transport ; the Kwinte Bank and Buiten Ratel (zone II) are
exploitated by private companies.

Each sandbank is represented by two stations which are
sampled twice a year, in spring and in autumn. Basic
parameters such as density, diversity, species number,
evenness and dominance are used to compare the communities
and to investigate whether there is an impact of the sand
exploitation on the macrofauna. The four major groups are
the Polychaeta, Mollusca, Crustacea and Echinodermata.
Nemertini, Archiannelida and Oligochaeta have also been
counted.

A detailed inventory has been made but is not included in
this paper.

Material and methods

In this study we have concentrated on six stations located
on three sandbanks. Their position is given in Table 1 and
Figure 1.

Due to logistic problems the autumn samples of the Kwinte
Bank (40004-40009) and Buiten Ratel (44009) have not been
taken in October 1984 but in early December 1984. Samples
were taken by a Van Veen grab (0.1 m^2). They were immedia-
tely fixed with 40% neutralized formalin up to a final
concentration of about 7%. The fauna was elutriated in the
laboratory on a 1 mm sieve. Three replicates of each
station were examined (except for 44003-March '84 only one
sample was taken for 44002 and 40009-April '82 ;
44009-April '83 and 40004-September '83, only two samples
were used).
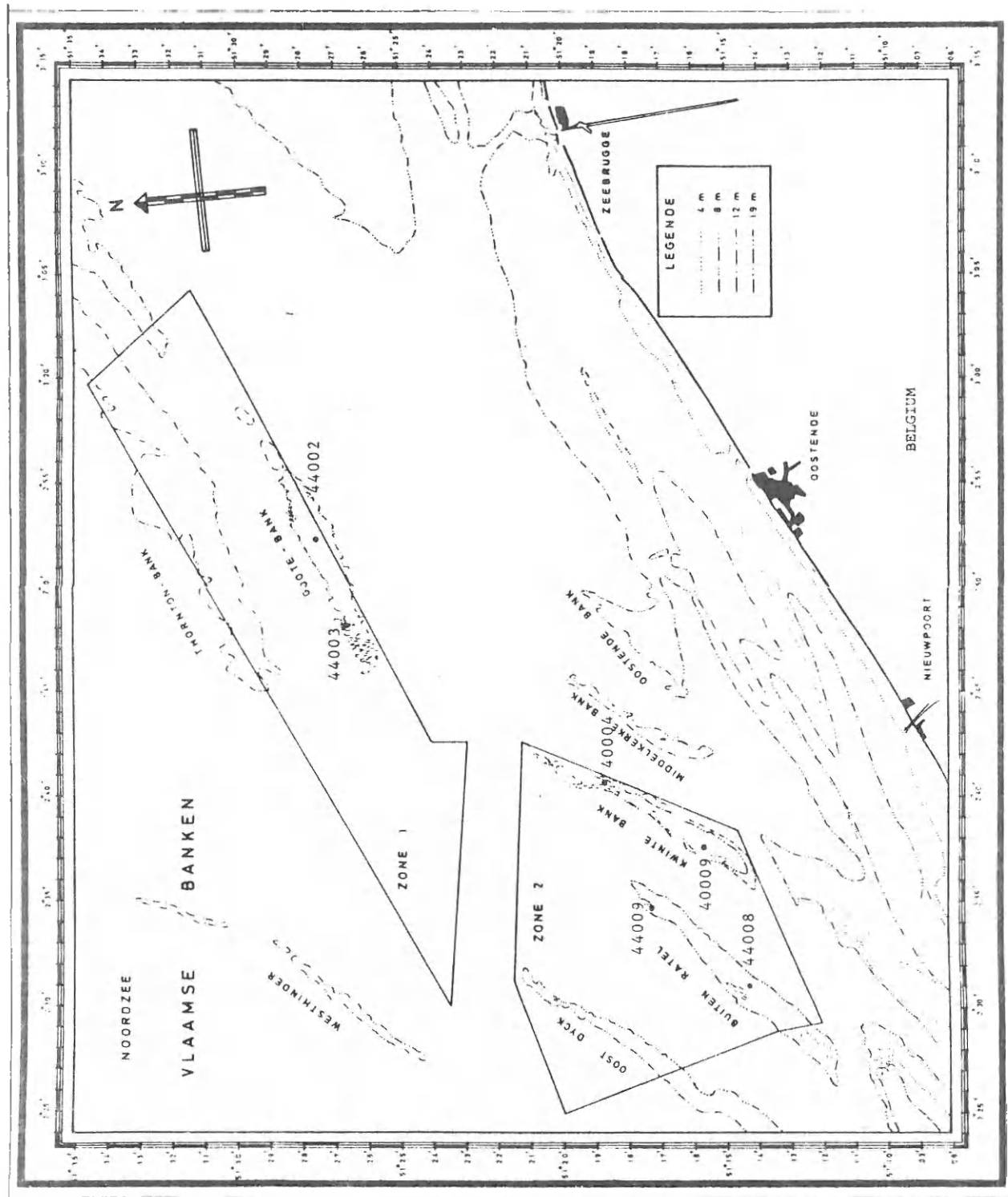
Fig. 1. Localisation of the six stations in the two exploitation zones.

All macro-organisms (Nemertini-Polychaeta-Archiannelida-Oligochaeta-Mollusca-Crustacea and Echinodermata) were identified where possible and counted.

Species diversity was estimated using the Shannon Wiener formula

$$H' = - \sum_i p_i \; \log_2 \; p_i$$

with $p_i = n_i/N_i$ the relative abundance of the i-th species.

Evenness - equitability of the allocation of individuals between the species - was calculated using the formula proposed by Heip (1974)

$$H_c = (e^{H'} -1)/(S-1)$$

with H' = species diversity and S = number of species. Evenness was also calculated using the formula

$$N_2 = 1/SI$$

in which SI = Simpson index $= \sum p_i^2 \quad 0 < SI < 1$.

Results and discussion

<u>Sediment analysis</u>

The location of the sampled stations and their sediment characteristics (mean values) are given in Table 1.

<u>Table 1.</u> Coordinates of the six stations and sediment characteristics over the 1980-1984 period. (Mean grain size of the sand fraction, mean % mud, sand and gravel).

Station	Lat. N	Long.E	Grain size Md mm	% mud < 63 µm	% sand	% gravel
40004	51.18'40"	2.40'45"	0.516±0.079	0.55±0.41	92.27±2.75	7.19±2.82
40009	51.15'35"	2.37'35"	0.260±0.011	0.09±0.02	99.91±0.02	0
44008	51.14'17"	2.31'29"	0.217±0.004	0.46±0.25	99.54±0.25	0
44009	51.17'30"	2.34.40"	0.651±0.105	0.46±0.28	92.51±3.64	7.03±3.69
44002	51.27'37"	2.52'20"	0.250±0.011	0.20±0.05	99.80±0.05	0
44003	51.26'37"	2.48'12"	0.328±0.015	0.18±0.03	92.06±2.21	7.76±2.21

The sediments are clean sands with a very low mud content. This can be explained by the very high hydrodynamic pressure on all three sandsbanks, a situation we also found on the other Flemish Banks (Oost Dyck and Middelkerke Bank) and Zeeland Bank (Thornton Bank). The perturbations are much stronger in the Northern part of the Buiten Ratel and Kwinte Bank which results in a coarser sediment (40004 and 44009 both coarse sand) with a relatively high amount of gravel than in the southern parts (40009 : medium sand and 44008 fine sand). This was already established on the Kwinte Bank by Bastin (1974) and brought into relation with

the macro- and meiofauna by Vanosmael et al., 1982b and
Willems et al., 1982a, b.

Remarkable are the relative great differences in grain size
(cfr. standard error) at stations 40004 and 44009.
According to De Moor (pers.comm.) there seems to be a
continuous sand transport from the northern part of the
Buiten Ratel to the northern part of the Kwinte Bank. This
can explain the greater fluctuations in grain size over the
five years. On the Goote Bank the differences between the
two stations are not so extreme. At the stations 44002 and
44003 we found respectively fine and medium sand. The
gravel content in 44003 (7.8%) is much higher than in 44002
(none). These observations are reflected in the faunal
composition of the sandbanks.

The fluctuations of the sediment characteristics within one
station are the result of three factors :
    - on the sandbanks there are many relative small
patches of different sediment compositions (cfr. surface
structure of the sandbanks (Bastin, op.cit.)).
    - because of strong turbulence and currents, often
large displacements of sand take place, for example after a
storm.
    - often it is very difficult to get the ship in the
right position because of heavy currents and stations are
not always sampled exactly on the same place.

Species composition

Species numbers are represented in Table 2 and Figure 2.

The total number of species in a station for the three
sandbanks varies between 9 and 34 species. Only 1 species
was recorded from the Goote Bank at station 44003 in April
1982. Seven months later we found at that station the
highest number of species (34) ever recorded. On the two
Flemish banks the highest means (20 species at 40004 and 19
species at 44009) are found at the nortern stations with
the coarsest sand and the highest amount of gravel. The
same is true on the Goote Bank where the mean number of
species is highest at the station with the coarsest sediment
(19 species at 44003 ; 17 at 44002).

Station 44008 is the most stable (cfr. low standard error
on the mean) while at station 44003 the number of species
varies most over the different years.

Table 2. Species number in sandbank stations.

	Kwinte Bank		Buiten Ratel		Goote Bank	
	40004	40009	44008	44009	44002	44003
03/80	22	12	11	15	12	14
09/80	25	15	13	13	17	16
03-04/81	21	9	16	13	17	19
09-10/81	23	13	15	22	12	27
04/82	19	20	15	30	10	1
10-11/82	27	9	14	19	—	34
04/83	17	17	15	10	26	28
09/83	12	11	18	17	30	20
03/84	25	20	16	30	14	9
10-12/84	9	12	13	24	13	19
mean	20.0±1.9	13.0±1.3	14.6±0.6	19.3±2.2	16.8±2.3	18.4±3.0

The species that are responsible for the differences in
species number between fine-medium and coarse sand stations
are mainly interstitial polychaetes. Coarse sediments offer
more interstitial space than fine sands.

Our findings confirm the observations of Wieser (1959) and
Fenchel (1978). Interstitial life only occurs when the
median grain size is larger than 200 µm. Interstitial
polychaetes get very abundant aboven 300 µm grain size,
although they can also occur in finer sediments.

At the coarse sand station 40004 of the Kwinte Bank, we
find several species that are restricted to that station.
The following species occur during at least three out of
ten investigated periods (regardless of dendity) : Pisione
remota, Microphthalmus similis, Streptosyllis arenae,
Sphaerosyllis bulbosa, Glycera capitata, Goniadella bo-
bretzkii, Orbinia sertulata, Aonides paucibranchiata, Ma-
crochaeta heloglandica, Polycirrus medusa, Nucula sp., My-
tilus edulis, Thia scutellata and Ophiura texturata. Very
abundant at 40004 are also Hesionura augeneri, Polygordius
appendiculatus and Spisula solida.

Species restricted to 44009 (coarse sand-Buiten Ratel) are
: Pisione remota, Eteone longa, Typosyllis armillaris,
Streptosyllis arenae, Sphaerosyllis bulbosa, Glycera capi-
tata, Goniadella bobretzkii, Aonides paucibranchiata,
Polycirrus medusa, Polygordius appendiculatus, Protodrilus
sp., Protodriloides chaetifer, Saccocirrus sp., Spisula
elliptica, Spisula solida, Bodotria scorpioides and Ophiura
sp. Abundant and occuring with a high frequency are :
Hesionura augeneri and Oligochaeta.

Table 3. Mean densities (N.m^{-2}) of the soft bottom macrofauna in three sand banks during the period 1980-1984.

KWINTE BANK

	1980 March 44002	1980 March 44003	1980 Sept 44008	1980 Sept 44009	1981 April 44002	1981 April 44003	1981 Oct 44008	1981 Oct 44009	1982 April 40004	1982 April 40009	1982 Oct 40004	1982 Oct 40009	1983 April 40004	1983 April 40009	1983 Sept 40004	1983 Sept 40009	1984 March 40004	1984 May 40009	1984 Dec 40004	1984 Dec 40009
NEMERTINI	20	30	110	107	10	40	157	87	27	25	40	3	57	7	5	30	30	37	60	10
POLYCHAETA	450	167	343	273	627	217	770	227	747	240	333	123	393	130	148	227	387	87	103	77
ARCHIANNELIDA	87	--	--	--	10	--	340	7	313	--	27	--	67	3	100	--	--	--	--	--
OLIGOCHAETA	67	7	5	--	17	--	73	7	40	--	30	--	80	--	--	--	20	--	--	--
MOLLUSCA	90	3	10	3	20	--	20	--	70	30	70	--	40	30	30	--	27	47	--	3
CRUSTACEA	3	27	143	47	7	--	17	40	--	225	37	60	10	937	85	43	307	107	3	17
ECHINODERMATA	10	--	17	7	--	--	--	--	--	3	--	--	--	3	--	--	20	--	--	7
TOTAL MACROBENTHOS	577	230	626	437	744	277	1377	368	1197	533	537	186	647	1110	360	300	791	278	166	114

BUITEN RATEL

	1980 Febr. 44008	1980 March 44009	1981 April 44008	1981 April 44009	1981 Oct 44008	1981 Oct 44009	1982 April 44008	1982 April 44009	1982 Oct 44008	1982 Oct 44009	1983 April 44008	1983 April 44009	1983 Sept 44008	1983 Sept 44009	1984 March 44008	1984 March 44009	1984 Oct 44008	1984 Dec 44009
NEMERTINI	+	+	7	53	17	183	10	60	--	110	7	10	7	110	--	153	23	197
POLYCHAETA	73	3083	223	280	63	1107	183	343	100	417	247	295	250	337	91	276	63	600
ARCHIANNELIDA	--	50	--	390	--	37	--	37	--	60	--	380	--	77	--	103	--	197
OLIGOCHAETA	7	197	--	53	--	70	--	3	--	10	--	35	--	20	--	40	--	120
MOLLUSCA	10	--	57	17	10	37	90	40	--	20	53	--	1	23	20	153	--	50
CRUSTACEA	120	7	150	--	147	50	63	37	220	33	87	--	237	63	27	83	83	13
ECHINODERMATA	--	--	23	--	7	--	57	10	--	--	17	--	7	--	--	10	77	3
TOTAL MACROBENTHOS	(210)	(3327)	460	793	244	1484	403	530	320	650	411	720	504	530	140	818	246	1180

GOOTE BANK

	1980 March 44002	1980 March 44003	1980 Sept 44002	1980 Sept 44003	1981 March 44002	1981 March 44003	1981 Sept 44002	1981 Sept 44003	1982 April 44002	1982 April 44003	1982 Nov 44002	1982 Nov 44003	1983 April 44002	1983 April 44003	1983 Sept 44002	1983 Sept 44003	1984 March 44002	1984 March 44003	1984 Oct 44002	1984 Oct 44003
NEMERTINI	+	--	+	+	10	23	17	60	--	--		217	23	73	57	90	37	240	7	113
POLYCHAETA	440	560	220	272	323	280	117	405	520	--		257	1027	223	1013	173	337	210	40	97
ARCHIANNELIDA	7	13	--	--	--	--	--	5	--	--		--	--	--	--	--	--	--	--	--
OLIGOCHAETA	3	217	7	87	7	--	3	15	--	--		7	--	10	83	7	2	150	10	33
MOLLUSCA	7	13	17	13	20	10	13	45	--	--		23	17	27	211	10	30	--	--	17
CRUSTACEA	20	20	107	140	60	30	240	820	210	57		187	267	467	211	77	47	10	70	77
ECHINODERMATA	--	--	--	7	--	--	--	10	--	--		13	10	87	3	20	--	10	3	3
TOTAL MACROBENTHOS	(477)	(1823)	(351)	(519)	420	343	390	960	730	37		704	1344	987	1369	377	453	620	130	340

Fig. 2. Species number (S) in six sandbank stations over the period 1980-1984.

KWINTEBANK

40008
40004

BUITEN RATEL

44009
44008

GOOTEBANK

44003
44002

SPECIES NUMBER

On the Goote Bank the difference in grain size is not so
great as on the other two sandbanks. Species number at
both stations (44002 and 44003) are very similar. Only
Polycirrus medusa, Spisula solida, Thia scutellata, Ophio-
thrix fragilis and Echinocyamus pusillus are restricted to
44003 (mean Md = 328 μm). H. augeneri and G. capitata
reach higher densities at 44003 than at 44002 and also
occur with a higher frequency at 44003.

Density (N/m^2)

The densities of the macrobenthic taxonomic groups on the
six stations in 1980-1984 are given in Table 3 and Figure 3.

The highest density in the investigated area is recorded at
station 44009 (Buiten Ratel) in March 80 (3337 ind./m^2 ;
Nemertini not included), the lowest at 44003 (Goote Bank) in
April 82 (37 ind./m^2)

The mean abundances of each station over the whole period
are :

Kwinte Bank		Buiten Ratel		Goote Bank	
40004	40009	44008	44009	44002	44003
742±116	383±89	326±37	1116±263	629±147	661±156

Not only species number but also density fluctuates very
little at station 44008 (Buiten Ratel).

- On the Kwinte Bank the highest densities are found at
station 40004 in October '81 (1377 ind./m^2) and April '82
(1197 ind./m^2) and at 40009 in April '83 (1110 ind./m^2).
At station 40004 polychaetes have the greates share in the
total population (resp. 55.9% and 62.4%), at station 40009
the Crustacea are dominant (84.4%).
Looking at species level, the interstitial polychaete
Hesionura augeneri (330 ind./m^2) and the interstitial
archiannelid Polygoridus appendiculatus (320 ind./m^2) are
responsible for the high density at 40004 in October '81.
In April '82 it is the interstitial polychaete Sphaerosyl-
lis bulbosa (220 ind./m^2), Goniadella bobretzkii (250 ind./
m^2). At station 40009 (April '83) Bathyporeia elegans is
the most abundant animal (885 ind./m^2).

- On the Buiten Ratel the macrofauna density at station
44009 is always higher than at 44008. The highest values
are 3337 ind./m^2 in March '80, 1484 ind./m^2 in October '81,
1030 ind./m^2 in April '82 and 1180 in December '84. In all
the periods polychaetes have the largest density (resp. 92%,
75%, 82% and 51%). On this sandbank we note the highest and
the lowest mean density of all three sandbanks over the
whole period : 326 ind./m^2 at 44008 and 1116 ind./m^2 in
44009.

In March '80 H. augeneri is very abundant : 2585 ind./m^2
Ophelia borealis reaches a density of 385 ind./m^2 In the
other periods (10/81, 04/82 and 12/84) H. augeneri is also
the most important species (resp. 955, 435 and 380
ind./m^2). In April '82 Scoloplos armiger has a density of
150 ind./m^2, which is high within the area.

- On the Goote Bank the overall means are very similar (629
ind./m^2 in 44002 and 661 ind./m^2 in 44003). The highest
densities are recorded at station 44003 (1823 ind./m^2 in
March '80) and station 44002 in April and September '83
(resp. 1344 and 1369 ind./m^2). In the three cases the po-
lychaetes are the most abundant (resp. 86%, 76% and 74%).
More in detail : the following species are responsible for
the high densities : Hesionura augeneri (920 ind./m^2), Spio
filicornis (240 ind./m^2) and Ophelia borealis (327 ind./m^2)
at station 44003 in March '80 ; Scoloplos armiger and Spio-
phanes bombyx (resp. 410 and 490 ind./m^2 at 44002 in
April '83) and S. bombyx (with a density of 740 ind./m^2
at 44002 in September '83).

It is clear that density is generally highest in the coarse
sediments (stations 40004 and 44009) where interstitial life
gets very important. This is reflected in the abundance of
the interstitial annelids at these stations.

On the Goote Bank, Buiten Ratel and Kwinte Bank, but also
on the Thornton Bank, Oost Dyck and Middelkerke Bank, the
share of the molluscs is very low, although they can raise
the biomass highly, especially when the genus Spisula is
present. At station 44009 (March '84) 146 ind./m^2 are
found. The crustaceans on the other hand are common on the
sandbanks. Sometimes they are more numerous than polychae-
tes (Table 3). The highest crustacean density is recorded
at station 40009 in April '83 (937 ind./m^2 of which 885
ind. belong to the species Bathyporei elegans. Not very
abundant are the echinoderms. The highest densities are
found at station 44008 in October '84 and at 44003 in April
'83 : resp. 77 ind./m^2 (all Echinocardium cordatum) and
87 ind./m^2 of which 77 ind. are Ophiothrix fragilis. It
must be said that O. fragilis is not common on the sand-
banks and is only recorded on the Goote Bank.

Diversity (H') - Evenness (H$_e$) - Dominance (SI)

The values are given in Tables 4, 5 and 6 and represented in
the figures 4, 5 and 6. The mean diversities per station
are varying between 2.59 bits/ind. at 44009 (Buiten Ratel)
and 3.11 bits/ind. at 40004 (Kwinte Bank).

Evenness mean values are fluctuating between H. = 0.33
(Buiten Ratel - 44009) and H$_e$ = 0.54 (Kwinte Bank - 40009).
The lowest mean Simpson Index : SI = 0.19 is found on the
Kwinte Bank (40004) and Goote Bank (44003). The highest
value (SI = 0.30) at 44009.

The highest and the lowest diversity are both recorded from the Goote Bank at station 44003 and are respectively H' = 4.15 (Nov. '82) and H' = 0.00 (April '82). The corresponding evenness values are H_e = 0.51 and H_c = 0.00. The intermediate evenness can be explained by the dominance of two species : <u>Ophelia borealis</u> (60 ind./m$^{\ell}$) and <u>Bathyporeia</u> <u>elegans</u> (83 ind./m$^{\ell}$). The Simpson index in November '82 is very low : SI = 0.09.

Worth mentioning are also the high diversity scores at 40004 (Kwinte Bank) in September '80 (H' = 3.90) and October '82 (H' = 3.84). Corresponding evenness values are respectively H_e = 0.58 and H_e = 0.51. In both periods the Simpson's dominance index is very low (resp. SI = 0.10 and 0.11). Low diversities are also recorded at 40009 (Kwinte Bank) in April '83 (H' = 1.30), at 44009 (Buiten Ratel) in March '80 and October '81 (resp. H' = 1.00 and 1.58). With the los diversities at the two stations corresponds a low evenness (resp. H_e = 0.09, 0.07 and 0.09) and high Simpson indices (resp. SI = 0.66, 0.69 and 0.61). As for th number of species we found respectively S = 17, 15 and 22 which are not extremely low values for the area.

Table 4. Species diversity (H') in six sandbank stations.

	Kwinte Bank		Buiten Ratel		Goote Bank	
	40004	40009	44008	44009	44002	44003
03/80	2.43	2.84	2.71	1.00	2.67	1.81
09/80	3.90	2.92	2.60	2.90	3.52	3.43
03-04/81	2.93	2.46	3.27	2.57	3.38	2.98
09-10/81	2.94	3.06	2.53	1.58	2.69	3.07
04/82	3.00	3.16	3.12	3.07	1.68	0.00
10-11/82	3.84	2.74	2.51	3.16	-	4.15
04/83	3.18	1.30	3.33	2.14	2.75	2.76
09/83	2.96	2.79	3.37	2.95	2.80	3.53
03/84	3.20	3.59	3.18	3.57	2.61	2.35
10-12/84	2.72	3.23	2.37	2.37	2.97	3.64
mean	3.11±0.15	2.81±0.19	2.90±0.12	2.59±0.25	2.79±0.18	2.77±0.37

Table 5. Evenness (H$_e$) in six sandbank stations.

	Kwinte Bank		Buiten Ratel		Goote Bank	
	40004	40009	44008	44009	44002	44003
03/80	0.21	0.56	0.56	0.07	0.49	0.19
09/80	0.58	0.47	0.42	0.54	0.66	0.65
03-04/81	0.33	0.56	0.57	0.41	0.59	0.38
09-10/81	0.30	0.61	0.34	0.09	0.50	0.28
04/82	0.39	0.42	0.55	0.25	0.24	0.00
10-11/82	0.51	0.71	0.36	0.44	–	0.51
04/83	0.51	0.09	0.65	0.38	0.23	0.21
09/83	0.62	0.59	0.55	0.42	0.21	0.55
03/84	0.34	0.58	0.54	0.38	0.39	0.51
10-12/84	0.70	0.76	0.35	0.29	0.57	0.64

Table 6. Simpson index (SI) in six sandbank stations.

|	Kwinte Bank		Buiten Ratel		Goote Bank	
	40004	40009	44008	44009	44002	44003
03/80	0.36	0.20	0.23	0.69	0.20	0.40
09/80	0.10	0.19	0.24	0.19	0.11	0.11
03-04/81	0.27	0.23	0.13	0.23	0.13	0.22
09-10/81	0.19	0.16	0.31	0.61	0.20	0.22
04/82	0.17	0.19	0.14	0.24	0.47	1.00
10-11/82	0.11	0.17	0.31	0.17	–	0.09
04/83	0.15	0.66	0.13	0.31	0.25	0.32
09/83	0.16	0.18	0.13	0.19	0.33	0.13
03/84	0.19	0.12	0.19	0.13	0.28	0.28
10-12/84	0.18	0.14	0.29	0.24	0.21	0.12
Mean	0.19±0.02	0.22±0.05	0.21±0.02	0.30±0.06	0.24±0.04	0.19±0.04

On the Kwinte Bank (40009-April '83) the amphipod Bathypo-
reia elegans is clearly dominant (80% of the total fauna).
Hesionura augeneri is dominant at station 44009 in March
'80 and October '81. The species reaches relative abun-
dances of respectively 77% and 64%. In March '80, Ophelia
borealis is also dominant (42%).

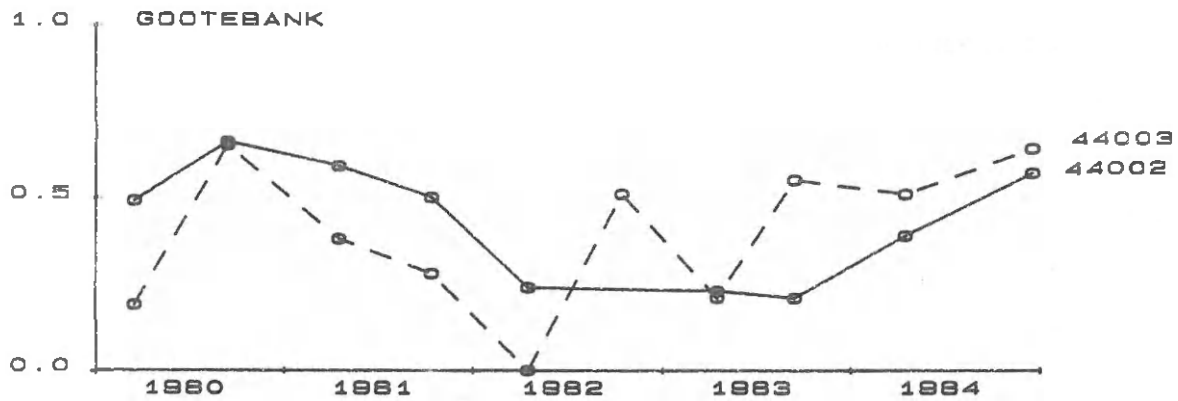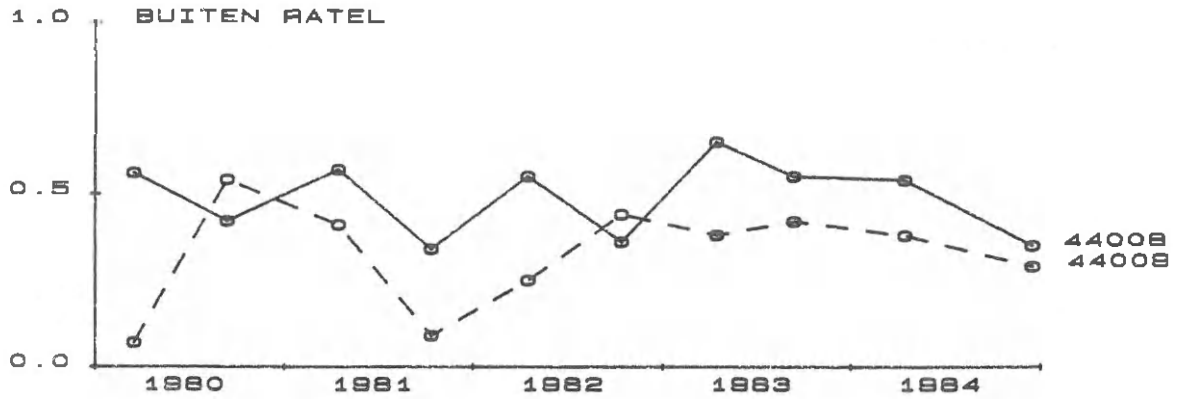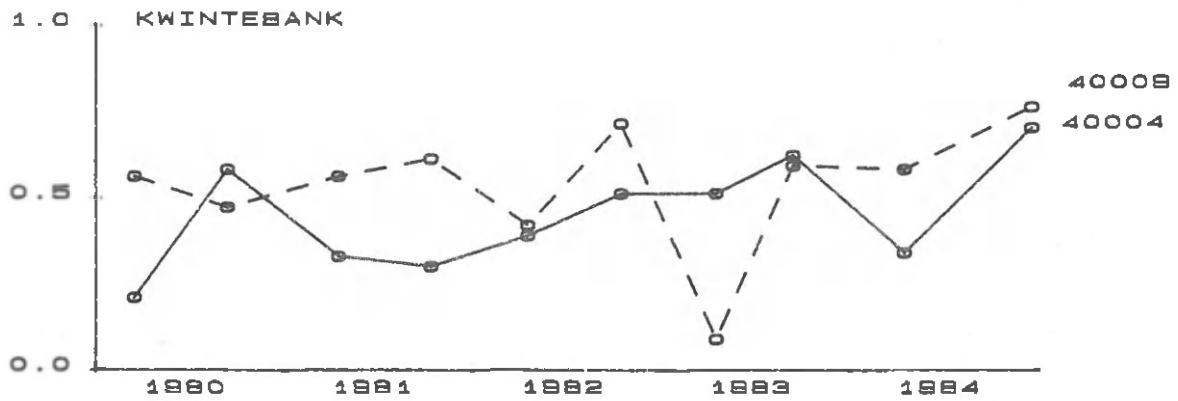Fig. 3. Fluctuation in density (N.m^{-2}) in six sandbank stations over a period of five years.
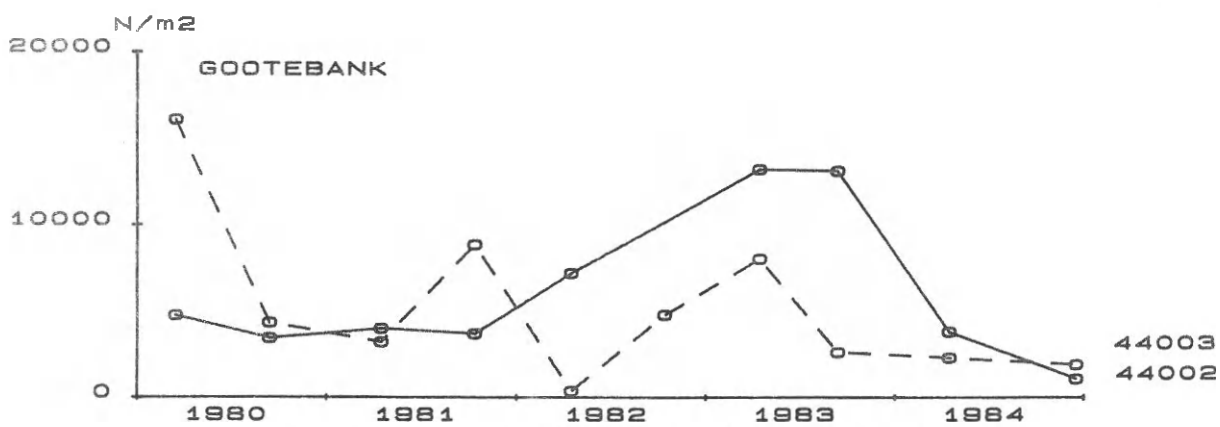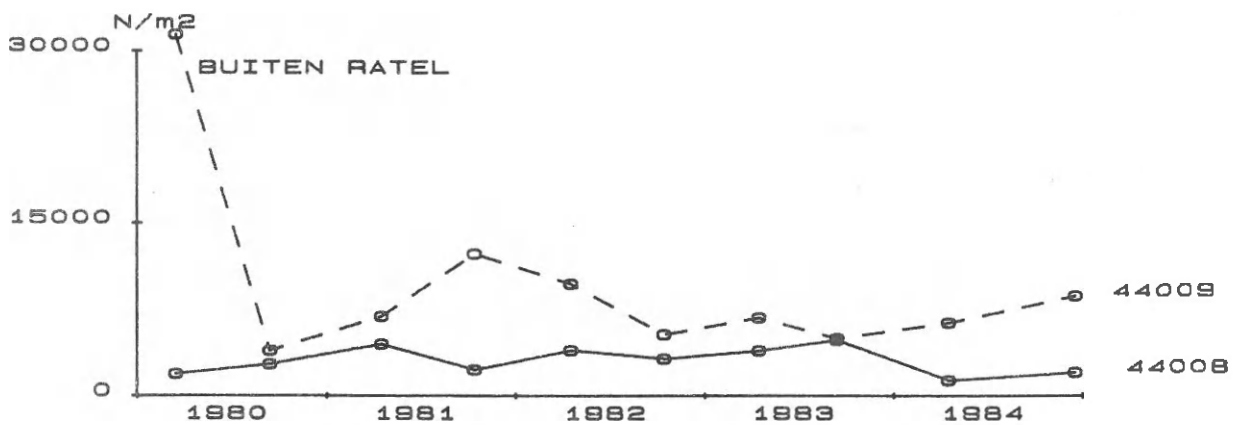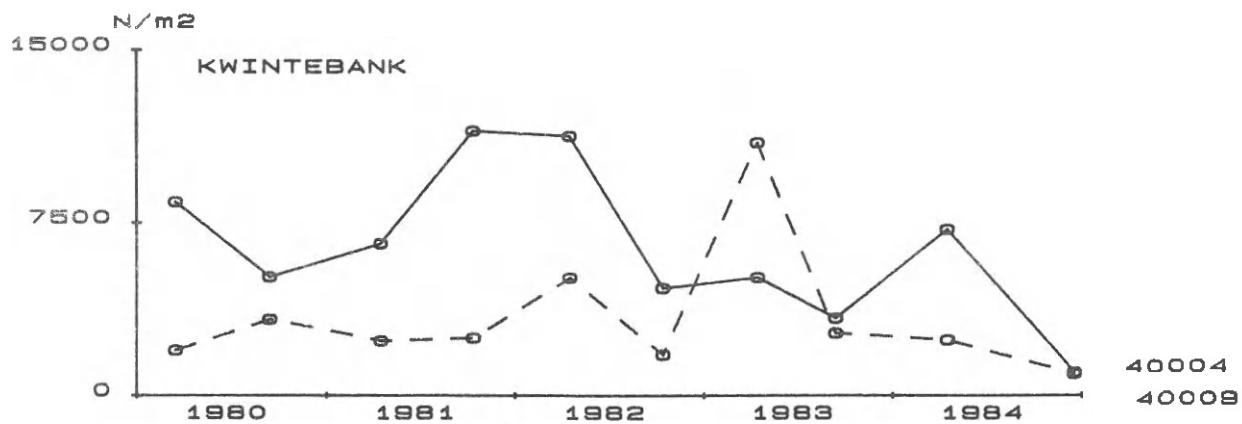
Fig. 4. Evolution of the diversity (H') in six sandbank stations during the period 1980-1984.
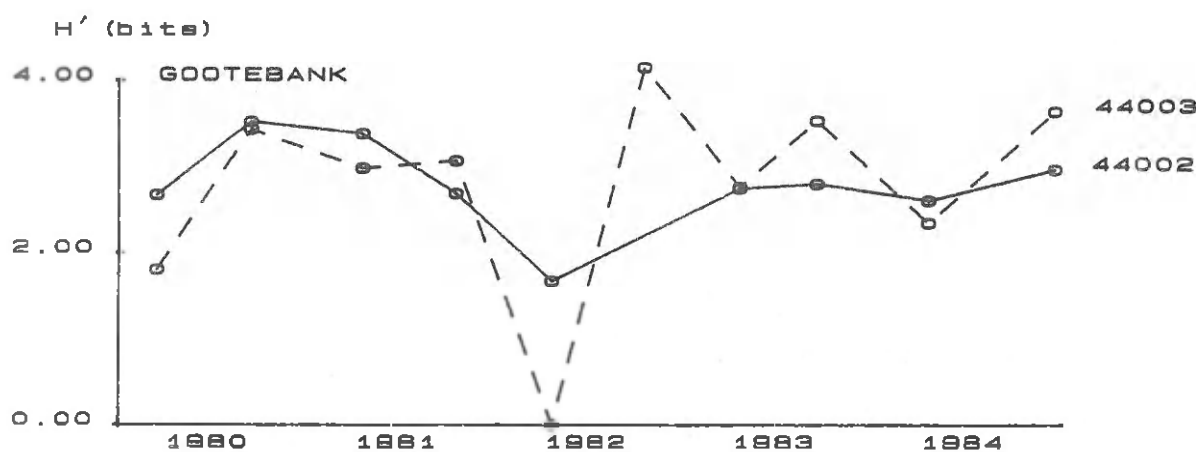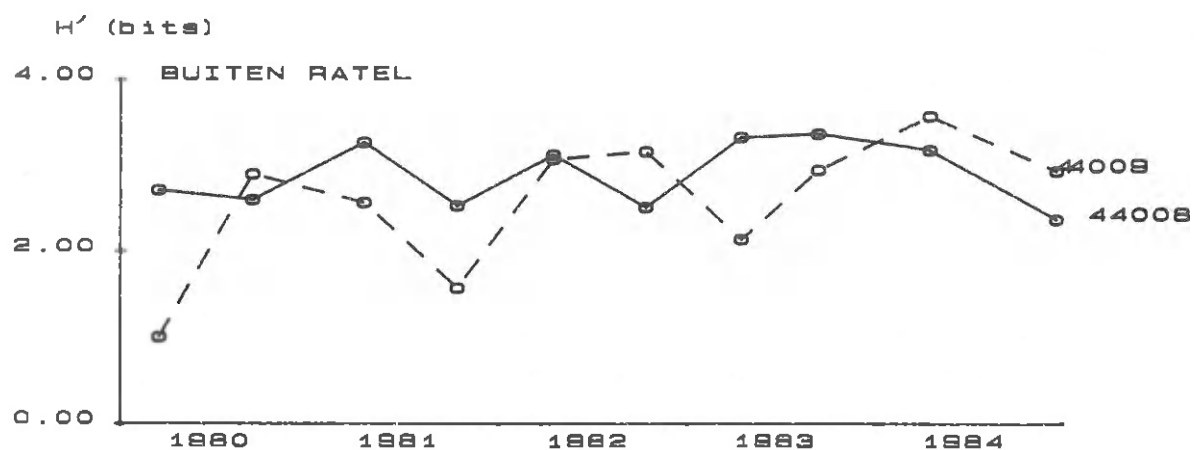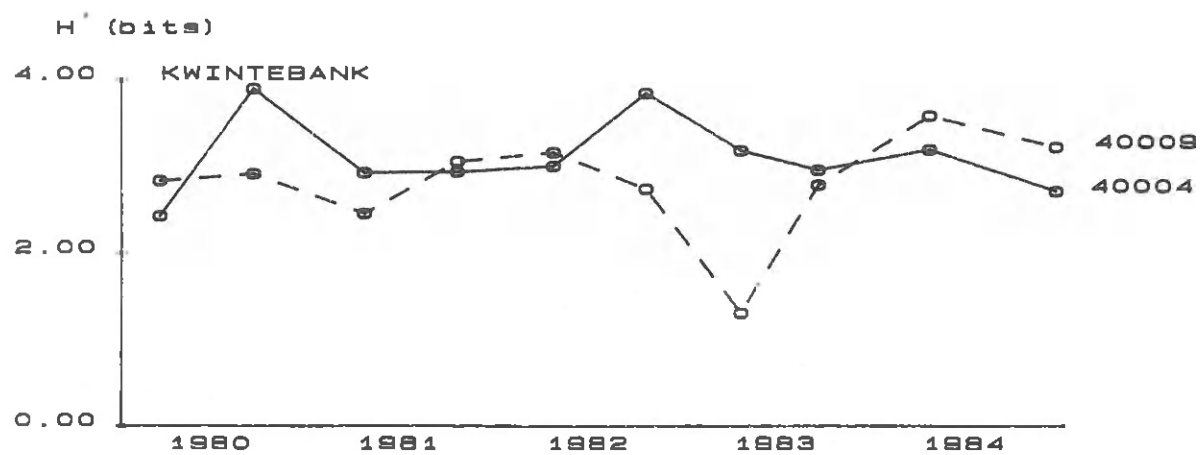
Fig. 5. Evolution of the evenness H_c in six sandbank stations during the period 1980-1984.

EVENNESS

Fig. 6. Evolution of the dominance SI in six sandbank stations
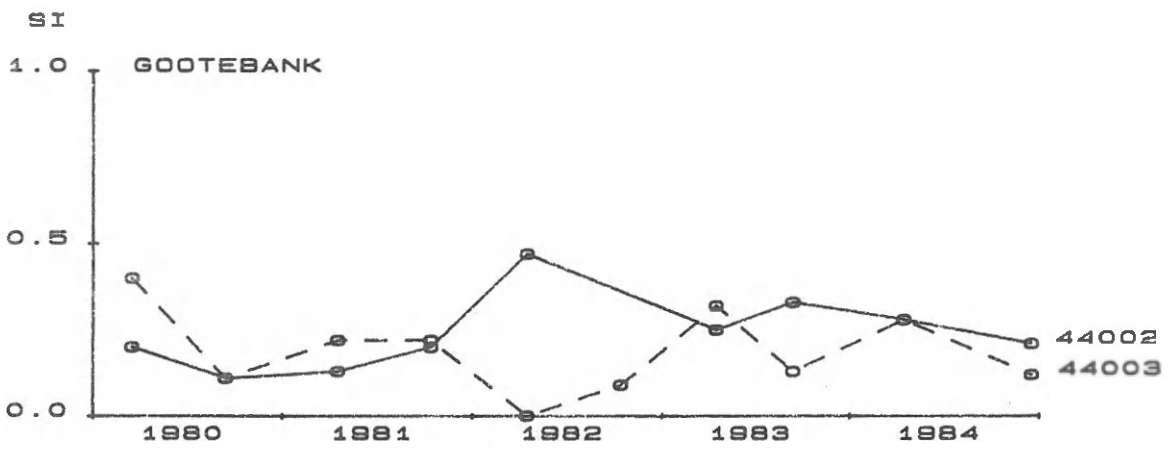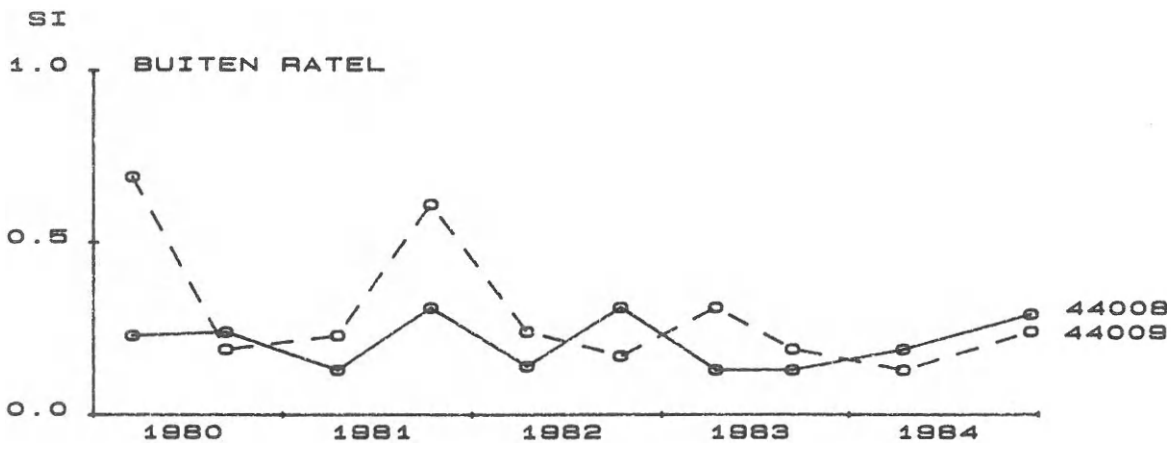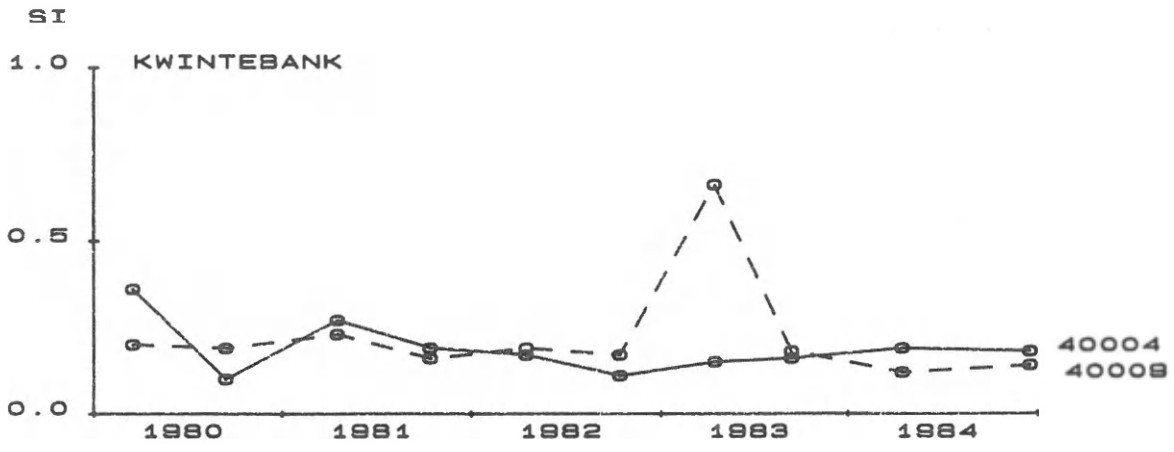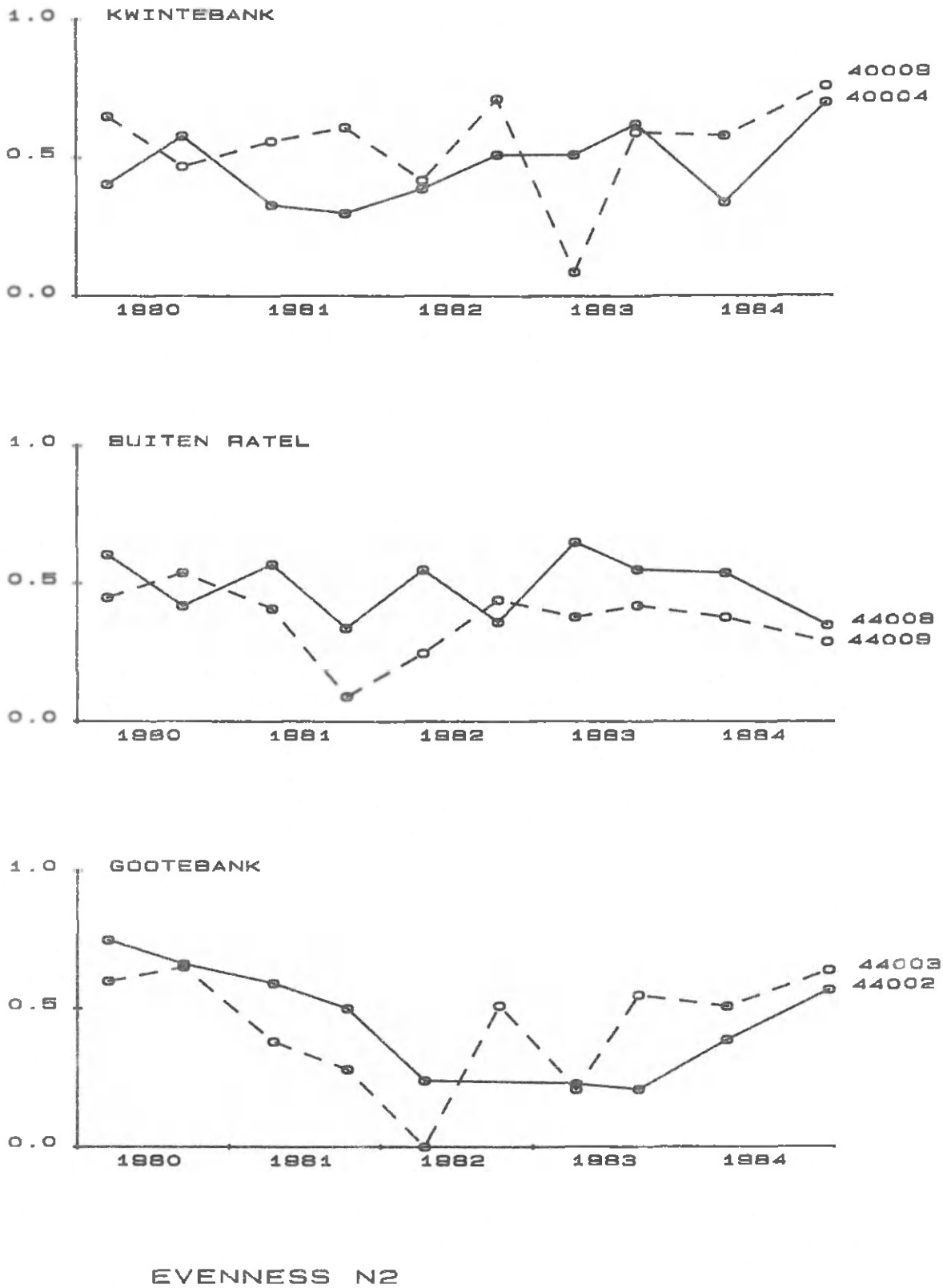during the period 1980-1984.

Fig. 7. Evolution of the evenness N_2 in six sandbank stations
during the period 1980-1984.

EVENNESS N2

The dominance of one or two species explains the obser-
vations mentioned above.

It is easy to correlate density (N), species number (S) and
species composition with the sediment characteristics of the
three sandbanks. This is not the case for the community
parameters diversity (H'), Evenness (H.) and dominanbce
(SI). Lacking at the overall means per station no trends
corresponding to the sediment compositon can be established.

General considerations

The species composition of the Kwinte Bank, Buiten Ratel
and Goote Bank is very similar. Among the Polychaeta, 26
species are common to the three sandbanks. In total 57
different species are recorded within the investigated area.
As to the Archiannelida, three species are incommon on a
total of four species ; for the Mollusca it is five species
on a total of 36 species and two Echinodermata species on a
total of six species.

Polychaeta species occuring on all three sandbanks are :
Pisione remota, Eteone longa, Hesionura augeneri, Anaitides
subulifera, A. mucosa, Eumida sanguinea, Microphthalmus
similis, Streptosyllis arenae, Nephtys ciriosa, N. caeca,
N. longosetosa, Glycera capitata, Goniadella bobretzkii,
Scoloplos armiger, Orbinia sertulata, Spio filicornis,
Spiophanes bombyx, Aonides paucibranchiata, Scolelepis
bonnieri, Magelona papillicornis, Chaetozone setosa, Ophe-
lia borealis, Capitella capitata, Notomastus latericeus,
Heteromastus filiformis and Polycirrus medusa.

For the Archiannelida : Polygordius appendiculatus, Pro-
todrilus sp. and Protodriloides chaetifer.

For the Mollusca : Mysella bidentata, Fellina fabula, Abra
alba, Spisula elliptica and S. solida.

For the Crustacea : Gastrasaccus spinifer, Pseudocuma lon-
gicornis, Diastylis rathkei, D. bradyi, Tanaissus lilljee-
borgi, Megaluropus agilis, Melita obtusata, Atylus falca-
tus, Urothoe poseidonis, Bathyporeia guilliamsoniana, B.
elegans, Pontocrates altamarinus, Panambus typicus, Thia
scutellata and Liocarcinus holsatus.

For the Echinodermata : Echinocyamus pusillus and Echino-
cardium cordatum.

Most of the species are typical clean sand inhabitants well
adapted to life in an environment with high hydrodynamical
stress. Many are mobile species, as settling is hampered
by the large displacements of sand after storms and even at
the turning of the tide.

In median and coarse sands, interstitial life gets very important. This is also reflected in the sandbank fauna. Nine interstitial polychaete species are recorded : <u>Pisione remota</u>, <u>Hesionura augeneri</u>, <u>Microphthalmus similis</u>, <u>Typosyllis armillaris</u>, <u>Streptosyllis avenae</u>, <u>S. websteri</u>, <u>Sphaerosyllis bulbosa</u>, <u>Autolytis</u> sp. and <u>Macrochaeta helgolandica</u>. Their densities are of course very much under-estimated since a 1 mm sieve was used.

A high density does not necessarily correspond with a high diversity. On the sandbanks the highest densities are recorded from the coarse stations. On these, interstitial species often dominate the community. A fall of the diversity is the result.

A positive correlation between median grain size of the sediment and number of species and density is striking. The same can not be said of the other community parameters such as diversity (H'), evenness (H.) and dominance (SI).

Mean H', H. and SI-values per station do not vary very much, species composition is also very similar on the three sandbanks. Meheus (1981) and De Rycke (1982) made the same observations for all the sandbanks in the area (Thornton Bank, Goote Bank, Oost Dyck, Buiten Ratel, Kwinte Bank and Middelkerke Bank). The sediment of the investigated sandbanks is similar and consists of clean sand with a very small amount of mud. Other conditions are also similar : same water masses, hight temperature, depth, etc.

At the Goote Bank an 'accident' occurred in April '82 at station 44002. The number of species fell to one, density to 37 ind./m^2., diversity and evenness to zero. However seven months later the old situation was reestablished. The number of species rose to the maximal value for the area : 34 species. Density reached 704 ind./m^2 and diversity came to a maximum value of H' = 4.15 (H. = 0.51 and SI = 0.09). It is clear that a major disturbance occurred. It is also clear that recovery of the fauna was quick with some overshoot of equilibrium values. Whether the unexpected fluctuations have someting to do with the sand extractions is impossible to say, since the exact localizations of exploitation on this sandbank is not known to us. However the disturbance did not occur on other sandbanks or on the other Goote Bank station.

Acknowledgements

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ECOTOXICOLOGY

Comparison of demographic (life- and fecundity table analysis) and biochemical (ATP and AEC) characteristics as sublethal pollution indices in the marine nematode *Monhystera disjuncta*

31323

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Abstract

Sublethal effects of cadmium and nickel on demographic characteristics
and adenylate metabolism of *Monhystera disjuncta*, a marine free-living
nematode, were studied during chronic exposure in culture conditions.
Mortality is a rather insensitive criterium to predict the environmental
impact of pollutants. MEC (minimal effect concentration) values based
on development rate and daily egg production were the most sensitive
criteria : values were up to two orders of magnitude less than the cor-
responding LC50 (96h). EC50's, effective concentrations resulting in
a 50% inhibitory effect on either the intrinsic rate of natural increase
(r_m) or on net-fecundity (R_O) were, on the one hand, less sensitive
than MEC's based on development and egg production, but on the other hand
more than one order of magnitude less than LC50 values.
Significant decreases of ATP content were observed at concentrations
considerably less than the LC50'S. However, compared with the demogra-
phic characteristics studied, this criterion is less sensitive. It is
argued that neither ATP concentrations, nor AEC measurements can give
ecological relevant information about detrimental effects caused by
long-term exposure to   sublethal concentrations of the metals tested.

INTRODUCTION

Up to now, the most widely used groups of marine invertebrates for bio-
assay tests are bivalves, crustaceans and polychaetes. Undoubtely, the eco-
nomic importance of crustaceans and bivalves was the main reason for their se-
lection.
Despite their ecological importance (Heip *et al.*,1979 ; Warwick & Price,1979 ;
Heip *et al.*, 1985), marine free-living nematodes have only recently been used
as test organisms in a few studies (Bogaert *et al.*, 1984 ; Howell, 1984 ;
Tietjen, 1984 ; Vranken *et al.*, 1984a, 1985, 1986). The aim of the present
study was to research the sublethal effects of cadmium and nickel on the free-
living marine nematode *Monhystera disjuncta* in laboratory conditions.

Monhystera disjuncta is previously used as test organism in several bio-
assays, by Vranken and co-workers. Vranken *et al.*,(1984) found that the juve-
nile stage was the most sensitive life-stage, when exposed to three different
mercury compounds. Short-term acute tests with cadmium as toxicant showed
that LC50 values are very time-dependent and that MEC (minimal effect concen-
trations) values, based either on mortality or on a developmental assay in
which success in attaining the adult stage was tested, are probably ecolo-
gically more meaningful than LC50 values (Vranken *et al.*,1985). Finally, a
comparison between mortality, developmental rate and fecundity as toxicity-
indices was made (Vranken *et al.*,1986). Based on a large data-base (seven
heavy metals, pentachlorophenol and γ hexachlorocyclohexane were tested),
fecundity turned out to be the most sensitive criterion, though, MEC values
remained substantially high.

In this study , we tested for sub-lethal changes in demographic and
biochemical characteristics. The demographic characteristics studied are
mortality as a function of age, generation time and fecundity. From these
figures we calculated the intrinsic rate of natural increase (r_m) and the
net-reproductivity (R_o). Several authors proposed to determine EC50 (r_m)
values, the concentration which has a 50% inhibitory effect on population
growth (Hummon & Hummon, 1975 ; Sabatini & marcotte, 1983). They believe
that such EC50 values are more reliable criteria than LC50 values, to deter-
mine so-called treshold-concentrations with regard to safe-guarding commu-
nities of organisms in the environment. The exact determination of these
parameters is tedious since at each level of intoxication, complete life
and fecundity tables (l_x and m_x) have to be constructed. In our study we
tested how the EC50 (r_m) relates to the LC50 (96h) and the so-called MEC
value (Vranken *et al.*,1985). The latter variables are much easier to de-

determine, but it is generally feared that lethal concentrations in short-term experiments) may seriously underestimate the influence of pollutants on a population.

Adenylate energy charge as a measure of pollution stress

In the late 1960s, Atkinson & Walton (1967) proposed the adenylate energy charge (AEC) as a means of expressing the metabolic energy status of an organism. It is given by :

$$AEC = (ATP + \tfrac{1}{2} ADP) / (ATP + ADP + AMP)$$

and varies between 0 and 1. The observation that its value decreases in response to stress, irrespective of the type of stress, led Ivanovici & Wiebe (1981) to propose AEC as a general biochemical index of sublethal stress : in optimal conditions, AEC ranges between 0.8 - 0.9, values between 0.5 - 0.7 point to suboptimal but still viable conditions whereas at values below 0.5 viability is lost (Ivanovici, 1980).

In several bio-assay studies, AEC determinations were used to assess "sublethal" effects of man-made pollutants on marine organisms (Bakke & Skjol-dal, 1979 ; Zaroogian *et al.*, 1982 ; Neuhoff, 1983 ; Haya *et al.*, 1983). A major drawback, however, is the fact that no information is available in multicellular organisms on how a decrease of AEC is related to ecological relevant parameters as growth, reproduction, etc... (Livingstone, 1985). Without such information, the predictive power of AEC as an early warning indicator of unfavarouble environmental conditions is limited. If a decrease of AEC is correlated with for example low growth rates and/or impaired re-production, than AEC would represent one of the most sensitive biochemical indices of stress available at present.

Another drawback of AEC measurements concerns the reliability of the me-thodology used. Verschraegen *et al.*(1985) described a reliable assay for ATP, ADP and AMP in two polychaetes (*Nereis diversicolor* and *Nephtys* sp.). In this study we tried to adapt this method. The main problem was the very small biomass of the nematodes (± 0.5 µg adult freshweight for *Monhystera disjuncta* against a mean of about 0.3 g for the two polychaetes.

ATP-concentrations as indicator for pollution stress

Due to these problems, we mainly concentrated on the determination of the ATP-concentrations in the nematodes. ATP-concentrations were determined in different life-stages of the nematodes under Ni as well as under Cd intoxi-cation. The objective was to establish a possible correlation between changes in ATP-concentrations in an early stage of chronic exposure experiments and

changes in demographic characteristics as net-reproductivity R_0 and intrinsic rate of natural increase r_m. This would considerably reduce time necessary to evaluate sublethal effects.

MATERIAL AND METHODS

Testspecies - culturetechniques

Monhystera disjuncta was sampled in the Suice dock of Ostend, a marine lagoon near the Belgian coast. M.*disjuncta* is a marine bacterivorous nematode with a cosmopolitan distribution. Adults have a mean length of ± 0.85 mm. The procedures for isolation and cultivation are described at full lenght by Vranken *et al.*(1984a,b) and Vranken *et al.*(1985). Stockcultures were maintained on 0.5% bacto-agar (Difco) plates with a mixed bacterial culture as food. The experiments were conducted in completely controlled monoxenic cultures. A monospecific bacterial isolate (belonging to the *alteromonas haloplanktis* rRNA branch) was added as food in a ring-formed excavation in agarplates (see Vranken *et al.*, 1985). In order to avoid pH-fluctuations, Tris buffer (5mM) was added to the culture medium (Vranken *et al.*, 1986).

Toxicity tests

Nickel and cadmium were added as $NiCl_2.6H_2O$ (Merck) and $CdCl_2.2\frac{1}{2}H_2O$ (Baker Chemicals BV Holland), both of analytical grade. The final concentrations tested ranged between 0.5 and 10 mg/1 Cd and 1 and 35 mg/1 Ni. for each concentration, 3 replicates were studied. All cultures were kept in the dark at a constant temperature (17°C) and salinity (30%).

I. Demographic characteristics

The demographic characteristics studied are mortality as a function of age, generation time and fecundity. For each concentration, 10 gravid females were allowed to deposit eggs during 24h (48h for 35 mg/1 Ni). Development was daily followed to calculate minimal generation time T_{min}, egg mortality, juvenile and total preadult mortality. The minimum generation time T_{min} is estimated as the period between identical stages of successive generations - this is almost equal to the development time (Vranken & Heip, 1983). Criteria for death were inactivity and the lack of movement even after prodding with the tip of a needle.

When females became adult (after a period T_{min}), eggproduction of 5 adult ♀♀ together with 3 ♂♂, was counted every 2 days for each test con-

centration. Every 4 days the adults were transferred to a new culture.
To test for adult survival, observations were done every 2 or 3 days on
40 ♂♂ and 40 ♀♀ per concentration. Every 6 days, surviving adults were
transferred to fresh cultures to distinguish between parents and offspring.
Dead organisms were eleminated from the cultures.

Calculation of the demographic parameters :

The intrinsic rate of natural increase r_m is calculated with the Euler –
Lotka equation.

$$\sum_{x=0}^{max\ age} e^{-r_m}\ l_x m_x = 1$$

   x = pivotal age, age of the females in the age-interval $(X, X+1)$

   l_x = age-specific survival rate, probability to survive from the egg-
      stage onwards until age x

   m_x = age-specific fecundity, number of female offspring produced per
      female alive in the age interval $(X, X+1)$

The age-specific fecundity m_x is estimated from the egg-counts as $m_x = Ne_x \cdot p$
where Ne_x is the number of eggs produced by a female of the parental ge-
neration with age x and p is the proportion of females in the adult popula-
tion.

The net-reproductivity R_0, the multiplication rate per generation, is cal-
culated as

$$\sum_{x=0}^{max\ age} U_x = \sum_{x=0}^{max\ age} l_x m_x$$

   U_x = net-fecundity, the realized number of female offspring per female
      of the preceeding generation, with the latter in the age-interval
      $(X, X+1)$

The mean generation time T is estimated as $T = (lnR_0)/r_m$

II. Biochemical characteristics.

The tests were executed simultaneously with the demographic assay.
The organisms were harvested from two of the three replicates of each test-
concentration studied. Procedures to determine ATP, ADP and AMP were based
on the method used for *Nereis diversicolor* and *Nephtys* sp. in previous stu-
dies (Verschraegen *et al.*, 1985). This method is based on the firefly
bioluminescence reaction. The (ATP dependent) light emission of the
luciferin-luciferase substrate-enzyme complex was measured with the integra-
tion method. A Constant Light Signal (CLS) reagent was used as

bioluminescence reagent. For a detailed description of the reagents and
equipment used, see Verschraegen *et al.*, 1985.

a. Extraction procedures

Preliminary experiments revealed that the results improved when the
nematodes in the extracts were fractionated by sonication. It appeared
that by doing so, the variance between the results decreased considerably.
A sonicator (Brown, Labsonic 1510) with a needle probe ($\emptyset$: 4mm) was used
to generate waves of 20 kHz (power 100W) during 60 sec. To avoid warming
up of the extracts, the test tubes were placed in an ice-bath. Furthermore,
extracts had to be diluted (with Tris-HCl buffer : Tris 0.02M, pH 7.75) as
much as possible to reduce possible interfering factors (Karl & LaRock, 1975)
in ATP measurements. Prolongation of the extraction time from 1 min to 10
min did not significantly affect the results.

Comparison of 7 different extracting agents

For each technique, 4 or 5 replicate extractions were made in Eppendorf
Test Tubes, using adult nematodes from stockcultures. The media used were
ice-cooled, except otherwise mentioned. Extracts were stored at -20°C until
determination of the adenylate concentrations.

EXTR 1 : TCA : 10 nematodes were transferred into 50 µl TCA extraction me-
dium (0.5M trichloroacetic acid Cl_3CCOOH and 0.25 Na_2HPO_4).
After neutralization with 50 µl NaOH (0.5N) and dilution to 1 ml
with Tris-HCl buffer (Tris 0.02M ; pH 7.75), the extracts were sto-
red at -20°C. Immediate before the ATP determination, extracts
were sonicated during 60 sec.

EXTR 2 : H_2SO_4 : Extraction as in EXTR 1, but with H_2SO_4 (0.5N) instead of
TCA extraction medium.

EXTR 3 : Formic acid : 10 individuals were transferred in 50 µl 10% for-
mic acid (HCOOH). Extracts were lyophilised (Chriss, Delta IIs)
to remove acids, and diluted to 1 ml with Tris-HCl.

EXTR 4 : PCA : Extracts consisted of 10 nematodes in 50 µl 6% PCA (perchlo-
ric acid). Upon neutralisation with 25 µl K_2CO_3 (5N), the extracts
were centrifuged. The supernatans was diluted to 1 ml with TrisHCl

EXTR 5 : Boiling Tris : 10 nematodes were transferred into 10 µl artifi-
cial seawater (Dietrich & Kalle, 1957). 490 µl boiling Tris-HCl
was added ; after 30 sec extracts were cooled-down, sonicated
and stored at -20°C.

EXTR 6 : Boiling ethanol : same procedure as in EXTR 5 but with boiling e-
thanol instead of Tris-HCl, and with 10-fold dilution just prior
to ATP-measurement.

EXTR 7 : NRB/NRS (Lumac) : The extracting medium was a mixture of 25 µl
NRB and 25 µl NRS (NRB is an extractant for bacteria, NRS for so-
matic cells ; composition is not mentioned by the manufacturer).

950 µl Hepes buffer (4(2hydroethyl) 1piperazin – ethansulfon acid) was added.

b. Determination of ATP

Extracts were thawed on ice ; for each extract, light emission is measured of :

1)   100 µl extract + 100 µl Tris + 200 µl CLS
2)   as in 1) but 100 µl internal standard (10^{-8}M ATP) is added in stead of Tris
3)   Blank : 200 µl Tris + 200 µl CLS

Extracts for the actual assay were made in TCA extraction medium (EXTR 1) but with 30 nematodes per extract. For each testconcentration, 4 or 5 replicate extractions were made.   ATP was measured in juveniles of about 3d old and in nematodes of about 8.5d old – this is when females became adult in the blank.

c. Determination af ATP , ADP , AMP

Extractions were performed according to EXTR 1 (TCA) but with 100 nematodes per extract. Preliminary experiments revealed that an extra amount of ATP, ADP and AMP had to be added to enhance the transformation of ADP and AMP into ATP.

The procedure is summarized in fig 1. The incubation mixture consisted of 50 µl extract and 25 µl Tris (containing, where necessary, the internal standard), added to 100 µl TrisA (Tris buffer + Mg^{++} and K^{+}), Tris B (Tris A + Phosphenolpyruvate, pyruvate kinase and co-factors Mg^{++} and K^{+}, for transformation of ADP into ATP) or Tris C (Tris B + myokinase to transform ADP and AMP into ATP) for the determination of ATP, ADP or AMP respectively. To each mixture, 25 µl ATP, ADP or AMP (each $1 . 10^{-7}$M) was added, according to which nucleotide was determined. To determine AMP, an extra amount of 25 µl ATP (5.10^{-7}M) was added. For ADP and AMP measurements, the mixture was incubated for 30 min at 30°C, 10 µl TCA and 5 µl pepsin were added, and incubation continued for 60 min at 35°C. After neutralization with 10 µl NaOH (0.5N), the solution is diluted to 300 µl with Tris-HCl buffer.

For each nucleotide, 3 measurements were done : one with and one without an internal standard, and one blank.

d. Determination of freshweight

ATP-content is expressed on freshweight-base. For each concentration, maximal length and width of 25 fixed individuals (4% formalin ; 80°C) was measured. Freshweight is calculated with Andrassy's formula (Andrassy, 1956) :

$$\frac{ab^2}{1600000} = \text{freshweight (ug)}$$

a = maximal length (um) ; b = maximal width (um)

RESULTS

I. Demographic characteristics

Minimum generation time

Tables 1 and 2 show that minimum generation times increased with increasing concentrations of Cd and Ni. This was very clear at 2.5 mg/l Cd and 15 mg/l Ni. A Games & Howell test (Sokal & Rohlf, 1981) revealed a MEC value (minimal effect concentration) of 1 mg/l (P=0.05) for both Cd and Ni.

In the whole experiment, males developed a little faster than females. For Cd, the sex ratio (measured as the percentage females in the adult population) increased with increasing concentration (except for 0.5 mg/l Cd) while for the Ni-assay, an opposite trend was observed.

Mortality as a function of age

For both Cd and Ni, mortality during the egg stage increased only slowly with increasing concentrations (table 1 & 2). The increase was very steep between 25 and 35 mg/l Ni, the latter concentration causing 100% mortality.

Concerning the juvenile mortality, a G-test (Sokal & Rohlf, 1981) showed that juvenile mortality was significantly influenced by the amount of metal added (P<0.001 : $G_{H/q}$ =1186 for Cd ; $G_{H/q}$ = 509 for Ni). The MEC values (P=0.05) are 2.5 mg/l for Cd and 5 mg/l Ni.

The preadult mortality, which compiles both egg- and juvenile mortality, showed about the same pattern as the juvenile mortality.

The adult survival of the females is represented in fig. 2. The mean adult female longevity in both the Ni and Cd assay was not significantly different (P=0.05) from the control (Games & Howell test : Sokal & Rohlf, 1981).

Fecundity
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Total eggproduction decreased with increasing Cd and Ni concentra-
tions ; the mean cumulative eggproduction per female alive is a linear
function of time (fig. 3). All regressions were significant ; the para-
meters of the regressions are given in table 3.

The slope b represents the mean daily egg production of a female
alive. It dropped significantly (in comparison to the control) at 1 mg/l
for Cd and 2.5 mg/1 Ni (P<0.001) ; these values are minimal effect con-
centrations for this criterion.

Other demographic characteristics R_O, r_m, T
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From the results above, several demographic parameters were calcula-
ted (table 4). Both the net-reproductivity R_O and the intrinsic rate of
natural increaser_m were clearly depressed by metal intoxication, mean ge-
neration time T was prolonged compared to the control (table 4). The va-
lues calculated for 0.5 mg/1 Cd are exceptional due to the very low sex
ratio (44.2% females against 81% for 1 mg/1 Cd) observed.

For both metals, EC50(R_O) and EC50(r_m) were calculated. These are
concentrations at which R_O, respectively r_m, are depressed with 50% com-
pared to the control. For Cd there was no clear correlation between R_O
(r_m) and the concentration of the metal. This is due to the aberrant va-
lue of R_O (r_m) at 0.5 mg/1 Cd (fig. 4a). Linear interpolation between
the control and 2.5 mg/1 Cd gave :

$$EC50(r_m) = 1.37 \text{ mg/1 Cd}$$
$$EC50(R_O) = 0.64 \text{ mg/1 Cd}$$

An exponential correlation was found between r_m (R_O) and Ni concentration
(table 5, fig. 4b). From the regressions, EC50 values were calculated as

$$EC50(r_m) = 12.29 \text{ mg/1 Ni}$$
$$EC50(R_O) = 3.48 \text{ mg/1 Ni}$$

II. Biochemical characteristics

Extraction procedures test
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Results were expressed on freshweight base and are represented in ta-
ble 6. A Bartlett's chi-square test showed that variances were heteroge-
neous. Therefore, the results were analysed with the Games & Howell test.
This revealed that there were no significant differences between the four
acidic methods (P=0.05). EXTR 1 gave the highest ATP-yield. All non-

acidic extraction techniques (EXTR 5,6,7) were significantly different
from EXTR 1 and 2. From this group, results with EXTR 5 approximated
these with formic acid (EXTR 4). EXTR 6 and 7 gave the lowest ATP-yield.

ATP concentration in the bio-assay

The ATP-content of 3d old juveniles decreased allometrically with
metal concentration (fig. 6 and 7). A similar relationship was found be-
tween freshweight and Cd (Ni) concentration (fig. 6 and 7).

Analysis of variance showed that the weight-specific ATP content was
significantly influenced by Cd (F_s = 4.717 ; 0.001< P <0.01) and by Ni
(F_s = 6.395 ; 0.001< P <0.01). Comparison limits (P=0.05) were calcula-
ted with the Gabriël-test (Sokal & Rohlf, 1981) and given in fig 4.
Values for 10 mg/l Cd were (marginally) significantly different of values
at 0.5 and 2.5 mg/l Cd. For Ni, a (marginal) significant difference
existed between 15 mg/l Ni and 1 mg/l Ni. Of more importance is that none
of the measured values differed significantly from the values in the con-
trol (P=0.05).

For 8.5d old organisms, Cd and Ni again affected the weight-specific
ATP content measured significantly (F_s = 43.988 , 0.001< P <0.01 for Cd
and F_s = 64.674 , P < 0.001 for Ni). Comparison limits (Gabriël test,
P=0.05) showed a significant decrease compared to the control at the high-
est concentrations of both metals tested (fig. 5) At the lowest concen-
tration, this difference was not significant except at 1 mg/l and 2.5 mg/l
Cd. At these concentrations, significantly higher values (P=0.05) were
calculated.

Adenylate Energy Charge

ADP and/or AMP were not always measurable. A few results are given
in table 7. The AEC was higher in "healthy" organisms than in starved
organisms (After 5 to 6 days starvation, the nematodes were barely alive).

DISCUSSION

Several studies, field studies as well as laboratory experiments,
show that nematodes commonly exhibit relative high resistance to pollu-
tants. LC50 values recorded after intoxication with inorganic and orga-
nic xenobiotics are regularly among the highest values noted for other
taxa, or even higher.

Vranken *et al.*(1986) studied the acute toxicity of seven heavy metals, PCP and ϑHCH on *Monhystera disjuncta*. For Ni and Cd, they found for the J2 juvenile stage a LC50 (96h) of 103 mg/l Ni and 37 mg/l Cd. Others (Haight *et al.*,1982)mentioned 50% mortality in the J2 juvenile stage of *Panagrellus silusiae* after 48h intoxication with 15.1 mg/l Cd and 105 mg/l Ni. In a mixed population of *Panagrellus* and *Rhabditis* the LC50(48h) value ranged between 35 and 40 mg/l Cd (Feldmesser & Rebois, 1965). In this study, no significant difference was found neither in adult survival, nor in mean adult longevity of *M. disjuncta*, at the different Cd and Ni concentrations studied. In the juvenile stage, which is more sensitive than the adult stage (Vranken *et al.*, 1984a), mortality increased significantly at 2.5 mg/l Cd and 5 mg/l Ni.

In table 8, a summary of the effects of Cd and Ni on the demographic criteria studied is given. Obviously, the LC50 (96h) values reported by Vranken *et al.*(1986) are much higher (almost one to two orders of magnitude) than all demographic citeria studied here. Also, the minimal effect concentration (MEC) for mortality during the juvenile stage, although less than the LC50, is higher than MEC's based on T_{min}, daily egg production, net reproductivity (R_o) and population growth (r_m).

When compared with the LC50 values, development rate and daily egg production are the most sensitive criteria. In the Cd-assay, this difference amounts to a factor 37. In the Ni-assay, treshold levels as measured by the daily eggproduction and development rate are 41 and 103 times less when compared with the LC50.
Similar results are reported in the literature. Reish & Carr (1978) and Petrich & Reish (1979) found a significant reduction of fecundity in polychaetes, exposed to a variety of heavy metals at levels almost two orders of magnitude less than the corresponding LC50(96h). For the nematode *Panagrellus redivivus*, Samoiloff *et al.*(1980) reported a difference of three orders of magnitude between the Cd level suppressing fecundity and the MEC as measured by juvenile mortality. Furthermore they showed that growth inhibition in this species is a more sensitive toxicity index than mortality. For *Diplolaimella* spec 1, Vranken & Heip (in press) found differences of two orders of magnitude (Cu & Pb) and 1.5 (Hg) between development time and the corresponding LC50. Other observations are however in variance with these findings. Vranken *et al.*(1984a) found no effect on the development rate of some specimens of *Monhystera disjuncta*, whereas for most individuals the mercury concentration tested was lethal. Haight *et al.*(1982) needed concentrations of 100 mg/l for e.g.

Cd to stop growth of *Panagrellus silusiae* whereas at 15 mg/1 Cd, 50% of
the J2 juvenile stage died.

A comparison with the results reported by Vranken *et al.*(1986) re-
vealed that, although the same test organism and an identical culture
technique was used, our MEC values, based on juvenile mortality, deve-
lopment rate and fecundity, are consistently less.This can be explained
by 1) differences in exposure time used in the experimental design :
Vranken and co-workers studied these criteria during a pre-set period of
time (96h) whereas in this study physiological standards (development
time and total lifespan) were used as time duration of the experiment,
and by 2) a higher susceptibility of the smallest juveniles as freshly
hatched juveniles (2.5 - 3d old) were excluded from their experiments
(they started with 4.5d old juveniles).

In the Cd-assay, there was a steep decrease of both the intrinsic
rate of natural increase (r_m) and the net-reproductivity (R_0) with in-
creasing concentrations. In the nickel-assay, the decrease of both pa-
rameters is less pronounced. R_0 can be considered as the most sensitive
life history parameter. EC50's based on R_0 are 1.64 mg/1 Cd and 3.48
mg/1 Ni. At these concentrations the production of female progeny drops
with 50% when compared to the control. EC50 values based on r_m are
2.37 mg/1 for Cd and 12.29 mg/1 for Ni. Consequently the effective con-
centrations based on r_m and R_0 are higher than MEC's based on development
time and fecundity.

Biochemical characteristics in pollution studies
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For a particular biochemical response to be acceptable as an index of
biological effect, it must fulfill two important criteria (Livingstone,
1985) : 1. The measurable change in biochemical processes must result
from, or be a response to, a change in the environmental conditions.
2. It must be possible to demonstrate that the change in biochemical
process(es) will have, either direct or indirect, a detrimental effect
on growth, reproduction or survival of the organism. Concerning the
use of the adenylate energy charge in multicellullar organisms, only
the first criterium is fulfilled implying that its use is limited.

Up to now, only a few papers have reported on the ATP content of
marine nematodes. Only Ernst (1970) and Goercke & Ernst (1975) made
measurements to study the relationship ATP - biomass.
Expressed in percentage of the total amount of organic carbon, Ernst
(1970) found 2.3% for *Panagrellus redivivus*, while for *Anoplostoma*

viviparum and *Adoncholaimus thalassophygas* values ranged from 0.9%
to 1.3% (Goercke & Ernst, 1975). In this study, the mean ATP content
in *Monhystera disjuncta* was 2.4% to 3.6% of the total amount of organic
carbon (calculated with an approximate conversion factor $C_{org} = \frac{1}{12}$ fresh-
weight).

The above mentioned authors used boiling Tris buffer as extracting fluid.
Our experience is, however, that the cuticle of *Monhystera disjuncta* re-
mained intact after boiling, probably resulting in less homogeneous and
less stable extracts. Our results improved when the nematodes were
fractionated by sonication. Nevertheless, the ATP-yield remained below
the values obtained with the four acid extraction procedures tested.
TCA was selected as extracting fluid as it gave the highest ATP-yield,
although it was not significantly different from the three other acid
extractions.

ATP as a measure of pollution stress in bio assay tests

The objective was to establish a possible correlation between changes in
ATP concentrations in an early stage of the experiment and changes in de-
mographic characteristics.

In juveniles (3d old), no significant alteration of weight-specific
ATP content was observed at each level of Cd and Ni intoxication compared
to the control. Yet, at this stage of the experiment, the impairement of
growth and development could be observed even at the lowest concentrations
tested. In a later stage of the experiment (8.5d old organisms), ATP con-
tent decreased significantly at the highest metal concentrations. At
lower concentrations, at which significant effects on R_O and r_m were mea-
sured, the ATP concentration retained the same level as in the control
(fig. 4 & 5), except at 1 and 2.5 mg/1 Cd. At these concentrations, a
(only marginally) significantly higher weight-specific ATP content was
calculated. We believe, however, that these two values are erroneous and
that the mean weight-specific ATP content should remain constant up to
2.5 mg/1 Cd inclusive, for two reasons. Firstly, it is very unlikely
that ATP concentrations would increase when organims live in stressful
conditions. Changes of ATP concentration as a consequence of harmful
conditions are more than once reported, but the change is always in the
reverse direction. Secondly, the mean ATP content per individual, which
is based on the measurement in 120 to 150 nematodes per concentration,
remained constant up to and inclusive 2.5 mg/1 Cd. It is possible that

the aberrations are a consequence of an inadequate biomass determination.
In fact, the determination of the mean body mass was based on its measure-
ment in only 25 individuals, which exhibited a very steep exponential growth
at the time of the observations (8.5d). The variance of the body weight
after 8.5 days was relatively high (and higher than at higher metal con-
centrations where growth stopped almost completely - results not shown) so
that it became difficult to pick the nematodes at random from the cul-
ture, possibly leading to erroneous estimates. Such errors would not
occur when body mass and ATP were measured in the same organism. In
this study, this was not possible as *Monhystera disjuncta* is too small
(± 0.5 µg freshweight per adult).

Summarizing we can say that : within the range of metal concentra-
tions tested, the ATP turn-over remained constant in an early stage (ju-
veniles), and also at the lowest Cd and Ni concentrations in a later
stage of the chronic exposure experiment. 2) developmental inhibition,
delayed and impaired reproduction already occured at metal concentrations
below those affecting the ATP concentration.
A possible explanation is that with increasing metal concentration and
probably with increasing exposure time, an increasing amount of energy
(potentially available in the adenylate system) is used in processes
related to adaptive responses such as avoidance reactions and active de-
toxification. As a consequence, less energy will be available for growth
and reproduction. For Cd, the level at which growth ceased completely
was observed at 5 mg/1 Cd (the organims are moribund, won't survive till
adulthood and consequently won't reproduce) and for Ni it is above
15 mg/1 Ni. The situation in the Ni-assay seems to be different from
in the Cd-assay as at the highest concentrations (5 and 15 mg/1 Ni)
the organisms still grew and reproduced despite the significant decrease
of the mean weight-specific ATP content measured in 8.5d old organims.
We believe however, that the ATP content in reproducing adults (although
less reproducing) may have remained constant and that the decrease may
have been a reflection of the proportion moribund animals (see the in-
creased juvenile mortality) at the time of (at random) sampling.

AEC as a measure of pollution stress in bio-assays with nematodes
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We encountered many problems in determining the AEC in *Monhystera*
disjuncta, using the same method as previously described for two poly-
chaetes (Verscraegen *et al.*, 1985). The major problem was, again, the

low biomass of the nematode and consequently the very low concentrations of the adenylates. Even with 100 nematodes per extract (which were manipulated one by one with a needle), transformation appeared to be too low to be measurable. In all different adaptations of the method tried, AMP was the least reliable factor. In the long run, the procedure became sufficiently complex, and still results were not always consistent. In spite of it, we determined AEC's in starved nematodes and in organisms from old neglected stock cultures (old nematodes in overcrowded and hypersaline conditions). Results showed that AEC in *Monhystera disjuncta* actually drops with increasing stress.

Haya & Waiwood (1983) summarized four possible ways in which the adenylate energy metabolism can be altered during sublethal intoxication with xenobiotics. These are : 1) AEC decreases due to an alteration in relative proportions of the adenine nucleotides while the level of total adenylates remains constant. 2) AEC remains constant while the level of total adenylates decreases. 3) AEC and total adenylates decrease. 4) Total adenylates and AEC remain constant, but precursors or endproducts of adenylate energy metabolism are altered.
Applied to our own data, this means that if a decrease of AEC could have been measured, this would only have been possible in organisms of 8.5d old, at the highest metal concentrations tested (at 5 and 10 mg/l Cd and possibly but not likely at 5 and 15 mg/l Ni). Indeed, if AEC were decreased at concentrations where the ATP concentration is constant, this would imply that, the more the individuals were stressed, the larger their total adenylate pool would be. Such a respons type has never been observed (Haya & Waiwood, 1983).

In conclusion, we think that neither ATP concentration or AEC measurements can give an ecological relevant idea about harmful effects caused by long-term exposure to sublethal concentrations of heavy metals. Both criteria are less sensitive than the demographic characteristics studied. These findings strengthen our previous idea that the usefulness of AEC as an index in pollution monitoring in the field is questionable (Verschraegen *et al.*, 1985). The hypothesis was that the maintenance of a stable population is impossible when the individuals constantly have low AEC's, so that in polluted stations only pollutant-resistant species will be found with normal AEC's.

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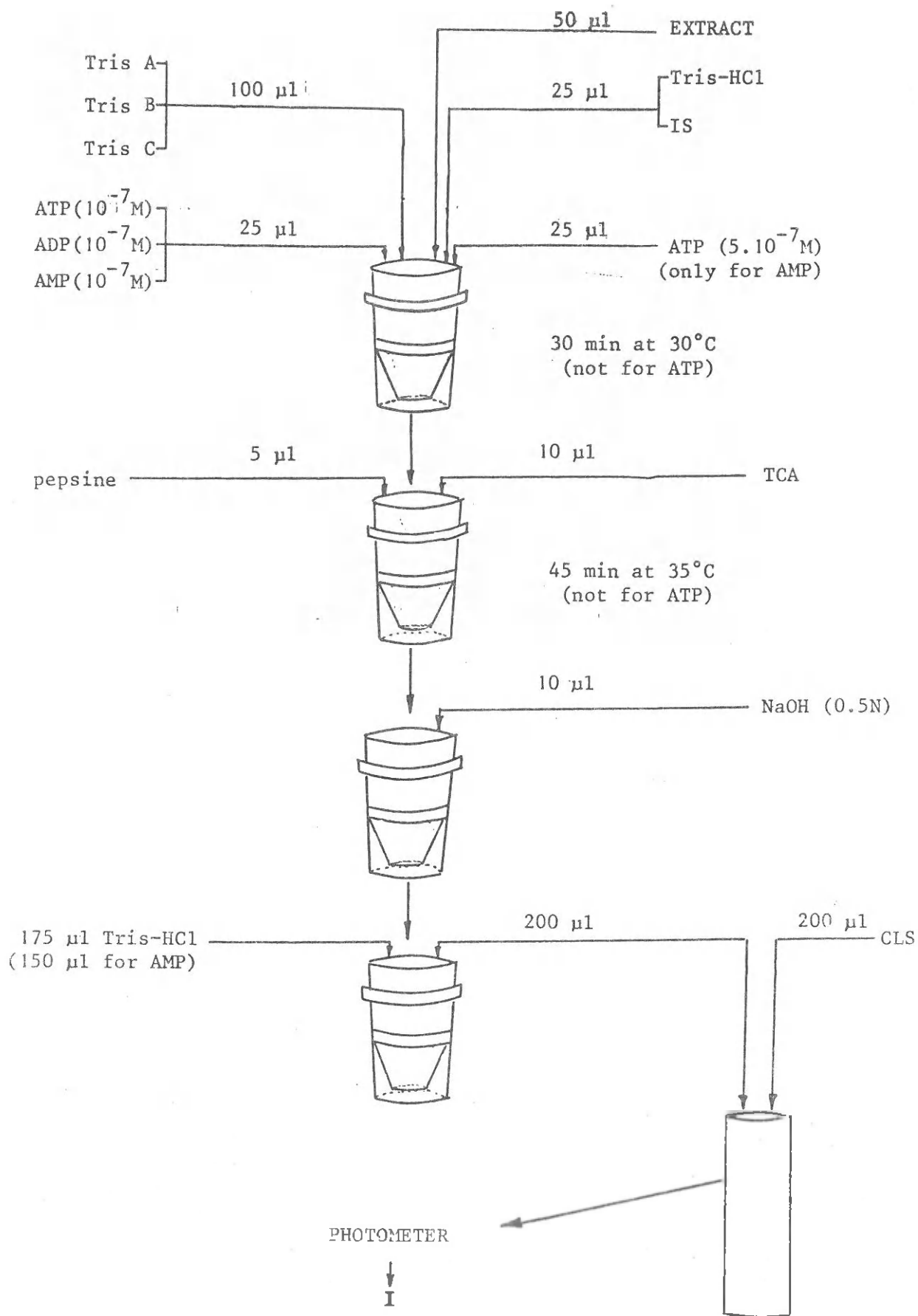
Fig. 1 : Protocol for ATP-, ADP-, and AMP-determination.
IS = internal standard

Cd ADULT SURVIVAL ♀♀

P

Cd (mg/l)		m.a.l. ± SE (± 95%CI)
□—□	control	22.90 ± 0.90 (± 1.84)
■—■	0.5	20.64 ± 1.84 (± 2.12)
▲—▲	1	21.96 ± 0.91 (± 1.86)
▼—▼	2.5	21.26 ± 0.98 (± 1.82)

time (days)

Ni ADULT SURVIVAL ♀♀

P

Ni (mg/l)		m.a.l. ± SE (± 95%CI)
□—□	control	22.90 ± 0.90 (± 1.84)
■—■	0.5	19.03 ± 1.39 (± 2.84)
▲—▲	1	19.13 ± 1.05 (± 2.14)
▼—▼	2.5	19.51 ± 1.16 (± 2.38)
▽—▽	15	15.82 ± 1.85 (± 3.99)

time (days)
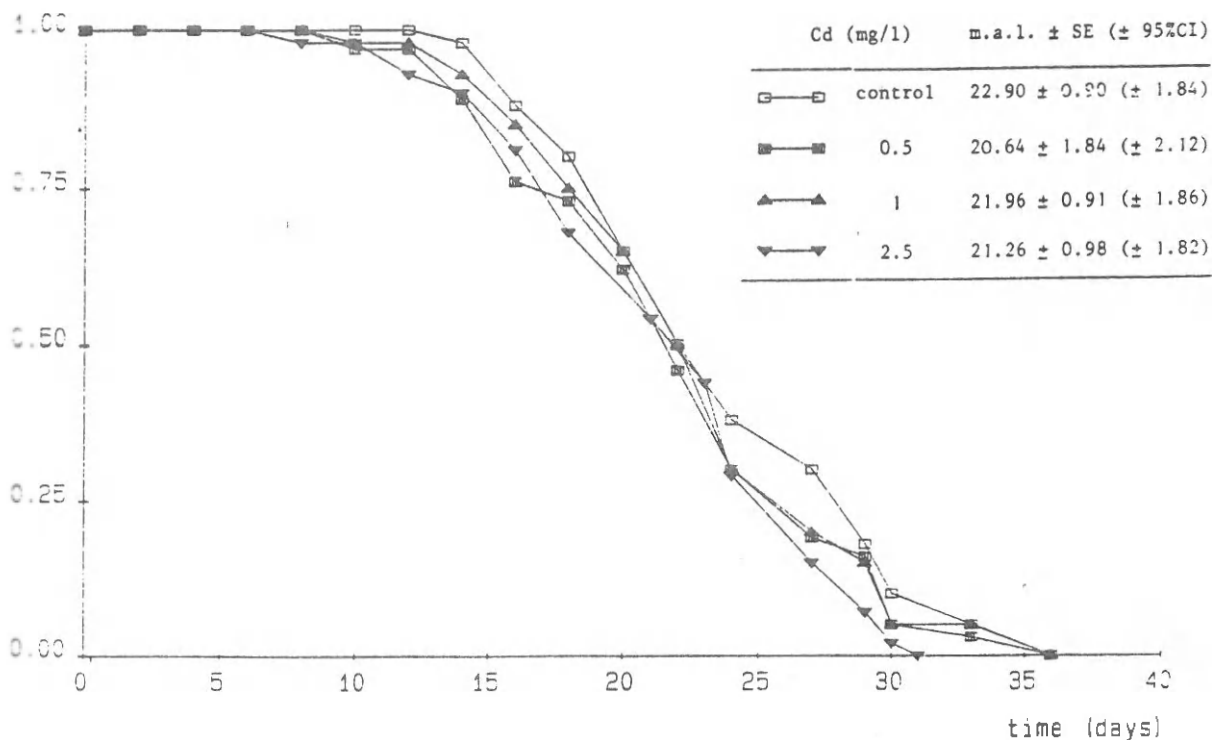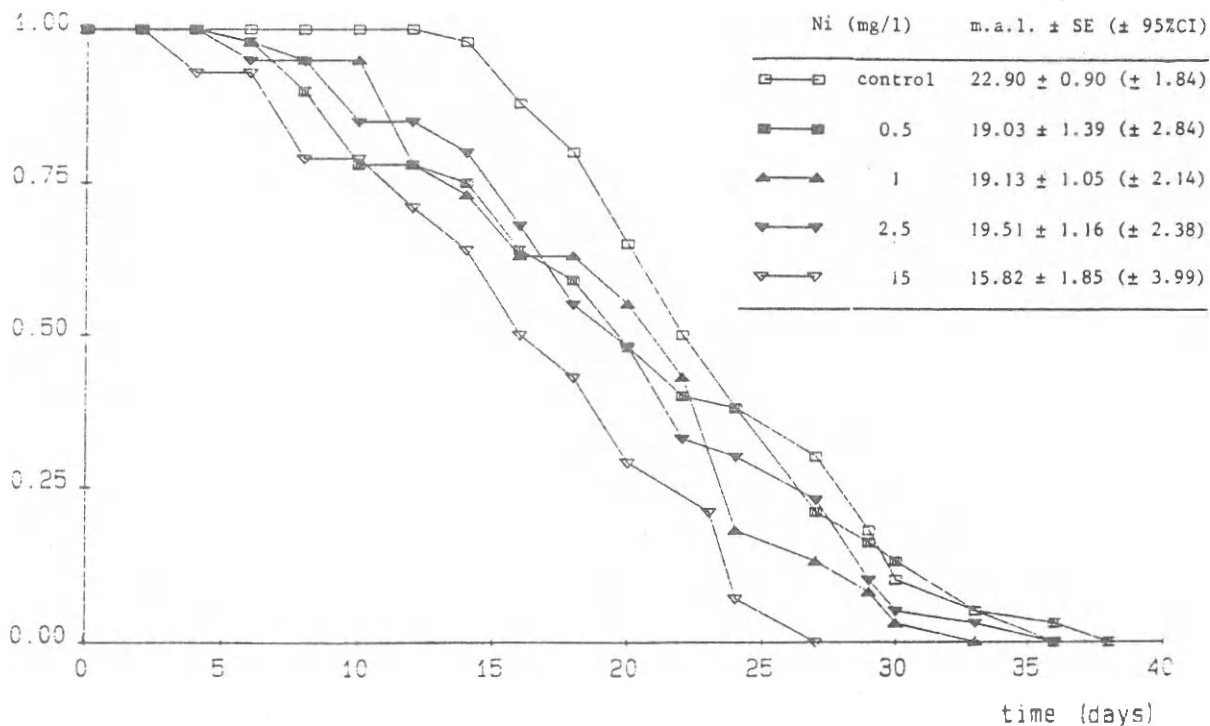
Fig. 2 : Female adult survival at different Cd (upper) and Ni (lower) con-
centrations. P is survival in proportions, m.a.l. is the mean adult longe-
vity (in days), SE is standard error. 95%CI between brackets.
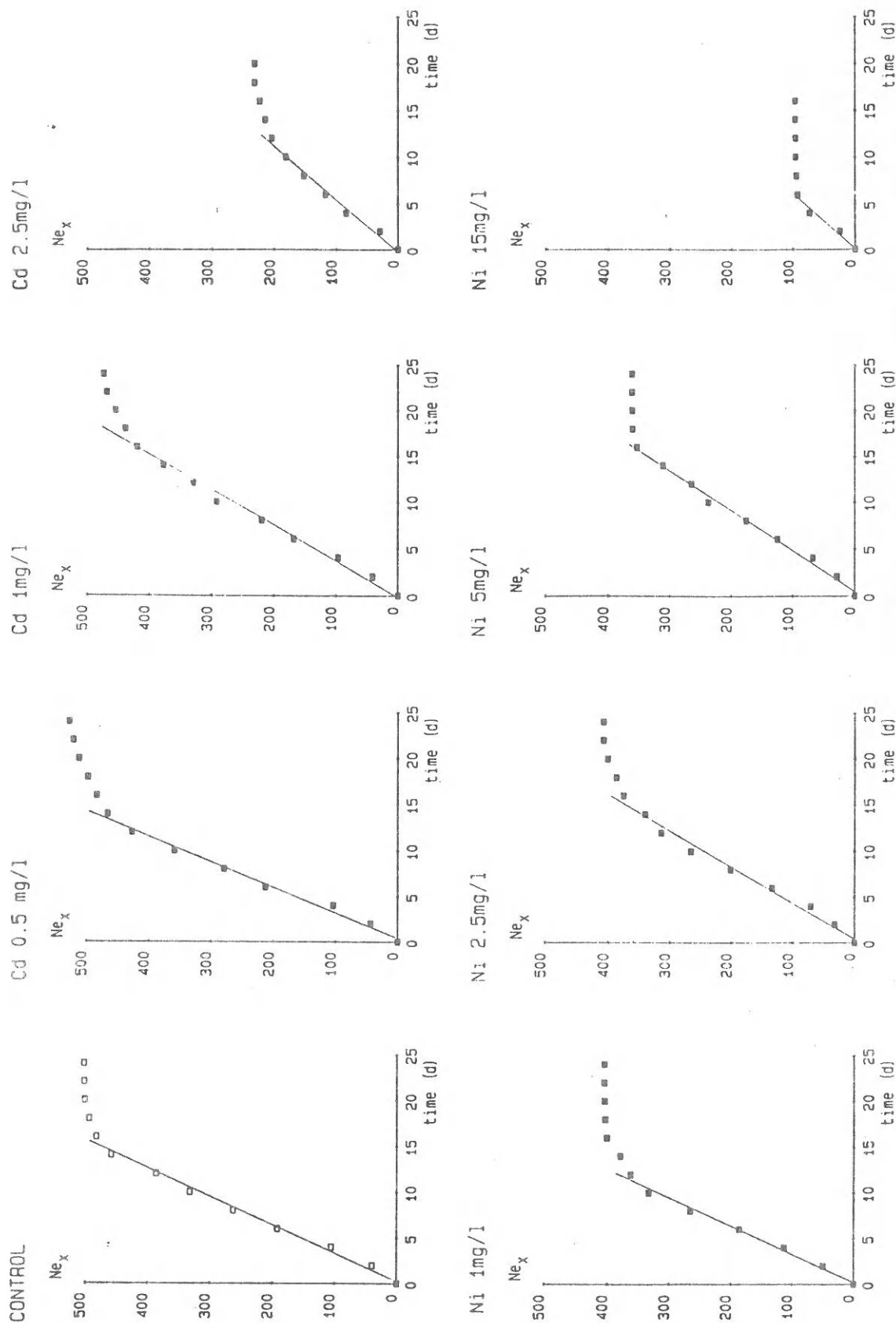
Fig. 3 : Eggproduction per female : cumulative in function of time at different Cd (upper) and Ni (lower) concentrations.

Fig 4a

WR$_0$, Wr$_m$, gATP.10^{-4}/gwwt

Fig 4b

WR$_0$, Wr$_m$, gATP.10^{-4}/gwwt
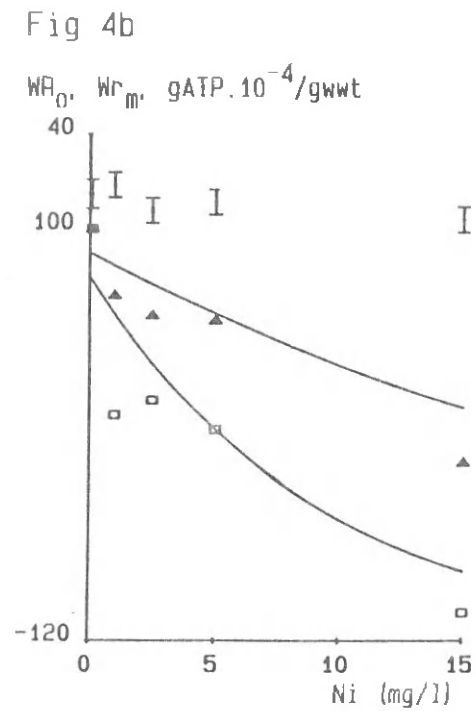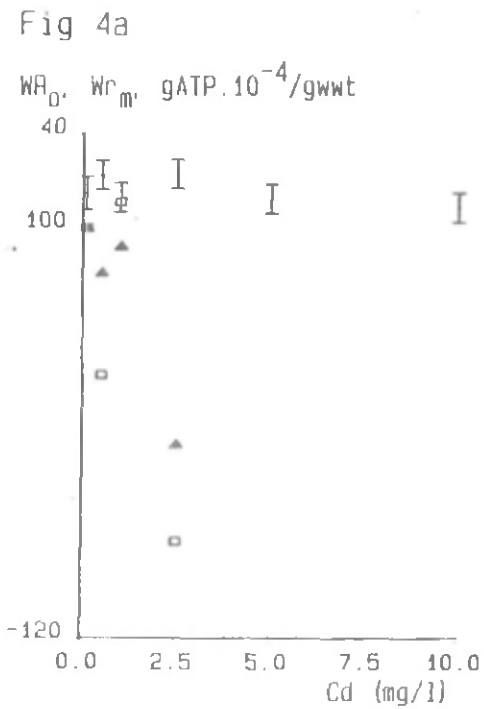
Fig. 4 : Comparison of demographic parameters and weight-specific ATP content of juvenile (3d old) at different Cd (fig. 4a) and Ni (fig. 4b) concentrations (mg/1). Ordinate : W, fitness relative to the the control; ▲ r$_m$ intrinsic rate of natural increase ; ▫ R$_0$ net-reproductivity ; · mean ATP content (in g ATP 10^{-4}) per g freshweight with comparison limits (P=0.05 ; Gabriël test).

Fig 5a

WR$_0$, Wr$_m$, gATP.10^{-4}/gwwt

Fig 5b

WR$_0$, Wr$_m$, gATP.10^{-4}/gwwt
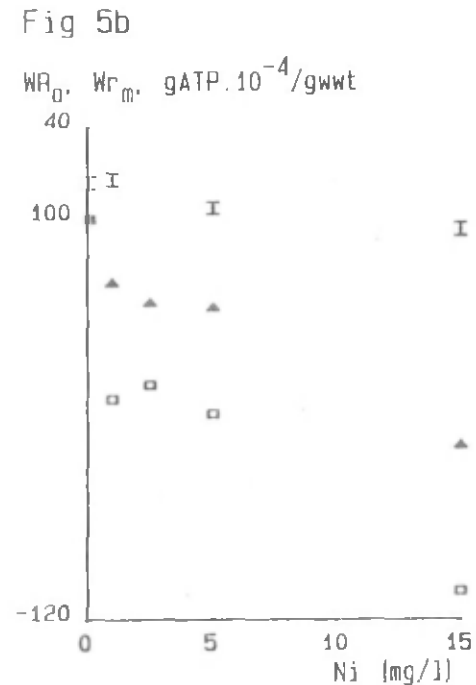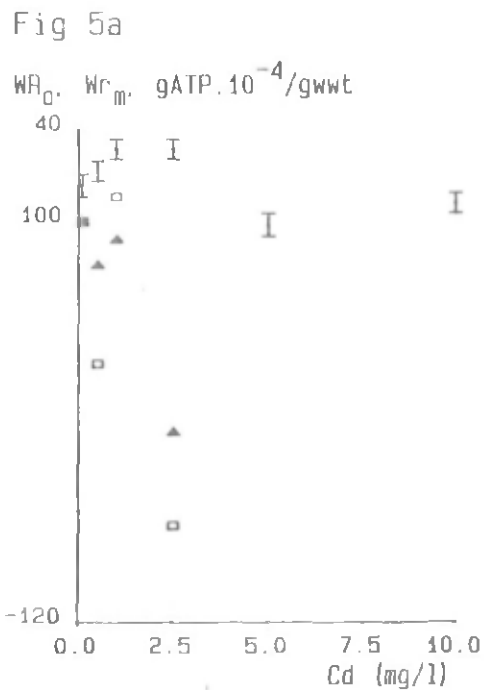
Fig. 5 : Comparison of demographic parameters (r$_m$, R$_0$) and weight-specific ATP content in nematodes of 8.5 d old at different Cd (fig. 5a) and Ni (fig. 5b) concentrations (mg/1). Abbreviations and symbols as in fig. 4.
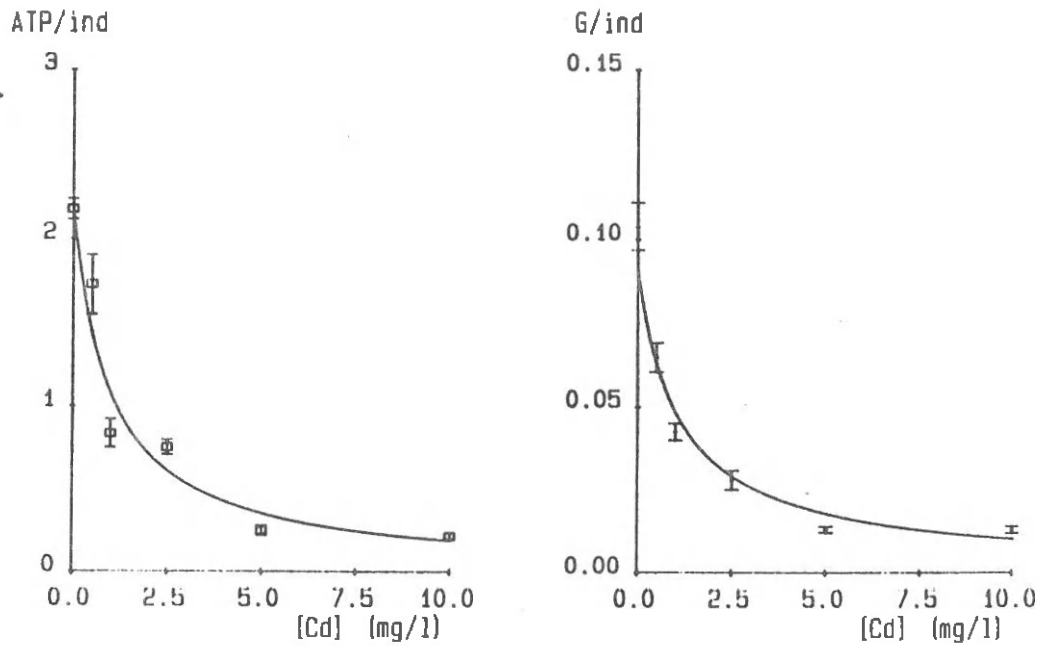
Fig. 6 : Cadmium : correlation between ATP content per juvenile nematode (in g ATP 10^{-10}) and Cd concentration (left) and between mean fresh weight perjuvenile nematode (in ug) and Cd concentration (right).
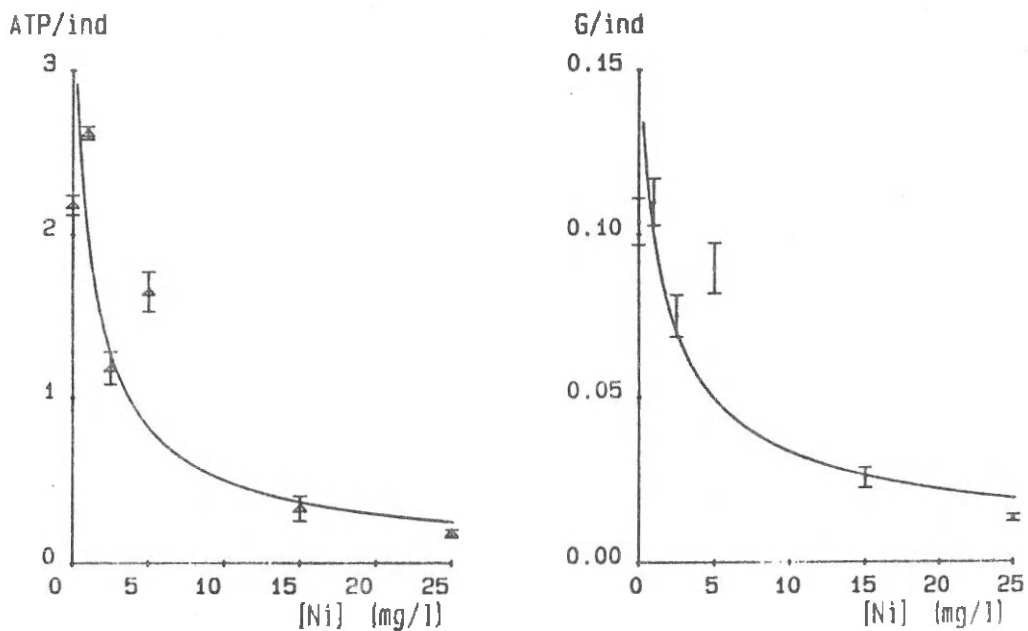
Fig. 7 : Nickel : correlation between ATP content per juvenile nematode (in g ATP 10^{-10}) and Ni concentration (left) and between mean fresh weight per nematode (in ug) and Ni concentration (right).

CADMIUM	control	0.5 mg/l	1 mg/l	2.5 mg/l	5 mg/l	10 mg/l
Ne	233	223	331	304	206	176
e(+)	6.01	6.28	6.04	6.25	7.30	14.18
j(+)	2.28	6.22	5.14	61.40	100	100
p(+)	8.15	12.10	10.80	63.80	100	100
N ♀♀	143	102	245	92	-	-
T_{min} ♀♀	8.14 ± 0.086 (± 0.173)	8.59 ± 0.105 (± 0.209)	8.86 ± 0.088 (± 0.176)	16.05 ± 9.234 (± 0.467)	-	-
N ♂♂	71	129	56	11	-	-
T_{min} ♂♂	8.01 ± 0.099 (± 0.199)	7.98 ± 0.065 (± 0.129)	8.84 ± 0.192 (± 0.384)	13.73 ± 0.407 (±0.906)	-	-
sex ratio	66.8	44.2	81	89	-	-

Table 1 : CADMIUM : Minimum generation time T_{min} ± standard error (with 95% CI between brackets), egg mortality e(+), juvenile mortality j(+) and preadult mortality p(+) at different Cd concentrations (mg/l). Ne is the number of eggs studied, N ♀♀ (♂♂) the number of adults and sex is the proportion females in the adult population.

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NICKEL	control	1 mg/l	2.5 mg/l	5 mg/l	15 mg/l	25 mg/l	35 mg/l
Ne	233	412	359	233	125	110	34*
e(+)	6.01	6.79	7.52	9.01	9.60	10	100
j(+)	2.28	6.51	3.92	11.32	33.63	100	100
p(+)	8.15	12.86	11.10	19.30	40	100	100
N ♀♀	143	185	184	111	29	–	–
T_{min} ♀♀	8.14 ± 0.036 (± 0.173)	9.61 ± 0.121 (± 0.243)	9.67 ± 0.129 (± 0.258)	9.49 ± 0.173 (± 0.347)	13.62 ± 0.291 (± 0.597)	–	–
N ♂♂	71	173	149	76	46	–	–
T_{min} ♂♂	8.01 ± 0.099 (± 0.199)	8.68 ± 0.091 (± 0.182)	8.84 ± 0.113 (± 0.226)	8.83 ± 0.174 (± 0.348)	12.22 ± 0.093 (± 0.188)	–	–
sex ratio	66.8	52	55	60	39	–	–

Table 2 : NICKEL : life-history features studied at different Ni concentrations. Abbreviations as in table 1. * after 48h.

CADMIUM (mg/1)	a (± 95% CI)	b (± 99% CI)	r^2	F_s	n
control	-8.14 (± 24.40)	32.30 (± 3.79)	0.992	888	9
0.5	-14.02 (± 28.89)	35.77 (± 5.23)	0.991	33	8
1	4.80 (± 24.62)	25.92 (± 3.35)	0.988	67	10
2.5	4.28 (± 16.61)	17.54 (± 3.62)	0.987	38	7

NICKEL (mg/1)	a (± 95% CI)	b (± 99% CI)	r^2	F_s	n
control	-8.14 (± 24.40)	32.30 (± 3.79)	0.992	888	9
1	-6.71 (± 25.23)	32.28 (± 5.49)	0.991	562	7
2.5	-10.42 (± 25.14)	25.36 (± 3.91)	0.987	516	9
5	-10.64 (± 14.49)	23.03 (± 2.25)	0.995	1281	9
15	-1.22 (± 31.97)	16.37 (± 19.1)	0.973	72^{*}	4

Table 3 : Egg production (cumulative) per female, at different Cd (Ni) concentrations (mg/1) : parameters of the regression $\sum_{x=0}^{max\ age} Ne_x = a+b.time$ (d) with b the mean daily eggproduction per female.
n is the number of observations, r^2 the coëfficient of determination, F_s the F-statistic of the linear regression (P<0.001 ; ** 0.01<P<0.05)

		R_o	$r_m(d^{-1})$	T (d)
CADMIUM (mg/1)	control	302	0.422	13.53
	0.5	194	0.376	12.48
	1	321	0.403	14.32
	2.5	72	0.199	21.40
NICKEL (mg/1)	control	302	0.422	13.53
	1	165	0.354	14.43
	2.5	176	0.333	15.52
	5	154	0.328	15.36
	15	21	0.182	16.85

Table 4 :   Demographic parameters at different Cd (Ni) concentrations (mg/1) R_o is the net-reproductivity, r_m is the intrinsic rate of natural increase (per day) and T the mean generation time (in days).

NICKEL	a	b	r^2	F_s	n
R_o	267	−0.164	0.953	61.1	5
r_m	0.398	−0.052	0.965	81.5	5

Table 5 : Nickel : R_o (respectively r_m) as a function of Ni concentration (mg/1). Parameters of the regression $Y=ae^{b[Ni]}$ with a and b constants, $Y=R_o$ (respect. $Y=r_m$) and $[Ni]$ in mg/1. Abbreviations as in table 3., $0.001<P<0.01$

Extraction procedure	n	ATP/gwwt ± SE
EXTR 1 (TCA)	5	26.771 ± 1.785
EXTR 2 (H_2SO_4)	5	22.782 ± 1.255
EXTR 3 (PCA)	5	19.599 ± 1.415
EXTR 4 (Formic acid)	4	12.115 ± 3.170
EXTR 5 (NRB/NRS)	5	11.303 ± 0.589
EXTR 6 (Tris 100°C)	4	3.559 ± 0.295
EXTR 7 (Ethanol ±80°C)	3	0.334 ± 0.143

Table 6 : Extraction procedures test : mean ATP content (in g ATP 10^{-4}) per g wet weight ± standard error. n is the number of replicate extractions

stress induction	repl. 1	repl. 2
control	0.86	AMP not measurable
overcrowded and hyper-saline conditions	0.70	--
1 day starvation	0.72	0.47
3 days starvation	0.47	AMP n.m.
4 days starvation	0.49	AMP n.m.

Table 7 : Artificial stress induction : AEC in old nematodes (in over-crowded and hypersaline conditions) and in starved organisms.

CRITERIUM	Cd	Ni
LC50 (96h)[*]	37	103
MEC J(+)	2.5	5
MEC T_{min}	1	1
MEC Ne_x	1	2.5
MEC m.a.1.	—	—
EC50 (R_o)	1.64	3.48
EC50 (r_m)	2.37	12.29

Table 8 : Compiled table of demographic criteria studied in *Monhystera disjuncta* under Cd (Ni) intoxication. MEC = minimal effect concentration, EC = effective concentration with 50% inhibitory effect, J(+) = juvenile mortality, T_{min} = minimal generation time, Ne_x = mean daily egg production, m.a.1. = mean adult longevity, R_o = net-fecundity, r_m = intrinsic rate of natural increase. [*] = data from Vranken *et al.*, 1986. concentrations in mg/1.

TOXICITY OF COPPER, MERCURY AND LEAD TO A MARINE NEMATODE

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Toxicity of copper, lead and mercury to the nematode Diplolaimella spec 1 is studied. Mortality responses obtained demonstrate high resistance to heavy metals. Population growth parameters as the intrinsic rate of natural increase and net-reproductivity are significantly depressed at copper-concentrations which cause no juvenile mortality. The lowest concentrations tested caused significant inhibition of development rate in both sexes. For this particular nematode species suppression of fecundity and developmental inhibition are more reliable criteria, determining non-exceedable limits with regard to environmental safety. Our tests show that nematode productivity may be significantly depressed at copper levels found in some areas of the North Sea.

INTRODUCTION

Marine nematodes are the most abundant animals in marine sediments and have several features that are advantageous in ecotoxicological research in the laboratory : they have a short life-span (Vranken & Heip, in press), a high fecundity (Vranken & Heip, 1983), represent several trophic levels (herbivores, bacterial feeders and carnivores) and at least some species are easily cultured (Heip et al., 1985). However, the effect of only a very limited set of chemical agents to only a few species has been tested up till now (Bogaert et al., 1984; Howell, 1984; Vranken et al., 1984b). From these few experiments it appears that nematodes are relatively resistant to pollutants. This is corroborated by evidence from field surveys which have shown that nematode density is not affected substantially by raw domestic sewage (Vidakovic, 1983), heavy metals (Tietjen, 1977, 1980) and acid iron waste (Lorenzen, 1974).

In this paper we present results from tests on the species Diplolaimella spec 1, a new species described by Jacobs & Vranken (in prep.) and misidentified as Monhystera microphthalma in previous papers. First, a series of acute (static) and sublethal (chronic) tests on the toxicity of copper, mercury and lead is described, in which both mortality and development rate are studied simultaneously. Nematodes moult four times before becoming adult and the success in reaching a particular stage has been proposed as a sensitive indicator of toxicity (Samoiloff et al., 1980). We used a slightly different approach and have determined generation time and preadult mortality as a function of metal concentration. These tests are presented as a supplement to the few data available and are intended to check whether the resistance of nematodes to toxicants is indeed true. Second, we will also report on sublethal effects of copper on fecundity, because mortality is not a

very sensitive index of toxicity. Fecundity, the number of eggs produced, has been used because it is known to be a sensitive criterion in toxicity testing (Bryan, 1980; Reish & Carr, 1978).

As a combination of fecundity and development rate (generation time) the intrinsic rate of natural increase r_m of the population can be calculated. This has been done by Tietjen & Lee (1984) to measure the impact of PCB's, PAH's and heavy metals on the nematodes Chromadorina germanica and Diplolaimella punicea. Because r_m values relate also to the productivity of the population, EC-50 values, defined as the concentration at which there is a 50 % inhibitory effect on population growth, are excellent tools to predict the environmental impact of toxicants (Hummon & Hummon, 1975; Sabatini & Marcotte, 1983; Vernberg & Coull, 1981). For several nematodes, a good correlation between r_m values and the rate of increase in the field has been found (Heip et al., 1978; Smol et al., 1980; Romeyn et al., 1983). Since nematodes are part of the diet of macrofauna, some fish and commercial crustaceans such as Crangon crangon, and since a significant part of the energy flow through benthic systems passes through the nematodes (Platt & Warwick, 1980),any factor influencing nematode productivity may be of more global importance.

MATERIAL AND METHODS

The species Diplolaimella spec 1, previously misidentified as Monhystera microphthalma, is a new species not as yet described. It was sampled in the Dievengat, a polyhaline water pond, situated near the Nature Reserve 'het Zwin' in north-western Belgium. Adults are about 1 mm long and weight circa 0.5 µg wet weight. Stock-cultures were maintained agnotobiotically on 0.5 % bacto-agar (DIFCO) enriched with 1 % Vlasblom-medium (Vranken et al., 1984a) and 1 % silicate

$(Na_2SiO_3.9H_2O$; 0.053 M stock-solution). Stock-cultures were kept in vented petri-dishes of 5 cm diameter.

Copper $(CuSO_4.5H_2O)$, lead $(Pb(C_2H_3O_2)_2.3H_2O)$ and mercury (Hg_2Cl_2) toxicity was studied using egg-, juvenile- and pooled pre-adult mortality as criteria. Experiments were performed in the dark in vented petri-dishes (∅ : 3.5 cm) at 20°C, 20°/$_{oo}$ salinity and an initial pH = 7.5. Salinity was measured with a refractometer. The medium used for the experiments consisted out 0.5 % bacto-agar (DIFCO) made in Dietrich & Kalle (1957) seawater, enriched with 1 % Walne-Provasoli medium, plus 1 % amino-acids solution with glucose enrichment and 0.5 % silicate (see Vranken et al., 1984a for details). At each concentration 5 replicate experiments were run in which 125 gravid females were allowed to deposit eggs for 1 day. An unidentified bacterial mixture grown separately on bottoms free from metals was given as food. The number of eggs studied, varied between 268 and 348 for copper, 248 and 427 for mercury and 248 and 407 for lead.

Fecundity was studied under similar conditions as the lethal tests. At copper concentrations of 100 μg/l and 1 mg/l, 60 females and males which matured in the previously done acute tests, were divided at random over 5 replicates. Every 6 days eggs were counted and adults transferred to fresh cultures. At 500 μg/l copper no mortality-assay was performed. The adults (60 ♀♀ and ♂♂) for the fecundity-test at this concentration were taken from the 100 μg/l experiment.

Population growth r_m was calculated with Lotka's equation :

$$\sum_{x=0}^{\infty} e^{r_m x} . U_x = 1 \qquad (1)$$

U_x is the number of female-newborns produced per female of the preceeding generation, when that generation is in the age-interval (x, x+1). U_x is calculated as $U_x = Ne_x.p.l_x$, where Ne_x is fecundity of a female aged x, p the proportion females in the population (= 0.5) and

1_x the survival probability form the egg-stage onwards until age x.
Net-reproductivity R_0, the multiplication rate per generation is
calculated as :

$$R_0 = \sum_{x=0}^{\infty} U_x \qquad (2).$$

Mean generation time T is determined as $T = \ln R_0/r_m$ (3).
Minimum generation time, T_{min} is estimated as the period between
identical stages of successive generations, equalling approximately
the sum of embryonic and postembryonic time.

RESULTS

Acute toxicity tests

Copper (Table 1)

    The lowest concentration inducing a significantly different
hatchability (P = 0.05) whem compared with the blank (= MEC : minimum
effective concentration) is 100 µg/l. For the juveniles MEC is 10 mg/l
and for the pooled pre-adult mortality MEC is 100 µg/l. At 50 mg/l Cu,
5 eggs were deposited; none of these hatched.

Mercury (Table 1)

    For both egg- and pre-adult mortality, MEC = 100 µg/l. At 1 mg/l
0.3 % of the juveniles did not reach adulthood. For the juveniles,
MEC = 5 mg/l. At 10 mg/l pre-adult mortality is 42 % : 31 % of the
eggs did not hatch and 16 % of the juveniles died before reaching
maturity.

Lead (Table 1)

    The MEC for non-hatching and pre-adult mortality is 5 mg/l. For
juvenile mortality MEC is 10 mg/l.

Developmental assay

Development time prolongs significantly (P < 0.001; NESTED ANOVA) with increasing metal concentration (Table 2). The smallest levels tested caused a significant lengthening for the three metals. Developmental retardation of both sexes, with an exception at 20 µg/l Cu (0.001 < P < 0.01; ANOVA) is similar. Development time at the highest Cu level is significantly longer when compared with the other two metals.

Fecundity and demographic analysis

Egg-production is significantly influenced (0.025 < P < 0.05; ANOVA) in the range tested. Maximum production was reached after 31 days. Total fecundity drops from 147 eggs per female in the blank to 97 at 1 mg/l Cu (Table 3). Daily egg-production at 0.5 mg/l and 1 mg/l is significantly less (P = 0.05) when compared with the blank (Table 3). Both net-reproductivity (0.005 < P < 0.01; ANOVA) and r_m (0.025 < P < 0.05; ANOVA) decreased significantly with increasing concentrations. r_m decreased 9.7 % at 100 µg/l and 29 % at 1 mg/l Cu. At 1 mg/l this corresponds with a 43 % decrease in net-reproductivity (Table 4).

DISCUSSION

Three different responses : mortality during the pre-adult stages, development retardation and suppression of fecundity, were studied. For the first two responses a physiological standard, development time, was choosen as the duration of the experiment. As generation time increases with metal concentration (Table 2), exposure time in our experiments increases simultaneously. Using mortality as a toxicity ranking criterion, Diplolaimella spec 1, appears rather insensitive,

even when compared with larger marine invertebrates such as

polychaetes (Reish, 1984), crustaceans (Ahsanullah et al., 1981) and

bivalve molluscs (Bryan, 1984). For copper an LC 50 of 12 mg/l

(95 % CI : 10.4-13.7), exceeding considerably its seawater solubility,

was calculated from the pooled pre-adult mortality with the Reed-Muench

method (Woolf, 1968). For mercury (I) the LC 50 is close to 10 mg/l

and for lead it is higher than 10 mg/l, a value also more than 1 order

higher than its maximal solubility. These figures are in contrast with

those presented by Howell (1984) for two large free-living marine

omnivorous/predacious nematodes, Enoplus brevis and E. communis. For a

similar exposure duration as in our experiments (312 to 430 h) the

LC 50's of Cu, Pb and Hg(II) of Enoplus brevis, the most resistant of

the two enoplids, lies somewhere between 10 and 100 µg/l. Howell (1984),

however did not mention whether he fed his animals during exposure.

For other organisms such as Daphnia magna metal-toxicity drops by adding

food (Biesinger and Christensen, 1972). We fed our animals on bacteria

previously grown on bottoms without metals. This may reduce toxicity

as it is known that some bacteria accumulate metals (Patrick & Loutit,

1976; Doyle et al., 1975; Berk & Colwell, 1981). The uptake of other

bacteria,on the contrary, like Escherichia coli (Doyle et al., 1975)

and gutbacteria of the nematode Mesorhabditis monhystera (Doelman et al.,

1984) is very limited. In additional experiments with the closely

related nematode species Monhystera disjuncta we could enhance mercury

(II) toxicity when juveniles were fed E. coli cells previously exposed

to 2.5 mg/l Hg(II). Mortality in this experiment was 56 %. When

mercury was only added to the substratum mortality was 6 %. Hence it

is clear that microbial activity influences toxicity in the Diplolaimella

assays and contributes almost certainly to the discrepancies found with

the Enoplus assay.

Several studies indicate that the free metal ion is the toxic species

(see Borgmann, 1983; Moore & Ramamoorthy, 1984 for a review).
Canterford & Canterford (1980) have demonstrated this with the marine
diatom Ditylum brightwellii : Cu, Zn, Cd & Pb toxicity decreased as
the concentration of EDTA, a strong chelating agent, increased.
Mercury-toxicity, on the other hand was independent of EDTA, which
is expected as in seawater Hg(II) froms strong covalent compounds
with chloride ions (Moore & Ramamoorthy, 1984). In freshwater mercury
complexation by EDTA lowers its toxicity (Whitton, 1967). The medium
used to culture Diplolaimella spec 1 is rich in substances, such as
phosphates, nitrates and organics like amino-acids, vitamins and
sodium EDTA (Vranken et al., 1984a) which bind a reduce toxicity of
heavy metals (Ramamoorthy & Kushner, 1975; Knezovich et al., 1981).
With the mussel Mytilus edulis, on the contrary, prior complexation of
cadmium enhances its uptake (George & Coombs, 1977) and therefore
probably its toxicity. Concerning the uptake of dissolved organics by
nematodes, conflicting results exist in the literature. Two predacious/
omnivorous nematodes Pontonema vulgaris (Chia & Warwick, 1969) and
Adoncholaimus thalassophygas (Lopez et al., 1979) are able to assimilate
labeled glucose, whereas the bactivorous Pellioditis marina is not
capable of doing it (Tietjen & Lee, 1975). Howell (1983) suggested
that, besides food intake, a significant route of metal uptake is via
the cuticle. Without having quantitative knowledge on the influence of
complexation on metal-toxicity to nematodes and without knowing the
exact route of metal-uptake we cannot assess the importance of these
factors in relation to the low toxicity-levels observed. Nevertheless
we may conclude that Diplolaimella spec 1 is a rather insensitive
species. Experimental evidence exists that this also holds for
other nematode species (Pitcher & McNamara, 1972; Samoiloff et al.,
1980; Haight et al., 1982). Field observations again corroborate this
statement as in highly polluted sediments in the North Sea, representatives

of the same feeding-type as Diplolaimella spec 1 become extremely
dominant (Heip et al., 1984).

Compared with mortality, development inhibition is a much more
sensitive toxicity index for Diplolaimella spec 1, which is similar
to what Samoiloff et al. (1980) reported for Panagrellus redivivus.
Data obtained by Haight et al. (1982) for P. silusiae are in variance
with the previous observations. The effective concentrations
inhibiting growth of P. silusiae were much higher than the LC 50's.
Vranken et al. (1984b) studying mercury toxicity to Monhystera disjuncta
noticed an 'all-or-none' response : some individuals developed as
fast as those in the blank, whereas the others died very quickly. So
far it seems advisable to supplement developmental tests by other
short-term assays. A possible alternative is to count the numbers
reaching adulthood in a fixed period of time, e.g. when at least 50 %
of the individuals matured in the blank. These figures are typical for
two different responses : mortality and developmental inhibition. When
using this as measure we obtain EC 50's (concentrations which induce a
50 % maturation inhibition when compared with the blank) of 28 µg/l
copper, 93 µg/l mercury (I) and 60 µg/l lead.

For Diplolaimella spec 1 significant reduction in fecundity occurs
at levels more than one order of magnitude less than the LC 50, e.g.
for the net-reproductivity the EC 50 is 1.4 mg/l Cu^{2+} (95 % CI : 0.7-
3.0 mg/l Cu^{2+}). Reish & Carr (1978) found for two polychaetes
differences of the same magnitude between these two types of tests,
except for copper and mercury where the differences were rather small.
Analogous small differences were obtained by Biesinger & Christensen
(1972) and Winner et al. (1977) for Daphnia magna. For this species
the 72 h LC 50 value of copper was not much different from the
concentration which inhibited the intrinsic rate of natural increase.
This is highly in contrast with our findings. Others (Blaylock et al,

1985) working with <u>Daphnia</u> <u>magna</u>, under copper stress also, observed large differences between mortality and reproduction, with the latter the most sensitive biological endpoint. Similar results were obtained by Moraitou-Apostolopoulou & Verriopoulos (1979) for the marine copepod <u>Acartia</u> <u>clausi</u> again under copper stress.

When pre-adult mortality is not too high, r_m approximates daily weight-specific productivity, namely the production-biomass ratio (P/B) (Vranken & Heip, in press). Mean annual temperature during the yearly growth period of <u>Diplolaimella</u> spec 1 is about 16° C. At this temperature daily P/B equals 0.112 Joule.Joule^{-1}.day^{-1}, resulting in a yearly P/B of approximately 22. At a Cu concentration of 1 mg/l, a level found in some sediments of the Belgian coastal area of the North Sea (Heip <u>et al.</u>, 1984), yearly P/B reduces to 15.5. Consequently it is clear that heavy-metal load may lower the productivity of bottom-dwelling organisms such as nematodes.

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Table 1. <u>Diplolaimella</u> spec. 1. Egg mortality (e), juvenile mortality (j) and preadult mortality in percentages at different metal concentrations (mg/l)

metal	significance (G/q test)	life stage	concentration (mg/l)					
			0	0.1	1	5	10	50
copper	★★★	e	3	12*	17	-	25	100
	★★★	j	1	0	0	-	10*	
	★★★	preadult	3	12*	17	-	33	100
mercury	★★★	e	3	10*	11	15	31	
	★★★	j	0	0	0	5	16	
	★★★	preadult	3	10*	11	19	42	
lead	★★★	e	3	5	8	10*	17	
	★★★	j	0	0	0	0	9*	
	★★★	preadult	3	5	8	10*	25	

★★★ : P < 0.001

★   : MEC-value (P ≤ 0.05)

-   : not tested

Table 2. Diplolaimella spec. 1. Development time (T_{min}) in days
of females and males with 95 % confidence interval (CI)
in parantheses at different copper, mercury and lead
levels; N = number of individuals studied, the
concentrations are in mg/l

	metal (mg/l)	♀♀	N	♂♂	N
copper	0	12.9 (+ 0.23)	156	12.8 (+ 0.20)	180
	0.02	14.0 (+ 0.27)	114	14.6 (+ 0.22)	131
	0.04	15.3 (+ 0.28)	121	15.4 (+ 0.29)	134
	0.06	14.8 (+ 0.23)	142	15.0 (+ 0.22)	149
	0.1	14.8 (+ 0.24)	120	14.8 (+ 0.21)	121
	1	17.0 (+ 0.42)	107	17.0 (+ 0.46)	116
	10	17.6 (+ 0.51)	102	17.9 (+ 0.44)	114
mercury	0	12.7 (+ 0.19)	213	13.0 (+ 0.22)	181
	0.1	14.3 (+ 0.29)	126	14.2 (+ 0.24)	140
	1	15.2 (+ 0.28)	186	15.1 (+ 0.25)	194
	5	15.0 (+ 0.35)	109	15.2 (+ 0.37)	119
	10	16.1 (+ 0.61)	69	16.3 (+ 0.60)	75
lead	0	12.7 (+ 0.19)	213	13.0 (+ 0.22)	181
	0.1	14.3 (+ 0.25)	183	14.0 (+ 0.23)	195
	1	14.5 (+ 0.32)	123	14.6 (+ 0.29)	129
	5	15.0 (+ 0.39)	115	15.3 (+ 0.33)	146
	10	16.3 (+ 0.44)	90	16.2 (+ 0.47)	96

Table 3.   <u>Diplolaimella</u> spec. 1. Total and daily egg-production per female at different copper concentrations (mg/1); R^2 = coefficient of determination; in parantheses : $\pm$ 95 % confidence limit, n = number of observations

Cu	Total egg-production	Daily egg-production	R^2	n
0	147	4.9 ($\pm$ 0.7)	0.99	6
0.1	132	4.2 ($\pm$ 0.9)	0.98	6
0.5	112	3.8 ($\pm$ 0.9)	0.97	6
1	97	3.5 ($\pm$ 1.0)	0.96	6

Table 4.   <u>Diplolaimella</u> spec. 1 : demographic characteristics at different copper concentrations (mg/1)

copper	Ro	estimated Ro $\pm$ 95 % CI	r_m (day^{-1})	estimated r_m $\pm$ 95 % CI	T (days)	estimated T $\pm$ 95 % CI
control	71	70 $\pm$ 7.0	0.186	0.181 (0.158-0.207)	23	23.1 $\pm$ 2.6
0.1	58	60 $\pm$ 4.7	0.168	0.176 (0.156-0.198)	24	23.6 $\pm$ 2.3
0.5	50	49 $\pm$ 4.8	0.159	0.155 (0.141-0.171)	25	25.4 $\pm$ 1.9
1	41	41 $\pm$ 6.9	0.132	0.133 (0.112-0.158)	28	27.7 $\pm$ 3.3

Toxicity of environmental toxicants on the marine nematode
Monhystera disjuncta. A comparison between developmental rate,
fecundity and mortality as toxicity-indices. 31326

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ABSTRACT

The toxicity of heavy metals (Cd, Co, Cu, Cr, Hg, Ni, Zn), acid-
iron waste, pentachlorophenol and γhexachlorocyclohexane on Monhys-
tera disjuncta is studied in laboratory assays. High levels of
toxicants (1mg/l) are necessary to cause acute effects on J_2-
larvae. The relative toxicity as measured by a developmental test
and fecundity was compared with threshold concentrations obtained
from the acute tests. Fecundity is the most sensitive criterion.
When LC 50 values are ranked and compared with the other criteria,
they correlate significantly with minimum effect concentrations
causing mortality and developmental inhibition (when only the
metals are considered), which are also correlated between them.
Ranking of toxicity based on fecundity does not correlate with
ranking based on mortality or development. Monhystera disjuncta
exhibits high resistance to pollutants when compared with other
benthic organisms. Therefore toxicants causing harmful effects to
nematodes have to be considered as extremely dangerous.

INTRODUCTION

The goal of ecological surveys on natural faunal assemblages is to
relate scarcity or absence of sensitive taxa to pollution. To
demonstrate the causality of such relationships, however, is not a
simple task. As presence/absence of species is the result of a
variety of physical, chemical and biological processes (Andrewartha
& Birch, 1954), adverse effects caused by pollution are not easily
distinguished from natural variability. This is certainly the case
for nematodes where effects of pollution and sediment-texture are
very difficult to separate (Heip et al., 1985). In the same
paper, Heip et al. (1985) concluded that the interpretation of
observed changes in stuctural community parameters such as diversity
is difficult, especially because the pre-pollution situation is
mostly unknown. In nature, pollution-induced changes in community
structure are probably the result of a complex set of multiple
interactions, such as synergystic and antagonistic effects between
constituents of effluents (Babich & Stotzky, 1983) influenced in
turn by fluctuating abiotic parameters (Bryant et al., 1984) and
by biological activity, e.g. bioaccumulation (Slowik, 1981) and
biodegradation (Doelman et al., 1985). Therefore it is hard to
study simple dose-response effects in natural conditions. Neither
do such studies yield quantitative information abouth no effect
levels (NEL's) of individual contaminants. Consequently the
effects of single chemical agents, such as heavy metals, on nematode
viability can only be determined properly under laboratory condi-
tions.
Experiments dealing with the adverse effects of pollutants on
organisms can be divided into two categories: 1) lethal short
term tests in either 48 or 96 h and 2) assays in which sublethal
effects over a longer period (several weeks) are studied. Acute
tests end with the determination of LC 50 values (concentration
of toxicant at which 50% of the organisms studied die). Mostly
these levels are much higher than those found in nature, implying
that short-term acute tests are inappropriate to assess and predict
long-term biological effects in moderately polluted situations.
Recently, Ward (1984) has shown that LC 50's are bad predictors
of ecological effects and that acute toxicity is only a minor

factor in structuring a seagrass faunal community. Brown's (1981)
critisism goes even further. He simply states that the determina-
tion of LC 50's is irrelevant for purposes of environmental pro-
tection. To predict 'safe' concentrations from 96 h LC 50's, an
application factor of 0.01 is often used (Reish & Carr, 1978).
This, of course, is rather thricky, unless the factor corresponds
to all or nearly all possible differences between acute and sub-
lethal toxicity indices. Most investigators, however, have publis-
hed only on acute effects and therefore it remains unknown whether
there exists a correlation between the two types of response.
When not, the usefulness of the arbitrary application-factor con-
cept is highly questionnable.
Another flaw of short-term tests is that standard procedures which
have been developed for easily cultured, so-called 'weed' species
usually are not suitable to test important indigenous species.
From an ecological point of view, the latter are without doubt
more important, especially in the case of point-discharges. Never-
theless, short-term tests using standard-species remain an important
tool for fast toxicity-screening and for inter-species comparisons.
The application of LC 50's is limited to these purposes and their
use in hazard assessment is not at all advocated.
In sublethal tests an array of possible responses can be studied
in relation to different toxicants-levels: respiration rates
(Bakke & Skjoldal, 1977), colonization ability (Chapman & Long,
1983), alternations of an organisms physiological condition (O:N
ratio) (Widdows, 1985), changements in adenylate energy charge and
ATP-ase activity (Verschraegen et al., 1985), scope for growth
(Widdows, 1985). Two toxicity-criteria, often used, are of major
interest in this context: the life-history parameters development
time until adulthood and fecundity. They are the key-factors
determining a species' potential productivity, and are thus re-
lated to the intrinsic rate of natural increase, r_m, which is con-
sidered as a measure of fitness (Snell, 1978). The exact determi-
nation of r_m requires the construction of the age-specific survival
rate (l_x) and age-specific fecundity schedules (m_x), which is a
tedious and very time-consuming task. To reduce time and con-
sequently cost of the experiments, we will study the influence of
environmental toxicants on daily fecundity and success in rea-
ching adulthood. These criteria are obviously related to m_x and
l_x-data. The results obtained from the fecundity and developmental

assay will be compared with those obtained from acute tests.
The nematode species studied, Monhystera disjuncta, is a bacterivo-
rous cosmopolitan species which can easily be cultured under labo-
ratory conditions (Vranken et al., 1984a). The advantages of
M. disjuncta for an in vivo toxicological assay have been dis-
cussed by Vranken et al. (1984 a&b). Procedures presented are also
applicable to other brackish-water nematode species (Vranken et al.,
in press) and even terrestrial nematodes. The test procedure
described by Coomans & Vanderhaeghen (1984) for Caenorhabditis
elegans is in fact based on our work on M. disjuncta.

MATERIAL AND METHODS

Monhystera disjuncta has been isolated from the Sluice Dock of
Ostend, a marine lagoon near the Belgian North Sea coast. For
isolation and simple agnotobiotic maintenance techniques we refer
to previous studies(Vranken & Heip, in press and Vranken et al.,
in press).
Medium
Small vented petri-dishes (35 X 10mm, Falcon) are filled with
4 ml 0.5% sterile bacto-agar (DIFCO) suspension in buffered artifi-
cial seawater (ASW) ($30°/_{oo}$S, 5 mM Tris buffer) after Dietrich &
Kalle (1957) and mixed with 0.2 ml of a sterol-mixture, with the
following constituents (Van Fleteren, 1980): 0.2 g cholesterol
(Fluka AG Buchs SG); 0.2 g ergosterol (Fluka AG Buchs SG), 0.2 g
β-sitosterol (Merck); 0.2 g stigma sterol (Merck); 0.2 g dehydro-
sterol (Merck) and 100 ml ethanol. The sterol-mixture is prepared
by adding 10 ml of the above mixture to 100 ml distilled water.
Hereafter the ethanol is evaporated and the mixture is autoclaved
during 20 min. at 1.2 bar.
After the medium has cooled down, a central ring-shaped excavation
(Ø : 15 mm) is made in the culture medium by pushing the top of
a sterile test-tube through the agar towards the bottom of the
petri-dish. The excavation is filled with ± 0.02 ml of an
Alteromonas haloplanktis suspension containing 10^{11}cells/ml.
Food preparation
Erlenmeyer flasks (100 ml) were filled with 50 ml heart infusion
broth suspended in artificial seawater (ASW) and than sterilized.
The medium is inoculated with the bacterial strain Alt. haloplanktis

under a horizontal laminar air flow bench and incubated during 48 h
at room-temperature and rotated in a rotary shaking machine at 125
rpm. Bacterial cells were harvested by centrifugation at 6000 rpm
during 15 min. The pellet is resuspended in sterile ASW and ad-
ded to the cultures.

Toxicity tests

Juvenile stage 2 larvae of M. disjuncta (4.5 days old) were exposed
to cadmium($CdCl_2$.2 1/2 H_2O, Baker Chemicals BV Holland); chromium
($Na_2Cr_2O_7$.$2H_2O$, Merck); copper ($CuCl_2$.$2H_2O$, Merck); cobalt ($CoCl_2$.
$6H_2O$, Baker Chemicals BV, Phillipsburg); mercury ($HgCl_2$, UCB Bel-
gium); nickel ($NiCl_2$.$6H_2O$, Merck) and zinc ($ZnCl_2$, UCB Belgium)
salts, to titanium dioxide waste water (pH = 1, NL Chemicals Sa-nv
Ghent, Belgium), the biocide pentachlorophenol (PCP, Merck) and
to the insecticide γhexachlorocyclohexane (γHCH, Sigma). For the
metals, stock-solutions of 1000 mg/l (as metal-ion) were prepared
in 1 liter distilled water using analytical grade salts. Then a
series of 100 ml solutions with concentrations ten times higher
than the final test-solutions were prepared. 5 ml of each soluti-
on is mixed with 42.5 ml 0.6% sterile (buffered) bacto-agar (60°C)
and 2.5 ml of a sterol-mixture. For cadmium the medium was prepa-
red immediately at the desired test-concentrations.

Stock-solutions of PCP and γHCH were prepared by solving 100 mg
in 0.5 ml analytical grade acetone, which was then added to 999.5
ml ASW and diluted to the final test-concentration. For TiO_2-waste,
two parallel experiments were run. In the first, the waste was
added to natural seawater (NSW) and the bacterial cells used as
food were grown previously during 48 h in identical waste-concen-
trations. In the second set, the toxicity of the waste was tested
in buffered (5 mM Tris) ASW. For practical purposes, the food
was grown in metal-free medium, because at pH = 7.5 to 8, the
iron present in the waste-water precipitates as ironhydroxide
($Fe(OH)_3$). This precipitate has a brown colour and hampers obser-
vation. Except with the iron-waste assay in ASW, the Alt.
haloplanktis suspension (containing $\pm$ 10^{11} cells/ml) added to the
cultures was grown during 48 h at the same drug doses as used in
the tests.

Active J_2-larvae (4.5 days old) sampled at random from a synchro-
nous cohort of M. disjuncta were tested in groups of 120 worms
(per concentration) equally distributed among 4 replicates. After

96 h the number of dead juveniles and adults were counted in all
test-cultures. Death is operationally defined as lack of movement
after stimulation with a needle. The daily egg-production of at
least 10 adult females, obtained from the different test-concen-
trations was thereafter determined by direct counts. Fecundity assays
were run during a period of 96 hours. All experiments were done
at 17°C and in the dark.

Statistical analysis.

LC 50 values were calculated by minimum logit Chi-square procedure
(Hewlett& Plackett, 1979). After fitting a linear relation between
the logit of the mortality response and the log_{10}dose, predicted
frequencies were compared with experimental frequencies by goodness
of fit analysis (Pearson's Chi-square). When the data were hetero-
geneous, the variances of both LC 50 values and the slopes were
corrected by the heterogeneity factor proposed by Hewlett & Plackett
(1979). In the γHCH-test, mortality could not be linearized by
logit nor probit transformation. LC 50 values for this compound
were estimated by inverse prediction from a linear least squares
regression of mortality, transformed into arc $sin\sqrt{proportion}$,
against the logarithms of the concentrations (Sokal & Rohlf, 1981).
Minimum effective concentrations (MEC's) based on mortality and
development were estimated by a log likelihood test (G-test, Sokal
& Rohlf, 1981). The raw data to determine the MEC's based on
development were obtained by counting the number of new adults in
all test-cultures when at least 50% of the juveniles reached adult-
hood in the blanks (after 96 h). The lowest drug concentration
at which the first significant difference appears, when compared
with the blank, is the MEC. Fecundity-data were examined by ANOVA.
MEC's based on this criterion were determined by a posteriori
tests by calculating 95% comparison intervals (Sokal & Rohlf, 1981).

RESULTS

Acute Toxicity

Acute toxicity to M. disjuncta of metals, TiO_2-waste, the biocide
PCP and the insectide γHCH are presented as LC 50 values (Table
3 &4). The mortality responses at the individual concentrations
tested are compiled in Table 2. The responses of M. disjuncta
J_2-larvae ranged only over one order of magnitude. Except mercury
and copper, none of the agents tested caused substantial mortality
at concentrations lower than 10 mg/l. The most toxic metal is cop-
per (96 h LC 50= 2.4 mg/l), followed by mercury (96 h LC50 = 5.6

mg/l). Zinc, cadmium, hexavalent chromium and cobalt are less to-
xic than the first two. Their LC 50 values ranged between 10 mg/l
and 100 mg/l. Nickel is the least toxic metal, with an LC 50 of
103 mg/l. This metal did not have an effect at concentrations
lower than 30 mg/l. Acute short-term toxicity testing results in
the following rank order of,toxcity: $Cu > Hg > Cr > Zn > Cd > Co > Ni$.
Values of MEC based on juvenile mortality result in the following
rank order of toxicity: $Cu > Hg > Co > Cr > Zn > Cd > Ni$. Except for the
position of cobalt, both sequences are the same (Spearman's $r_s = 0.79$;
$P < 0.05$; N=7). The concentration of Co which causes a significant
increase in mortality is 10 mg/l whereas its LC 50 is 94 mg/l.
This illustrates the very smooth dose-response pattern for this
particular metal (slope f=3.2; Table 3). For copper a very steep
relationship was found between mortality and concentration (slope
f=11.8 $\pm$ 1.0; Table 3). This means that deleterious effects caused
by Cu are situated in a very small range: between 1 mg/l and 2.5
mg/l. The slopes of the dose-mortality curves of Zn, Hg, Cr and
Ni have intermediate and nearly identical values (Table 3).
Acid-iron waste, tested in NSW, did not cause mortality up to a
dilution of 0.3 ml waste/ 1 NSW. At 0.4 ml waste/1 NSW mortality
steeply rose to 90%. This corresponds with a sharp decline in
pH : at 0.3 ml waste/l, pH is 6.7, whereas at 0.4 ml waste/l, pH
is 5.2, which demonstrates that an increase in acidity significant-
ly influences mortality ($F_s = 1706$; df= 1,10; $P < 0.001$). The estima-
ted LC 50 value is 0.36 ml waste water/l. The pH equivalence of
this LC 50 is 5.7 (95% CI: 5.5 - 5.9). The acid-iron waste water
is 100% toxic at a dilution of 0.5 ml waste water/l. The dose-
response relation caused by the waste water diluted in NSW is extre-
mely steep. No dose response relationship was observed when the
acid-iron was diluted in buffered ASW. Concentrations up to 10 ml
waste water per liter buffered ASW were tested.
The toxicity of the biocide PCP and the insecticide ƔHCH is compa-
rable to that of the most toxic metals (Cu and Hg). Concentrations
of 1 mg/l HCH and 2.5 mg/l PCP did not cause any mortality. The
LC 50's of PCP& ƔHCH are respectively 4.8 and 6.7 mg/l. The steep-
est dose-response relationship was found for the highly toxic PCP.
As a result the MEC of ƔPCP as measured by the percentage mortality
is somewhat higher than the LC 50.

Developmental assay.

Developmental inhibition of J_2 larvae caused by the metals, acid-iron waste water and the organics is shown in Table 5. Hexavalent chromium, although only intermediately toxic in the acute tsts, is extremely effective in inhibiting development. Chromium is the only metal studied which is toxic in the μg/l range. The relative toxicity rank order of the metals as measured by developmental inhibition is comparable to the sequence obtained when using mortality (LC 50 and MEC) as toxicity-index (r_s = 0.829; P=0.05). The concentrations which cause a significant developmental inhibition are at least similar (Cu and Hg) and for most metals (Zn, Cd, Cr and Ni) smaller than those found in the acute tests. The ratio's of the MEC's based on mortality and development ranged between 1 and 20. The ratio is maximal for Cr, intermediate for the intermediately and relatively non-toxic metals (Zn, Cd and Ni) and identical to 1 for Cu and Hg.

Similar results were obtained for PCP, $\int$HCH and the acid-iron waste. Lindane inhibits growth at 1mg/l, a concentration 2.5 times less than the acute toxicity threshold.

The toxicity thresholds of acid-iron waste tested in NSW are identical for both development and mortality. As for mortality, developmental rate is influenced by pH, the MEC is 5 times smaller in NSW than in buffered ASW. The EC 100 of the waste water diluted in buffered ASW is more than 25 times higher than in NSW. The relative toxicity rank order of all substances tested as determined by developmental inhibition only correlates significantly with the rank order obtained using MEC mortality as a criterion.

Fecundity

In Table 6 the daily number of eggs produced per female in the first 4 days of the fertile period is shown for all chemicals. Except for copper, the concentration range tested caused a significant reduction in fecundity (ANOVA, Table 8). At the highest test-level of Cu, a concentration identical to the MEC as measured by mortality, reproductive impairment was only 24%. For copper and mercury MEC's as measured by reduction in egg-production, are identical to the MEC's obtained in the acute and developmental assays. The position of Cu and Hg with regard to their relative toxicity as measured by suppression of fecundity is

intermediate to low. Chromium and zinc are the most toxic metals
followed by cadmium. Ni requires, as for the other criteria, the
highest concentration to inhibit reproduction. Toxicity ranking
of the metals based on reduction of fecundity does not correlate
with any of the other criteria studied (Table 9 & 10). For Zn
and Ni EC 50's were estimated (Table 7). Their values were almost
(Ni) and more (Zn) than one order of magnitude less than the cor-
responding LC 50 values. For PCP and γHCH the MEC is 1 mg/l. In
buffered ASW a concentration of 0.1% acid-iron waste significantly
depressed fecundity. The EC 50 was estimated roughly as 0.09% of
the undiluted effluent.

DISCUSSION

The toxicity of heavy metals, acid-iron waste, PCP and γHCH to
Monhystera disjuncta, as measured by mortality, developmental in-
hibition and suppression of fecundity was studied. Relatively
high levels of these substances, exceeding 1 mg/l are necessary to
cause immediate effects on J_2-larvae of the nematode. Fecundi-
ty is the most sensitive criterion for all substances tested.
Large discrepancies between the three toxicity-indices were only
found for the agents which are intermediately or relatively non-
toxic on an acute basis. For the most toxic drugs (Cu, Hg & PCP)
on an acute basis, there exists no difference in sensitivity be-
tween the three parameters studied. For the metals, development
(MEC), correlates with mortality (LC 50 and MEC) when these
effects are ranked. For all the substances tested, however,
development (MEC's) only correlates with MEC's as determined by
mortality. Fecundity does not correlate with mortality (MEC),
nor with development. Hexavalent Cr is most toxic, both in the
developmental and fecundity-test. Zn and Cd reduce reproduction
more strongly than Cu and Hg. Ni is the least toxic metal for
all criteria tested. These results are similar to those reported
by Reish and Carr (1978) for two polychaetes (Ctenodrilus
serratus and Ophryotrocha diadema): Cr, Zn, Cd and Pb were extre-
mely effective in suppressing reproduction, when compared with
96 h acute tests. For Cu and Hg, on the contrary, the difference
between the 96 h LC 50 data and reduction of reproduction was
rather small. Samoiloff (1980) reported for the nematode
Panagrellus redivivus that significant reduction in production

of offspring caused by Cd occurred at concentrations three orders
of magnitude lower than the MEC as measured by survival. For
Daphnia magna conflicting results are available concerning this
aspect of Cu-toxicity. Biesinger & Christensen (1972) and Winner
et al.(1977) found only small differences between the two criteria,
whereas Blaylock et al.(1985) found considerable differences in
Cu-toxicity depending on the criteria studied. For Diplolaimella
spec 1 significant reduction in fecundity occurs at levels more
than one order of magnitude less than the LC 50, which is highly
in variance with the present observations.
Haight et al.(1982) studied the influence of 7 heavy metals on the
length-growth and mortality of the free-living terrestrial nema-
tode Panagrellus silusiae. Concentrations necessary to block
growth ranged between 50 mg/l for Cu, Cd & Cr and 500 mg/l for
Zn, and were considerably higher than the 72 h LC 50 data. Ni and
Pb were without effect at the highest soluble concentrations and
mercury was either ineffective in blocking growth or lethal. For
this species effective growth inhibitory concentrations are con-
siderably higher than those found for M. disjuncta for all metals
tested. Samoiloff et al. (1980) present completely different
results for the nematode Panagrellus redivivus. For this species
growth inhibition is a more sensitive toxicity index than mortali-
ty. This also holds for the free-living marine monhysterid
Diplolaimella spec 1 (Vranken & Heip, in prep.). The difference
between the 2 toxicity-measures was 2 (Cu & Pb) and 1.5 (Hg)
orders of magnitude. Vranken et al. (1984 a) studying mercury
toxicity to M. disjuncta, found, as did Haight et al. (1982) that
the three mercury-compounds tested were either without effect on
development or lethal. More or less identical results were
obtained by Bogaert et al. (1984) for the nematodes Diplolaimello-
ides bruciei and Diplolaimella spec 1. Only the percentage of
successful moults of stage 4 larvae to adults is reduced at Hg
levels which cause no mortality.
Athough relevant information is lacking on the uptake and loss by
the nematodes of the substances tested, their bioavailability,
their concentration in the food offered and on the complexation
capacity of the growth-medium, we can conclude that M. disjuncta
is relatively resistant to pollution. Similar high resistance of
nematodes, especially to heavy metals is reported in other studies.

Feldmesser & Rebois (1966) reported LC 50 values for mixed popula-
tions of Panagrellus and Rhabditis between 53 and 40 mg/l. De
Maeseneer (1968) found that 200 ppm Cu caused 80% mortality with
three longidorid species. However, the density of other nematode
species such as Pratylenchus crenatus, Rotylenchus robustus and
unidentified saprozoic species was not influenced, even in very
acid soils. For three plant-parasitic nematodes, Xiphinema
diversicaudatum, Aphelenchoides ritzemabosi and Pratylenchus
penetrans, the 18 - 24 LC 50's of Cu are respectively 0.1, 4.1
and 2.6 mg/l (Pitcher and Mc Namara, 1972). A rather low LC 50
value for Cu of 0.06 mg/l is given by Hafkenscheid (1971) for the
nematode Trichodorus pachydermus. The predatory nematode Mononchus
aquaticus remained highly active during the first six hours after
exposure to 63.5 ppm Cu (Bilgrami & Jairajpuri, 1984).
Although LC 50 values are only of limited importance in hazard
assessment studies, they are a convenient criterion to compare
on the hand the relative toxicity of harmful substances and on the
other hand the relative susceptibility of different organisms.
In Table 11 the toxicity of heavy metals to M. disjuncta as mea-
sured in acute tests is compared with 3 free-living nematodes
and with representatives of the major taxonomic groups living in
benthic and epibenthic marine faunal assemblages. The ranking
of the LC 50 values of M. disjuncta correlates only significantly
with Panagrellus silusiae (r_s=0.829; P=0.05). Although copper is
most toxic to both species the difference between the two species
is slight to 1 order of magnitude. This is readily explained as
in seawater the maximum solubility of coppersalts is 0.4 - 0.8
mg/l (Davenport & Redpath, 1984). At higher concentrations,
excess copper will be precipitated as malachite (Bengtsson, 1978).
For the other metals the differences between the two species range
between 6.3 (Cd) and 1.1 (Cr) times. Enoplus communis is much
more sensitive than M.disjuncta. The Blyth river population of
Enoplus brevis, on the contrary is almost as resistant as M. dis-
juncta. In contrast, E. brevis, from Budle Bay,a site which is
considered as relatively unpolluted, exhibits higher resistance
to Zn, Cd & Cu than M. disjuncta. The LC50 values of the enoplids
calculated by us are high exposure concentrations (Howell, 1984).
At low exposure concentrations E. brevis from the Blyth survives
better than Budle Bay animals. Howell (1984) explained this as

an adaptation to environmental realistic concentrations. Perhaps
the high resistance of M. disjuncta may have evolved as the result
of a similar adaptation as very high metal-levels are reported
for sediments in the vincinity of the Sluice Dock (Bouquiaux &
Herman, 1977). Monhystera disjuncta is also extremely abundant
in heavily polluted sediments of the Southern Bight of the North
Sea (Vincx, 1983). The harpacticoid Nitocra spinipes is except
for Cu, at least 17 times more sensitive. Whether this can be
used as a support for the use of the nematode/ copepod ratio for
risk assessment is open to question. However, it proves unequi-
vocally that the harpacticoid probably is the better in vitro
bioassay. As similar high sensitiveness of harpacticoids to
pollution, especially heavy metal-load, is also observed in the
highly polluted Westerschelde estuary (Van Damme et al., 1984),
the use of harpacticoids as bioindicators in survey studies merits
more consideratiion. Further the . interspecies comparison revea-
led high differences between on the one hand, larvae of Carcinus
and Crassostrea and on the other, M. disjuncta with the former the
most sensitive. The differences with the polychaete Nereis
diversicolor, except for Cu and Hg, are rather small.
The growth-medium used is relatively poor in nutrients (Vranken
et al., in press). The most active binding substances present in
standard microbial growth media (Ramamoorthy & Kushner, 1975) are
not included in our medium. Therefore the low toxicity of heavy
metals and other compounds tested to M. disjuncta is in our opini-
on indicative for the general resistance exhibited by this parti-
cular nematode feeding-type to effects of pollution. This
conclusion is corroborated by field-studies, which have shown
that nematode-density is not effected substantially by raw domestic
sewage (Vidakovic, 1983), heavy metals (Tietjen, 1977, 1980) and
acid-iron waste (Lorenzen, 1974).
Ernst (1984) reviewed the toxicity of pesticides and organic
chemicals to marine organisms. For γHCH, 96h LC50 values ranged
between 0.2 μg/l for the shrimp Penaeus duorarum and 0.1 mg/l for
the sheepshead minnow Cyprinodon variegatus. The least sensitive
organism with regard to Lindane is still some 50 times more sen-
sitive than M. disjuncta. For PCP, the median acute level is
smaller than 1 mg/l for most organisms tested. Crangon crangon

Palaemon elegans and the blue mussel Mytilus edulis exhibit
similar or higher resistance to PCP than M. disjuncta. In the
present assays, PCP and ɣHCH, were solved in acetone before adding
to the test-medium. The concentration of acetone at the highest
exposure-concentration of both PCP and ɣHCH is about 40 mg/l.
Although acetone toxicity to M. disjuncta is not tested, there is
good reason to believe that such acetone concentrations are with-
out effect: 18h LC50's of acetone are higher than 1 g/l
(Bouwman et al., 1981), for example the 96h LC50 of acetone to
Nitocra spinipes is 16.7 g/l (Lindén et al., 1979).
At a temperature of 17°C and in NSW of about 30°/$_{oo}$S, the LC50
value of the acid-iron waste tested (pH≈1), which contains about
20% sulphuric acid and 2% iron (Roekens and Van Grieken, 1983),
is 0.036% (pH=5.7; Fe= ± 7mg/l). At such a dilution Cr is the on-
ly metal present at a relatively high concentration of 120 µg/l.
The other metal-constituents probably occur at levels far below
their MEC : 14 µg/l Zn, 1 µg/l Cu, 1 µg/lPb and about 40 ng/l of
both Cd & Hg (simplified after Pickaver, 1981). Acidification
might of course increase metal-toxicity. In our opinion it is
only the sulphuric acid component of the effluent which is harm-
ful. To test this hypothesis pure sulphuric acid will be tested
in a future-experiment. Possible synergistic interactions
between the metal-constituents can then be evaluated. Under
such test-conditions, a dilution of 1/500 (Fe concentration=
40 mg/l) caused developmental inhibition. After dumping in the sea
the iron present in the effluent is oxidized and precipitated.
The metals are coprecipitated forming mainly complexes with Fe
and Ti (Lehtinen et al., 1984), which probably reduce their
toxicity. The acid discharge is completely neutralized within
less than 1h (Roekens & Van Grieken, 1983). After 20 min. the
waste is 80,000 times diluted which is 160 times higher than the
developmental threshold against M. disjuncta, and 80 times
higher than the MEC as measured by fecundity. In the sea, the
reduction in pH is only limited to the first seconds after
discharge (after 1 min : pH⟩6). Therefore the assay done in
buffered ASW is probably more useful for risk-evaluation of acid-
iron waste with regard to nematodes. Newell et al. (1983) studied
the benthos-community in the vincinity of TiO$_2$ outfall and

they found no evidence of faunal impoverishment although some
stations were poor in meiofauna, but the differences were not
considered significant in view of meiofaunal variability in num-
bers. However, Huys et al. (1984) report a significant decrease
in taxonomic diversity in dumping areas of the Dutch coastal water.
Temperature also seems to have a significant impact on the acid-
waste toxicity. Lehtinen et al. (1984) reported at 21°C and 7°/oo
salinity a 96h LC50 to adult Nitocra spinipes of 0.013% of the
undiluted effluent, whereas at 4°C, 0.09% of the undiluted effluent
results in 50% mortality. The results of their 13 day fecundi-
ty test (EC50=0.024 to 0.033% of the undiluted waste-water)
can not be compared with the data of the Monhystera disjuncta
 fecundity assay because in the present work the egg-production is
studied under buffered conditions. We suppose however that
under natural conditions the effect of TiO_2 outfall have only
a minor effect on the viability of M. disjuncta. In conclusion
we may say that environmental toxicants that reduce the nematodes'
viability at levels occurring in nature have to be considered
extremely dangerous, since M. disjuncta is a relatively resistant
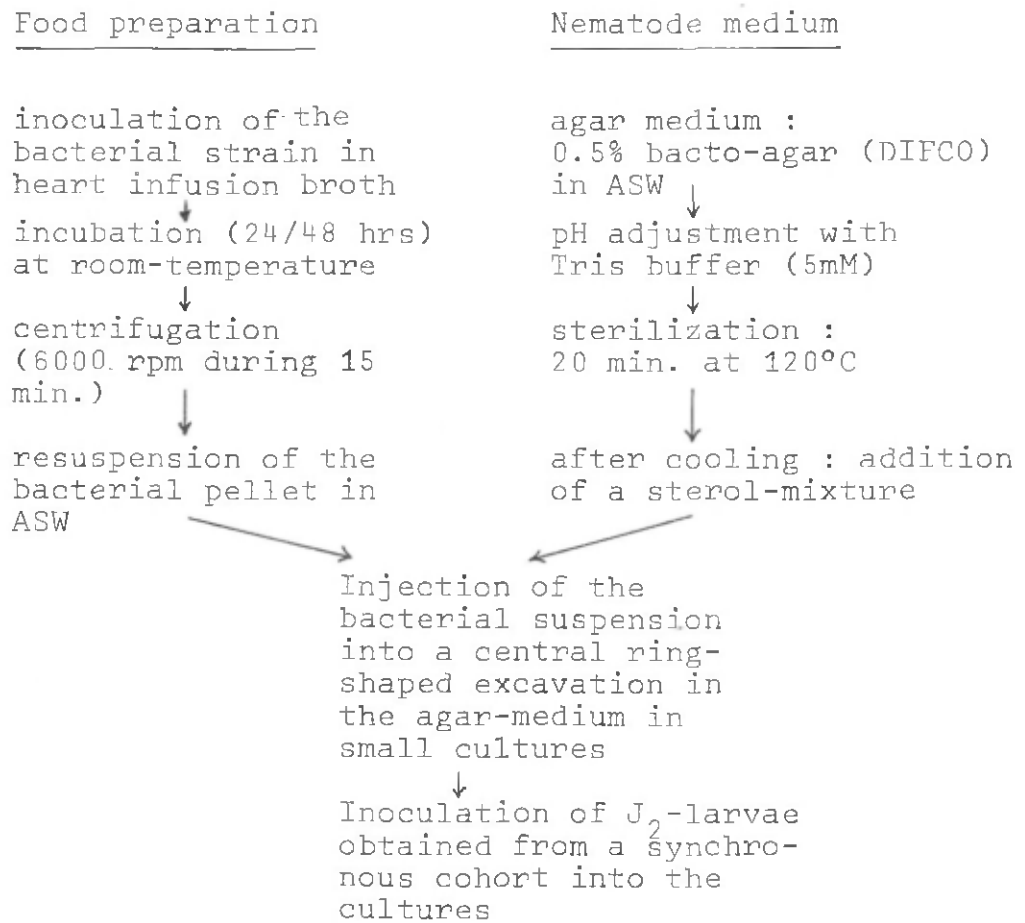species.

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Table 1. Diagram of the experimental procedure used in the tests.

Food preparation

Nematode medium

inoculation of the
bacterial strain in
heart infusion broth
↓

agar medium :
0.5% bacto-agar (DIFCO)
in ASW
↓

incubation (24/48 hrs)
at room-temperature
↓

pH adjustment with
Tris buffer (5mM)
↓

centrifugation
(6000 rpm during 15
min.)
↓

sterilization :
20 min. at 120°C
↓

resuspension of the
bacterial pellet in
ASW

after cooling : addition
of a sterol-mixture

Injection of the
bacterial suspension
into a central ring-
shaped excavation in
the agar-medium in
small cultures
↓

Inoculation of J_2-larvae
obtained from a synchro-
nous cohort into the
cultures

sterol-mixture: for composition see text

Table 2 : Percentage mortality (in parenthesis) after 96 hours at different metal/toxicant concentrations (mg/L) of 4.5 days old individuals of Monhystera disjuncta : the G/q statistic and an unplanned test : responses of underscored concentrations are homogeneous .

NSW : natural seawater ; ASW : artificial seawater

Metal/Toxicant	G/q	concentration and % mortality (in parenthesis)								
Zn²⁺	825***	0.75(0)	5(0)	0(0)	1(4)	10(4)	20(37)	30(67)	50(86)	70(99)
Cd²⁺	57***	0(0)	1(0)	5(0)	10(2)	25(22)				
Cu²⁺	779***	0(0)	0.75(1)	1(2)	1.75(10)	2.5(60)	5(97.5)	10(100)		
Hg²⁺	538***	0.5(0)	0(0)	1(0)	2.5(8)	5(36)	10(81)	7.5(82)		
Co²⁺	936***	0(0)	1(0)	3(0)	6(0)	10(6)	200(65)	300(94)	400(100)	
Cr⁶⁺	427***	0(0)	0.5(0)	0.75(0)	1(0)	5(0)	10(3)	15(13)	20(71)	30(71)
Ni²⁺	284***	0(0)	5(0)	15(0)	25(0)	35(0)	50(7)	70(21)	90(44)	110(51)
PCP	866***	0(0)	0.5(0)	1(0)	2.5(0)	5(60)	7.5(97)	10(100)		
γ-HCH	497***	0(0)	0.1(0)	0.25(0)	0.5(0)	0.75(0)	1(0)	2.5(40)	5(37.5)	7.5(50)
TiO₂-waste ° (NSW)	812***	0.3(0)	0.1(0)	0.2(0)	0(0)	0.4(90)	0.5(100)			
TiO₂-waste ° (ASW+buffer)		no mortality; highest concentration tested : 10 ml waste / L medium								

° : concentrations in ml waste / L medium

Table 3 : Minimum logit chi - square analysis : regression of the logit of the
mortality response (1) against the logarithm of the concentration
(C) : $1 = t + \beta \log C$; t : intercept ; β : slope ; m : 96 h LC 50
in mg / L ; SE : standard error ; CI : confidence interval ; χ^2 :
Pearson's chi - square for goodness of fit .

Metal/Toxicant	t	f (±SE)	m (95 % CI)	Pearson's χ^2
Zn^{2+}	-9.70	6.97(0.63)	24.6(22.7-26.6)	7.1(dℓ=5; NS)
Cu^{2+}	-4.47	11.78(1.00)	2.4(2.2- 2.5)	6.6(dℓ=4; NS)
Hg^{2+}	-5.30	7.06(0.98)*	5.6(5.0- 6.4)*	10.3(dℓ=4;0.025<P<0.05)
Co^{2+}	-6.27	3.18(0.81)*	93.5(49 - 180)*	34.8(dℓ=5;P<0.001)
Cr^{6+}	-11.38	8.64(2.09)*	20.7(17.5-24.6)*	28.4(dℓ=6;P<0.001)
Ni^{2+}	-15.70	7.79(1.12)	103.3(94.4-113.1)	5.7(dℓ=6; NS)
PCP	-11.77	17.37(2.25)	4.8(4.5- 5.0)	1.4(dℓ=4; NS)
TiO_2-waste (NSW)	22.15	50.47(8.88)	0.36(0.35-0.38)°	2.0(dℓ=3; NS)

° : ml waste / L medium

Table 4 : Regression of mortality (arc sin $\sqrt{\text{proportion}}$) against the logarithms
of the γ-HCH concentration .
a : intercept ; b : slope (+ SE) ; R^2 : coëfficient of determination ;
P : evaluates the significance of regression (F - test)

Metal/Toxicant	a	b	LC 50 (95 % CI)	R^2	P
γ - HCH	7.52	45.24(5.47)	6.7(2.6 - 19.1)	0.97	P<0.001

Table 5 : Percentage maturation (in parenthesis) after 96 hours at different metal / toxicant concentrations (mg/L) of 4.5 days old individuals of Monhystera disjuncta ; the G/q statistic and an unplanned test : responses of underscored concentrations are homogeneous.

NSW : natural seawater ; ASW : artificial seawater

Metal/Toxicant	G/q	concentration and % maturation (in parenthesis)								
Zn^{2+}	1024***	0(91)	0.75(97)	1(91)	5(60.5)	10(2.5)	20(0)	30(0)	50(0)	70(0)
Cd^{2+} ⊕	194***	1(69)	5(69)	0(67)	10(11)	25(0)				
Cu^{2+}	331***	0(61)	1(54)	1.75(42)	0.75(40)	2.5(0)	5(0)	10(0)		
Hg^{2+}	584***	0.5(87)	0(85)	1(82)	2.5(24)	5(10)	7.5(1)	10(0)		
Cr^{6+}	892***	0(97)	0.5(96)	0.75(79)	1(74)	5(0)	10(0)	15(0)	20(0)	30(0)
Ni^{2+}	680***	0(91)	5(87)	15(63)	25(39)	35(16)	50(0)	70(0)	90(0)	110(0)
PCP	703***	0.5(94)	0(92)	1(91)	2.5(87)	5(24)	7.5(0)	10(0)		
γ-HCH	1066***	0.1(96)	0(95)	0.25(93)	0.75(88)	0.5(87)	1(73)	2.5(16)	5(0)	7.5(0)
TiO_2-waste (NSW) °	692***	0.3(94)	0.1(93)	0.2(93)	0(89)	0.4(0)	0.5(0)			
TiO_2-waste (ASW+buffer) °	101***	0.1(92)	0(89)	1(88)	2(70)	10(43)				

° : concentrations in ml waste / L medium

⊕ : experimental time period : 120 hours

Table 6 : <u>Monhystera disjuncta</u> : daily fecundity per female during the first 96 hours
of the fertile life – period at different levels of metals / toxicants ;
CI : confidence intervals ; Comp I : comparison intervals : non-overlapping
intervals include significantly different fecundities ; Exp : experimental ;
Est : estimated (when not specified experimental values are given) .

Metal/Toxicant	Concentration (mg/L)	Daily fecundity	95% CI	95% Comp I
Zn^{2+}	control	62.1	53.7 – 70.5	60.9 – 63.3
	0.75	47.4	44.8 – 50.0	46.2 – 48.6
	1	49.6	44.2 – 55.0	48.4 – 50.8
	5	5.0	3.4 – 6.6	3.8 – 6.2
Cu^{2+}	control	43.2		
	0.75	34.1		
	1	43.8		
	1.75	33.0		
Hg^{2+}	control	45.4	34.3 – 56.5	39.4 – 51.4
	0.5	35.1	28.2 – 42.0	29.1 – 41.1
	1	41.1	30.9 – 51.3	35.1 – 47.1
	2.5	32.0	24.5 – 39.5	26.0 – 38.0
Cr^{6+}	control	52.4	39.0 – 65.8	46.7 – 58.1
	0.5	44.5	34.4 – 54.7	38.9 – 50.2
	0.75	30.7	23.5 – 37.8	25.0 – 36.3
	1	36.9	25.7 – 48.1	31.3 – 42.6

Ni^{2+}		Exp.	Est.	Est.(95% CI)
	control	65.7	65.7	–
	5	65.3	65.2	59.2 – 65.7
	15	19.8	31.4	12.2 – 51.7
	25	5.8	5.4	1.1 – 19.2
	35	1.4	1.1	0.1 – 7.3

	Concentration	Daily fecundity	95% CI	95% Comp I
PCP	control °	65.8	59.5 – 72.2	59.9 – 71.8
	0.5 °	58.7	48.8 – 68.5	52.7 – 64.6
	1 °	44.0	35.9 – 52.1	38.1 – 50.0
	5 °	10.2	2.1 – 18.3	4.2 – 16.1
PCP	control •	44.1	30.6 – 57.6	33.9 – 54.3
	0.5 •	49.9	33.9 – 65.9	39.7 – 60.1
	1 •	34.0	19.7 – 48.3	23.8 – 44.2
	5 •	9.6	0.0 – 21.5	0.0 – 19.8
γ-HCH	control	60.4	47.4 – 73.3	48.8 – 72.0
	0.5	44.4	31.8 – 57.0	32.8 – 56.0
	1	33.5	15.2 – 51.9	21.9 – 45.2
	2.5	26.2	4.4 – 47.9	11.2 – 41.2

o : first 96 h period of the fertile life-period
• : second 96 h period of the fertile life-period

Table 7 : 96 h minimum effect concentrations (MEC : concentrations at which
the first significant differences, compared with the blanks, appear)
of different metals / toxicants obtained from mortality responses, a
developmental assay and fecundity suppression.

Metal/Toxicant	Criterion		
	Mortality (MEC : mg/L)	Development (MEC : mg/L)	Fecundity (MEC : mg/L)
Zn^{2+}	20	5	0.75
Cd^{2+}	25	10	1 ✻
Cu^{2+}	1.75	1.75	1.75
Hg^{2+}	2.5	2.5	2.5
Co^{2+}	10	-	-
Cr^{6+}	15	0.75	0.75/1
Ni^{2+}	50	15	15
PCP	5	5	1/5
γ-HCH	2.5	1	1
TiO_2-waste ᵒ (NSW)	0.4	0.4	-
TiO_2-waste ᵒ (ASW+buffer)	>10	2	1 +

✻ : Boffé (pers. comm.)

ᵒ : in ml waste / L medium

+ : Verschraegen (pers. comm.)

NSW : natural seawater

ASW : artificial seawater

Table 8 : Influence of metals / toxicants, examined by ANOVA (F_s - statistic),
on fecundity during the first 96 hours of the fertile life - period .
P : significance level ; EC 50 (mg/L) : effective concentration reducing
fecundity with 50 % when compared with the blank ; df : degrees of free-
dom ; NS : not significant .

Metal/Toxicant	ANOVA : F_s (df)	P	EC 50 (95% CI) (mg/L)
Zn^{2+}	3661 (3,4)	P<0.001	1.9 (0.8-4.3)
Cu^{2+}	0.8 (1,2)	NS	>1.75
Hg^{2+}	4.4 (3,12)	0.025<P<0.05	>2.5
Cr^{6+}	15.0 (3,8)	0.001<P<0.005	>1
Ni^{2+}	117 (1,2)	0.005<P<0.01	15 (7.0-28.8)
PCP			
first period	70 (3,16)	P<0.001	2.1 (0.5-7.0)
second period	12.5 (3,16)	P<0.001	-
γ- HCH	6.6 (3,14)	0.005<P<0.01	1.6 (0.6-4.9)

Table 9 : Relative toxicities of metals to <u>Monhystera disjuncta</u> as measured by
different toxicity criteria.
MEC : minimum effective concentration at P<0.05
D : development ; M : mortality ; F : fecundity

LC 50	:	Cu > Hg > Cr > Zn > Cd > Co > Ni
MEC (M)	:	Cu > Hg > Co > Cr > Zn > Cd > Ni
MEC (D)	:	Cr > Cu > Hg > Zn > Cd > Ni
MEC (F)	:	Cr = Zn > Cd > Cu > Hg > Ni

Table 10 : Correlation between the relative toxicity rank order of metals to
Monhystera disjuncta as measured by different toxicity indices :
LC 50 ; MEC (M) ; MEC (D) and MEC (F) .

Criteria	Spearman's r_s	P	N
LC 50 / MEC(M)	0.786	P 0.05	7
LC 50 / MEC(D)	0.829	P=0.05	6
MEC(M) / MEC(D)	0.829	P=0.05	6
LC 50 / MEC(F)	0.116	NS	6
MEC(M) / MEC(F)	0.116	NS	6
MEC(D) / MEC(F)	0.464	NS	6

Table 11 : Toxicity of heavy metals to Monhystera disjuncta (mg / L), measured as 96 h LC 50 's in comparison to other organisms.

Metals	Monhystera disjuncta 96 h LC 50 (J2-larvae)	Panagrellus silusiae 72 h LC 50 (J2-larvae)	Enoplus communis 96 h LC 50* B.B.	Enoplus brevis 96 h LC 50*		Nitocra spinipes 96 h LC 50 (A)	Carcinus maenas 48 h LC 50 (L)	Nereis diversicolor 192 h LC 50* (L)	Crassostrea virginica 48 LC 50 (L)
				B.R.	B.B.				
Zn^{2+}	24.6	20.0	0.38	>100	>100	1.45	1.0	30	0.31
Cd^{2+}	∿ 37	5.85	0.2	10	>100	1.8	–	100	3.8
Cu^{2+}	2.4	0.28	∿ 0.1	1.6	5	1.8	0.6	0.27	0.103
Hg^{2+}	5.6	2.81	<0.01	5	0.08	0.23	0.014	>0.1	0.0056
Cr^{6+}	20.7	18.5 +	–	–	–	–	–	10	10.3 +
Ni^{2+}	103.3	28.6	–	–	–	6.0	–	–	1.18

* : stage not mentioned

+ : Cr^{3+}

L : larvae ; A : adults ; B.B. : Budle Bay (unpolluted) ; B.R. : Blyth River (polluted).

References : P.silusiae in Haight et al. (1982) ; E.communis & E.brevis in Howell (1984) ; N.spinipes in Bengtsson (1978) ; C.maenas in Connor (1972) ; N.diversicolor in Bryan (1980) ; C.virginica in Calabrese et al (1973).