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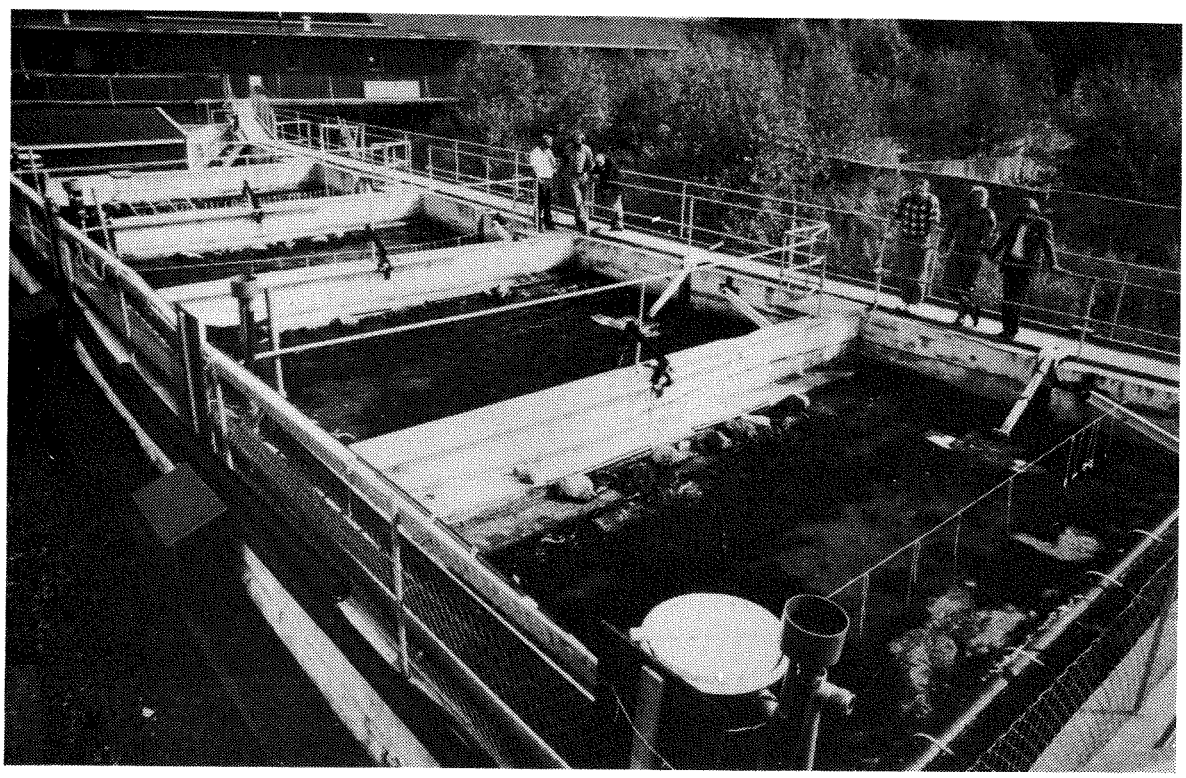
Solbergstrand Experimental Station



Long term effects of oil on marine benthic communities in enclosures

Section I
Littoral Rock Community Project
Progress Report no. 5

Section II
Sublittoral Soft Bottom Project
Progress Report no. 2

December 1984

Norwegian Institute for Water Research NIVA

University of Oslo

NIVA – REPORT

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Abstract:

The report presents results achieved during 1982-84 in the subproject under the research programme "Long term effects of oil on marine benthic communities in enclosures".

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PREFACE

The BP/NIVA/UiO Joint Research Programme was started in 1981 and will run to 1986. The aim of the programme is to investigate the effects of low level continuous pollution by oil hydrocarbons on two types of marine communities kept in large concrete basins. The programme contains two main projects; the Rock Littoral Project and the Soft Bottom Sublittoral Project, each covering a series of subprojects performed by Norwegian and foreign scientists. The programme is funded by BP Petroleum Development Ltd., Norway.

The present status report contains the compiled contributions from these subprojects, and covers the research done up and to November/December 1984. For the Rock Littoral Project this means that the whole oil exposure period (September 82 to September 84) is covered. Some contributions also report on the short term recovery after oil dosage termination. The Soft Bottom Report covers the subprojects performed by UiO and IMER (Warwich & Gee). Report on the soft bottom subproject performed by Kieler Meehresforschung (Ruhmor) has not been received.

The report will form the basis for an Advisory Board seminar arranged at Tømte, Hurdal (north of Oslo) at 10-12 March 1985. This seminar aims at giving a thorough scientific discussion of the results obtained in the programme.

Oslo 7 January 1985

Torgeir Bakke

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Programme Manager

C O N T E N T S :

SECTION I

SECTION II

SECTION I

LITTORAL ROCK COMMUNITY PROJECT

1. INTRODUCTION

In the Rock Littoral Project the oil pollution phase was terminated on 27 September 1984. Two communities had by then been chronically exposed to diesel oil at two different levels for more than 2 years. The communities are at present left undisturbed for investigation of short and log term recovery from the pollution impact. The recovery phase will last until the end of summer 1985. At present 1985 is considered the final year of the Rock Littoral Project; but efforts are made to establish new projects which can utilize the experimental communities which have developed in the basins.

Due to time shortage it has not been possible to prepare a report on the recruitment,growth and metabolism of the rock microlayers (the "granite chips" studies of Bokn and Pedersen) for inclusion in the present volume. The authors aim to present some of the results during the seminar at Tømte. Furthermore, the volume does not contain reports on the population dynamics of mussels and barnacles. These projects are at present being worked up as students' thesis works.

2. CHEMICAL ANALYSIS OF HYDROCARBON CONTENT IN WATER

Torgeir Bakke and Kai Sørensen

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INTRODUCTION

The Rock Littoral Project aimed at exposing two enclosed littoral communities to two different chronic levels of diesel oil hydrocarbons. The levels originally aimed at were 200 and 50 µg/l total hydrocarbons (dissolved and as droplets).

The purpose of the hydrocarbon analysis within the hard bottom project was to determine the levels of hydrocarbons in the dosing system, the basins and the organisms. The substrate on the walls has also been sampled.

This report presents results from the analysis of levels of oil hydrocarbons in the water.

Two types of analysis are involved in this project:

Fluorescence spectroscopy is used to

- perform frequent and inexpensive estimates of the concentrations of oil hydrocarbons in the water/oil emulsions (water accomodated fraction, WAF) produced for dosing into the basins, and in the basin waters.

- produce results quickly for routine adjustment of the dosing system to keep a stable exposure at the desired levels in each test basin.

- improve the fluorecence technique for routine monitoring of oil concentrations in experimental set-ups.

- correlate basin fluorescence analysis with analysis of the WAF extracts and with analysis by high resolution GC or GC/MS.

High resolution gas chromatography is used alone or in combination with mass spectrometry to

- determine real concentrations of oil hydrocarbons in WAF and basin water samples and the relative composition of the different oil components in these samples.

- determine levels of oil hydrocarbons in plant and animal tissues from exposed and control basins, and from material sedimented to the bottom or adhered to the basin walls.

DOSING SYSTEM DESCRIPTION

The system for continuous dosing of oil hydrocarbons into the basins consisted of:

- a preconditioning unit to keep a steady temperature, particle load and flow of the sea water supply to the system,

- a mixing chamber where diesel oil and sea water (gravity fed) is mixed continuously by propellers,

- a separation unit where most oil droplets are skimmed off from the WAF,

- dosing pumps and pipelines for controlled feed of WAF to the basins,

- header tanks above each basin where the WAF is diluted continuously with the inflowing sea water to the basins.

The prototype of the mixing and separation unit has been developed at the Bermuda Biological Station for Research. The dosing system was improved in the course of the experiment but the basic design was the same as when the system was started. Main improvements have been installment of the temperature preconditioning unit and larger capacity pipelines and pumps. The sea water supply to the unit was taken from 40 m depth and the conditioning temperature have been in the range of 11 to 13^{0}C (operative since February 1983). A diagram of the system was presented in the previous status report.

SAMPLING AND ANALYSIS

Fluorescens analysis

Samples of the WAF for fluorescence analysis were taken at three points in the dosing system by filling a stoppered measuring cylinder with 45-50 ml. The samples were extracted immediately with 5 ml n-hexane (p.a. grade) in the cylinder and the UV fluorescence intensity of the extracts was measured with a Perkin-Elmer spectrofluorometer with the excitation at 265 nm (slit width 10 nm) and the emission at 322 nm (slit width 5 nm). Fig. 1 shows the emission spectra of diesel oil in n-hexan. The fluorescence intensity was converted to total hydrocarbon units (mg/liter) by use of a calibration curve made by analysing fluorescence intensity of a dilution series of the diesel oil in n-hexane. An example of the calibration curve of 4 different diesel oil standards is shown in Fig. 2. The WAF-sample extracts were diluted with n-hexan to a concentration of diesel oil below 7 µg/ml prior to analysis.

The sampling points were the outlet of the separation unit, and the inlets to the header tanks of the two exposed basins. Two parallells from each sampling point were taken once every Monday, Wednesday and Friday.

For quick control of the fluorescence levels of the WAF and basin waters, and hence of dosing levels, water samples were run directly on the spectrofluorometer without prior extraction (excitation 265, slit 10 nm, and emission 335 nm, slit 5 nm) and the readings compared to previous set values. Efforts were made to make this a daily routine.

Samples for fluorescence analysis of the basin waters were taken with 1 liter brown glass bottles without collecting the surface film. The samples were taken 10-30 cm below the surface in the south end of each basin, and extracted twice with 25 ml n-hexane in separation funnels. The pooled extracts were analysed for fluorescence intensity in the same manner as the WAF extracts. These samples were taken once every week (Wednesday) from both exposed basins and one control basin. Occational samples have also been taken from the surface film of the high oil basin and one control basin and from the bottom and the outlet of the high oil basin.

The precision level tied to sampling and to analysis of the hexane

extracts have been tested:

Source	Number of series	Replicate Samples	C.V.-range (%)
Hexane extracts	3	6	1-2
Sep. unit outlet	6	5-6	2-5
Basins (different pos.)	3	3-5	4-10
Basins (same pos.)	2	4	4-12

Gaschromatographic analysis

Samples for GC analysis have been taken from the WAF pipeline at the inlet to the high oil basin header tank, and from the waters in both exposed and one control basin. The samplers have been 1 liter brown glass bottles and the basin samples have been taken simultaneously and in the same manner and posision as those for fluorescence analysis. Some samples have been taken from the separation unit outlet, the basin surface film and the basin outlet. The samples have been extracted on site with 3x20 ml of redistilled methylene chloride, and the extracts stored at -20 to -30 ^{0}C for 0-9 months prior to further analysis. Before analysis the extracts were dried with NA_2SO_4 and concentrated to a volume of 10 ml (WAF) or 155 ul (basin samples).

The extracts have been analysed by use of capillary GC and the amount of diesel oil determined as total hydrocarbon content (THC). Quantitation is carried out by comparing the flame ionization detector response area with corresponding areas of known amounts of the diesel oil. The analyses are performed using a Carlo-Erba Model 2100 gas chromatograph equipped with a FID detector. The instrument is supplied with a 30 or 15 meter fused silica capillary column coated with DB-5. 2ul extracts are injected splitless into a Grob-type injector with hydrogen as carrier gas. The injector is helt at 280^{0}C. The oven is initially held at 30^{0}C for 1.5 min; then the temperature is elevated quickly to 50^{0}C. This temperature is held for 3 minutes and then the oven is programmed by 20^{0}C/min up to 280^{0}C which is held for 9 min.

GC samples have been taken regularly every week during most of 1983/84, but the budget has only allowed a fraction of the samples to be analysed to date.

RESULTS AND DISCUSSION

Dosing procedure

Hydrocarbon dosing ran continuously for about 25 months from 15 September 1982 to 27 September 1984. With the exception of a two weeks break due to a fire in February 1983 interruptions in the dosing have lasted only a few hours in connection to regular maintenance or repairs. The main elements of maintenance have been regular cleaning (weekly or more often) of the whole dosing system, skimming off surplus surface oil in the separation unit, propeller and flow adjustments, and technical maintenance of the pumps and pipelines.

Microscopic investigation of the microbial growth in the mixing chamber and pipelines showed mainly small rodshaped bacteria incorporated in an organic matrix.

Although the maintenance demanded much man-power, it must be concluded that the dosing system has worked to satisfaction.

WAF analysis

The fluorescence analysis has shown quite large fluctuation in the WAF concentration of oil even between samples taken the same day. Most of this variation can be described to fluctuation in the mixing efficiency of the propellers, in 1982 also to temperature variation since the preconditioning unit was not installed. The range in values throughout the 25 months (based on means of duplicate samples) was (in mg/liter = ppm):

Source	Maximum	minimum	mean $\pm$ 1 st.dev.	samples
Sep. unit (SU) outlet	43.4	6.3	18.4 $\pm$ 5.9	255
HO header tank inlet	39.1	4.2	15.8 $\pm$ 5.6	255
LO header tank inlet	39.1	3.5	13.6 $\pm$ 5.4	255

As expected there was a significant correlation between the concentrations in samples taken simultaneously from these three points in the dosing system (r^2 indicates how much of the variation is explained by the regression):

$HO = 0.8842(SU) - 0.4858$, r^2: 84.7%, $p<<0.01$
$LO = 0.7660(SU) - 0.5507$, r^2: 68.5%, $p<<0.01$

The regression equations indicated that 12% of the fluorescing compounds (mainly aromatic hydrocarbons) were lost from the separation unit to the HO header tank, and 23% were lost from the separation unit to the LO header tank.

As stated above only few of the GC extracts of WAF samples have been analysed to date. They cover the period from October 82 to June 83, and have been reported earlier:

Source	Maximum	minimum	mean ± 1 st.dev.	samples
HO header tank	37.0	13.5	24.0 ± 11.3	5

The gas chromatograms show that the diesel oil enters the basins mainly as oil droplets (oil in water emulsion).

In the previous status report a regression between corresponding GC and fluorescence values for the WAF was presented:

$$GC = 1.33F - 14.13$$

and it was suggested that the reason why the fluorescence readings were higher than the corresponding GC readings was a relative increase in the amounts of aromatics in the WAF compared to the diesel oil, which both GC and fluorescence was related back to. A GC analysis on total and aromatic hydrocarbons of samples taken 22 June 1982 showed a relative increase in the content of aromatics from the diesel oil to the basins:

source	% aromatics
diesel oil:	23
WAF:	37
HO basin water:	58

It must, however, be stressed that these values are based on only one set of samples. Nothing is known of the variability of the aromatic content in the WAF and basin waters.

Basin waters

Theoretical hydrocarbon concentrations in the basins have been computed from the WAF concentrations at the entrance to the headertanks and the flow rates of WAF and sea water to the basins. The weekly mean concentrations in HO and LO basins from these calculations are presented in Fig. 3. The nominal overall means are:

#1 HO basin: 207.8 ± 74.2 µg/liter (st.dev.)
LO basin: 42.4 ± 17.0 µg/liter (st.dev.)

mean ratio HO/LO: 4.9

Fluorescence analysis of the hydrocarbons in the basin waters (HO, LO, and C2) has been performed routineously once a week from August 1983 to the end of exposure. The results are presented in Figure 4 (dotted lines). Mean concentration were (HO and LO are adjusted for C2 mean):

HO basin: 127.3 ± 49.5 µg/liter (st.dev.)
LO basin: 27.9 ± 11.6 µg/liter (st.dev.)
C2 basin: 5.6 ± 2.9 µg/liter (st.dev.)

mean ratio HO/LO: 4.6

Regressions between the HO and LO basin water content of hydrocarbons and simultaneous and corresponding WAF sample concentrations were significant ($p<0.01$):

regression equation	variation expl. by regression	correlation coefficient
HO basin conc. = 5.9089 (HO-WAF) + 36.1245	35.6%	0.60
LO basin conc. = 1.8999 (LO-WAF) + 4.3675	45.5%	0.68

indicating that the basin concentrations reflected the fluctuation in the WAF concentrations . The regressions above were therefore used to improve the estimates of basin hydrocarbon concentrations on basis of WAF values throughout the 25 months exposure period (Figure 4, solid lines).This produced the following total mean concentrations:

#2 1982-1984: HO basin: 129.4 ± 33.3 µg/liter (st.dev.)
LO basin: 30.1 ± 10.3 µg/liter (st.dev.)

mean ratio HO/LO: 4.3

CONT.

1982:	HO basin:	154.9 ± 50.0 µg/liter (st.dev.)
	LO basin:	42.4 ± 15.9 µg/liter (st.dev.)
	mean ratio HO/LO: 3.7	
1983:	HO basin:	123.1 ± 27.5 µg/liter (st.dev.)
	LO basin:	28.8 ± 7.8 µg/liter (st.dev.)
	mean ratio HO/LO: 4.3	
1984:	HO basin:	127.7 ± 28.3 µg/liter (st.dev.)
	LO basin:	27.4 ± 7.2 µg/liter (st.dev.)
	mean ratio HO/LO: 4.7	

These figures show that the mean exposure was highest in 1982, just after the dosing system was started. They also show that the LO exposure became gradually smaller compared to that of HO. The gradual increase in the HO/LO ratio (from 3.7 in 1982 to 4.7 in 1984) was significant (regression analysis and runs test).

A comparison of the estimated means given by #1 and #2 shows that the latter are 38 and 29% less than the former for HO and LO respectively. Since the estimates under #1 would have been the mean basin concentrations if no loss of hydrocarbons occurred from the header tanks to the basins, and the estimates under #2 are based on real basin analyses, one can conclude that there has been a mean loss of hydrocarbons from the header tank to the sampling point in the basin of 38% in HO and 29% in LO.

The estimated mean total loss of hydrocarbons of 12%+38%=50% and 23%+29%=52% from the separation unit to the basins is slightly lower than the estimates given in the previous status report (11%+43%=54% and 25%+37%=62% for HO and LO respectively), but this difference is hardly significant.

The GC analysis of the hydrocarbon content of the basins covers the first year of dosing (6 reliable sets of samples analysed to date), and has been presented in the previous report:

#3	HO basin:	82.3 ± 64.4 µg/liter (st.dev.)
	LO basin:	22.7 ± 25.5 µg/liter (st.dev.)

mean ratio HO/LO: 3.6

The gas chromatograms showed that most of the oil was present as droplets.

The correlation between simultaneous GC and fluorescence values from the basins are positive and significant (r-sq = 96 %, $p<0.5$), but so far only based on four sets of samples. A linear regression through the points has the equation GC = 0.41F + 1.29 reflecting the GC values as being less than half of the fluorescence values, and also that a zero value by GC gives about zero value by fluorescence.

The basin fluorescence analysis is related back to the original diesel oil, although the aromatic fraction of that is less than in the basin "oil". Therefore the fluorescence values could overestimate the real levels of total hydrocarbons. If the basin fluorescence analysis results are corrected for the found difference in aromatics, the mean basin levels of hydrocarbons from #2 would be as low as 51.3 and 11.9 µg/liter for HO and LO respectively, which is far below even the GC values (#3). Such correction should therefore not be done until the figures of aromatic content are better based.

Surface water of basins

At the same time as samples were taken for fluorescence analysis of basin waters, samples were taken and analysed from the surface water and film of the HO basin (slurp samples). The relation between surface and deeper water concentrations of hydrocarbons could be described by the equation

$$\log(\text{surface}) = 0.973 \log(\text{deep water}) + 0.294$$

and the correlation was significant ($p<0.01$). This indicates that the hydrocarbons accumulated in the surface water of the HO basin to a level about 75% higher than in the basin water deeper down. The mean value from two GC analyses of the surface water was 180 µg/l, which is more than 100% higher than the mean GC levels deeper down (cf #3), also suggesting that the hydrocarbons accumulated to a large extent in the surface film.

After termination of oil exposure a few tests were run to see if there

was any leakage of oil components to the water from organisms and basin walls. The HO water inlet was closed off for 6 hours during which 4-5 water samples were taken at set intervals from the water and surface film. This test was done 1, 4 and 36 days after the dosing was terminated. In connection to the tests, samples were also taken from the LO basin, the control C2, and the fjord.

These tests will be reported fully when GC analysis of some of the samples has been performed, but the fluorescence emission spectra indicated a slight flux of hydrocarbons to the water after 1 day, but hardly so after 4 and 36 days. The spectra indicated that fluorescing biogenic compounds may have contributed to the fluorescence intensity, especially of the surface film samples.

Conclusions

The fluorescence analysis of the water accomodated fraction (WAF) produced in the dosing system and the basin waters has shown that the littoral communities during the period September 1982 to September 1984 were exposed to the following mean concentrations of total oil hydrocarbons:

HO-basin: 129.4 µg/liter
LO-basin: 30.1 µg/liter

Background fluorescence (oil and other components) was 5.6 µg/liter.

The analyses showed that the ratio between the HO basin exposure and the LO basin exposure was gradually higher during the 24.5 months when oil was dosed to the basins: HO concentrations were 3.7 times higher than the LO concentrations in 1982 and 4.7 times higher in 1984.

Gas chromatographic analysis of the concentrations of oil in the basins showed the mean values:

HO-basin: 82.3 µg/liter
LO-basin: 22.7 µg/liter

The GC-analysis also indicated an enrichment of aromatic hydrocarbons in the basin water relative to the original diesel oil applied (58 and 23% aromatics respectively).

Both the fluorescence and GC analysis showed that the surface film of the HO basin had higher concentration (75-100%) of hydrocarbons than the water below.

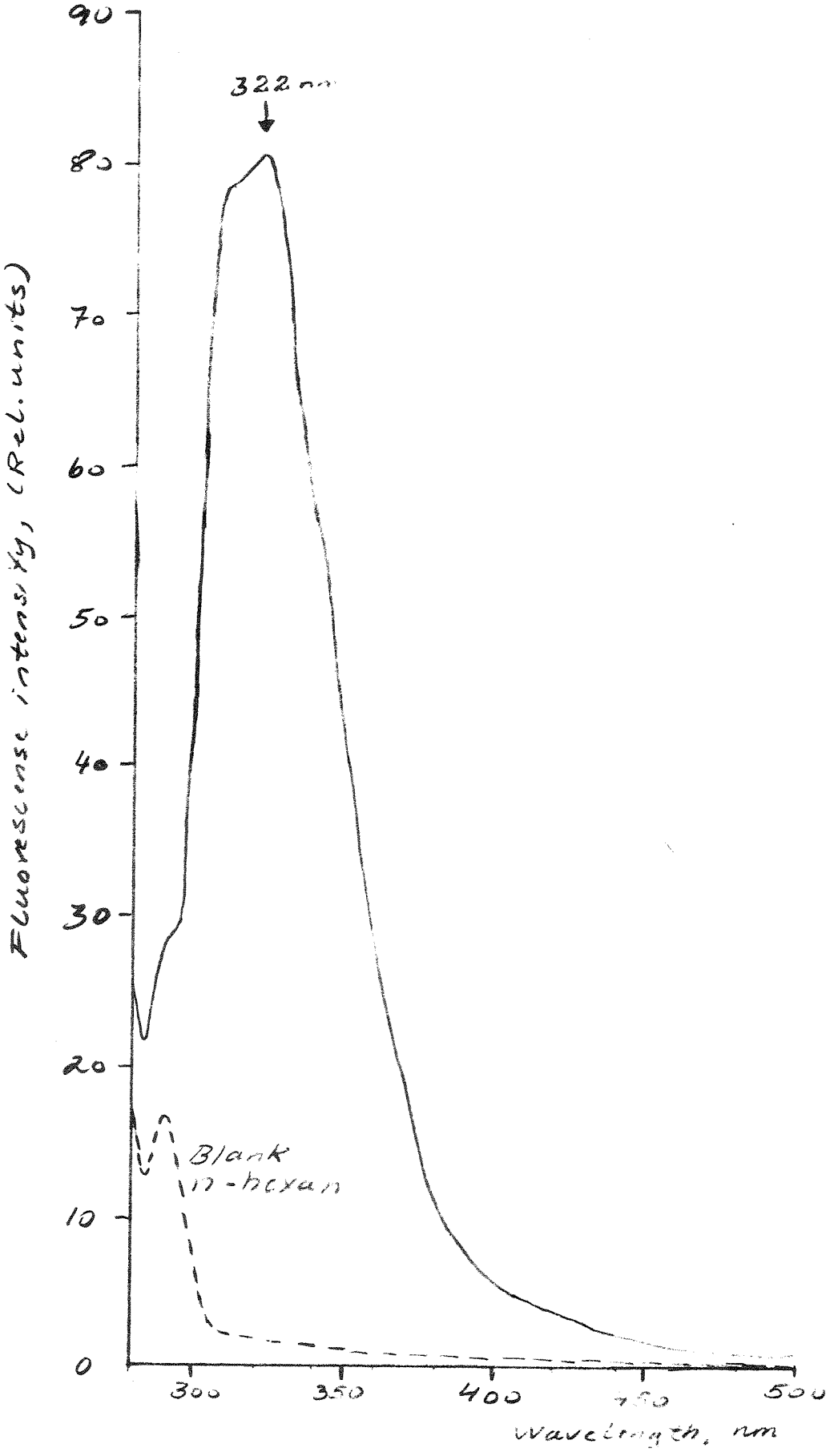

Figure 1 Fluorescence emission spectra of a diesel oil standard (1.74 µg/ml) in n-hexane.

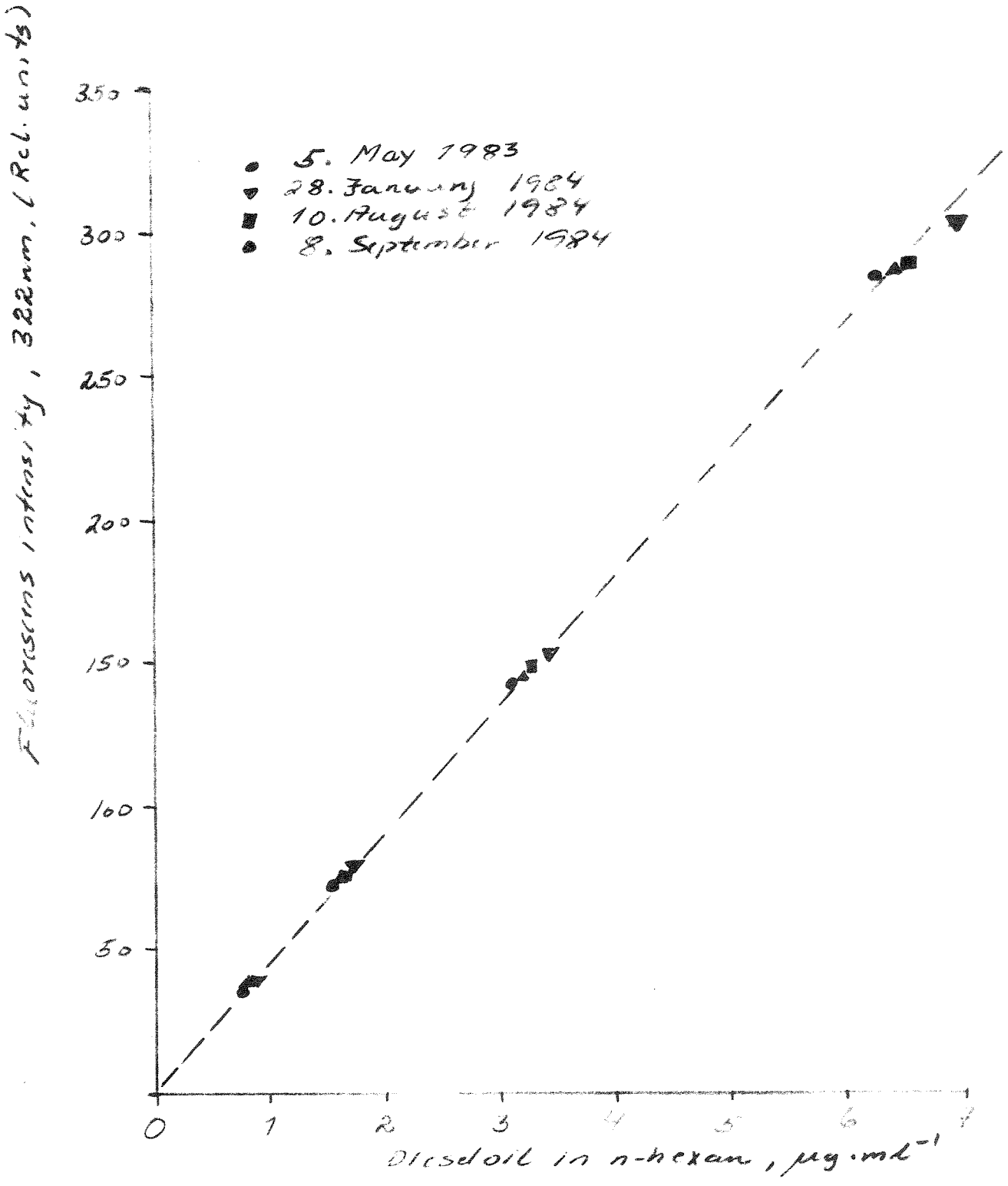

Figure 2 Calibration curve for 4 different diesel oil standards in n-hexane.

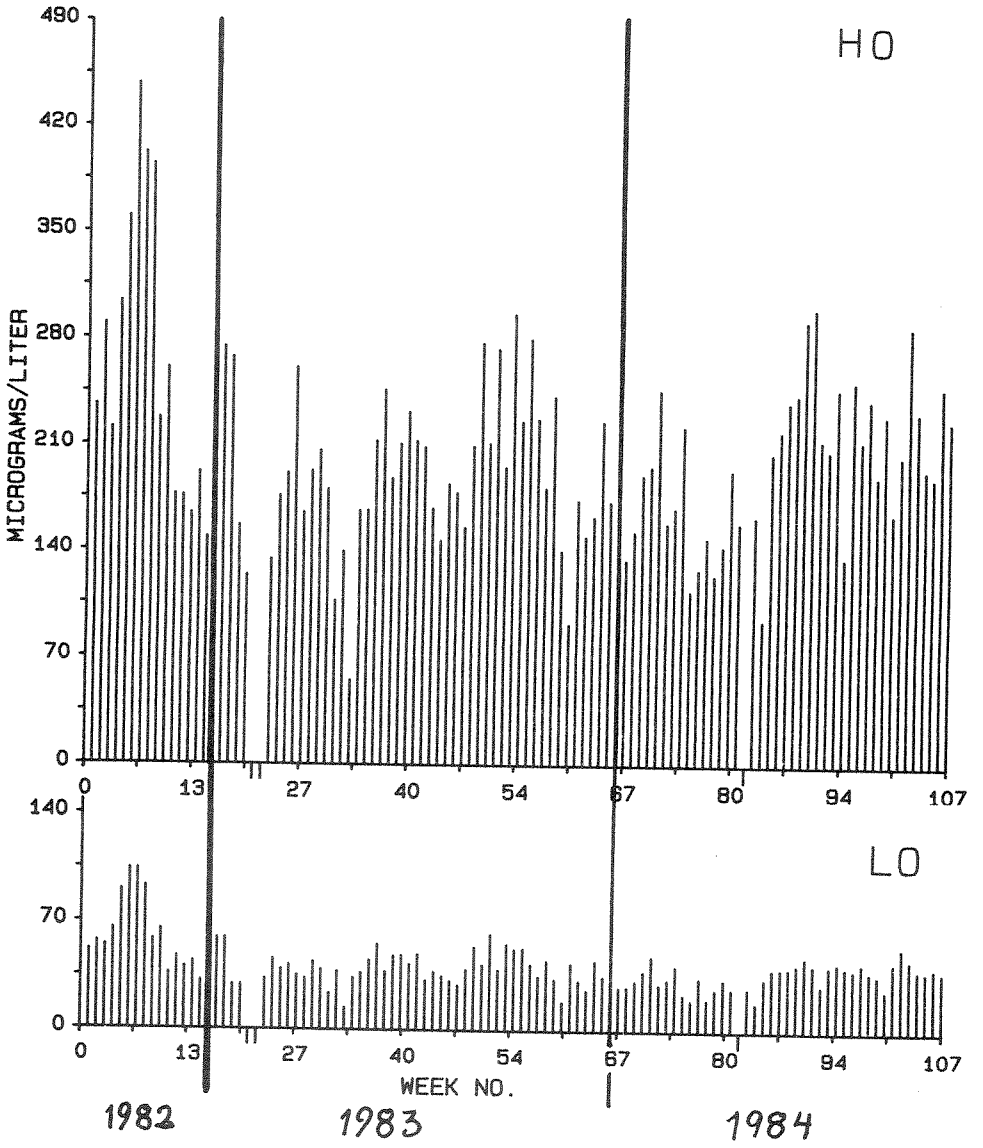

Figure 3 Nominal oil hydrocarbon levels of the HO and LO basin calculated from the fluorescence analysis of the WAF. The bars show weekly mean concentrations throughout the exposure period 1982-1984.

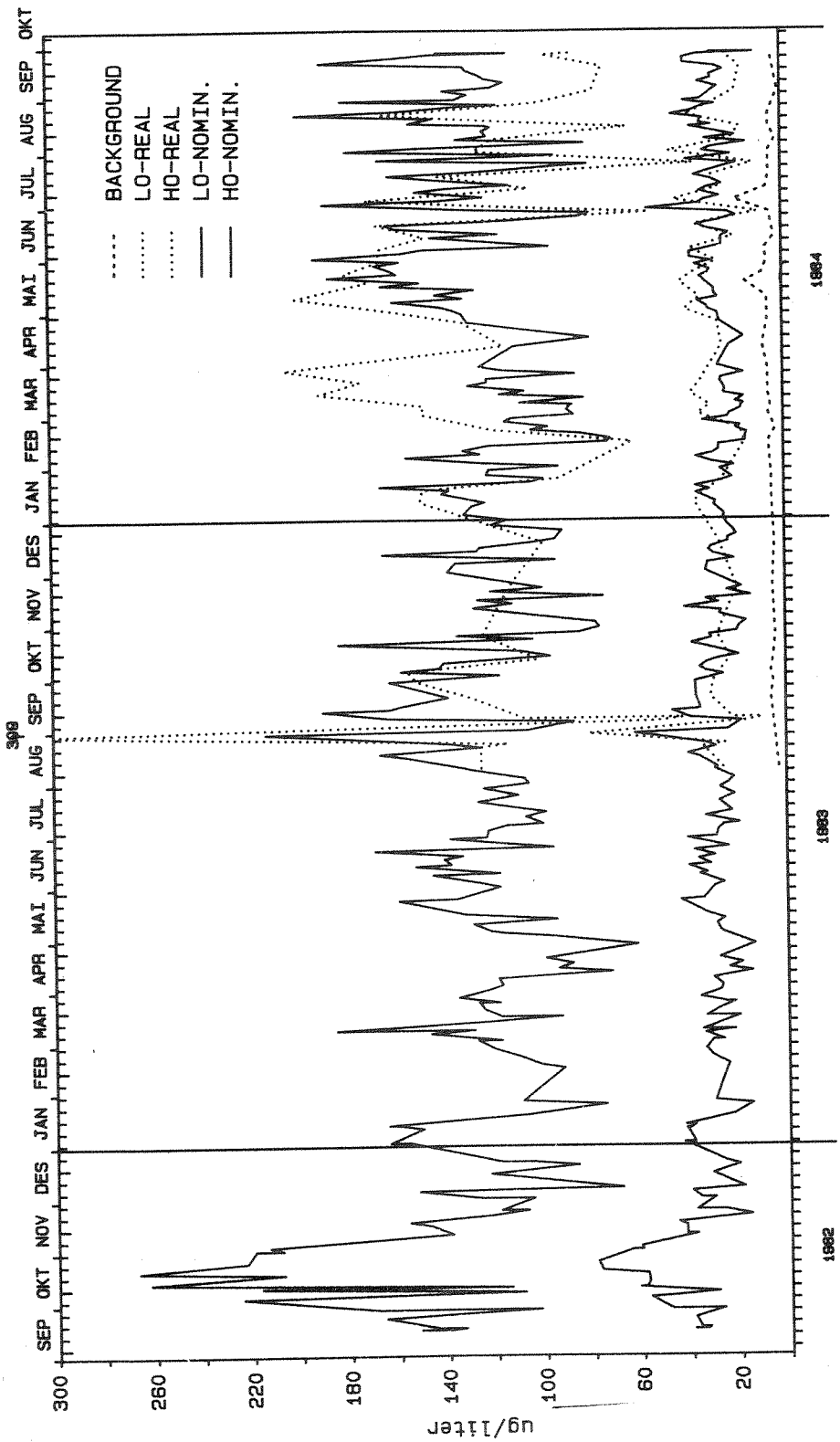

Figure 4 Real oil hydrocarbon levels in basins HO, LO, and C2 from fluorescence analysis of the basin waters between August 1983 and September 1984 (dotted lines). The solid lines show corresponding concentrations in the basins from 1982 to 1984 based on the regression between the WAF and basin fluorescence analysis.

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3. CHEMICAL ANALYSIS OF HYDROCARBON CONTENT IN ORGANISMS

by
Sigve Sporstøl and Frøydis Oreld

INTRODUCTION

The aim of the subproject presented here is to study the hydrocarbon load and dynamics of marine organisms contineously exposed to low concentrations of diesel oil.

MATERIALS AND METHODS

Sampling.

Four different species have been selected for tissue analysis; Fucus serratus, Ascophyllum nodosum, Mytilus edulis and Littorina littorea.
Samples have been collected both from the HO, LO and control basins, and covers the first 14 months of exposure. Ascophyllum nodosum have been collected at 2 different dates, while the other three species have been collected at 3 different dates (see table 1).

Chemical analysis.

The chemical analysis includes determination of both total hydrocarbon content (THC) as well as the amount of selected aromatic hydrocarbons (naphthalene, phenanthrene/anthracene, dibenzothiophene and their C_1, C_2 and C_3 alkylhomologs), 10-20 g of freeze dried and homogenized material is saponified in methanolic KOH after addition of deuterated internal standards (biphenyl-d_{10}, anthracene d_{10}, pyrene-d_{10}). The hydrocarbons are thereafter extracted into pentane and the pentane extract is concentrated to a volume of 0.5 ml. Polar components in this extract are removed by chromatographing with pentane on deactivated fluorisil. The eluate is concentrated to a volume of 150 µl.

Total hydrocarbon content (THC) is determined from gas chromatographic analysis of 2 µl of the concentrate. Quantitation is performed by comparing the total gas chromatographic respons within the boiling range of n-C_{10} alkane to n-C_{32} alkane to that of given amounts of the diesel oil applied at Solbergstrand.

The amount of the selected aromatic hydrocarbons (naphthalene, phenanthrene, dibenzothiophene and their C_1, C_2 and C_3 alkyl homologs) is determined from gas chromatographic/mass spectrometric analysis of the same extracts. The instrument is operated in the selected ion monitoring mode and the determination is based on the response of the molecular ion masses of the different compounds. Quantitation is performed by using deuterated internal standards which have been added to the sample prior to work up. The quantitation is based on multi level response factors.

RESULTS AND DISCUSSION

The results from the chemical analyses are given in table 1 and in figure 1 to 4. Both the sum of the selected naphtalenes, phenanthrenes/anthracenes and dibenzothiophenes (NPD) as well as the total hydrocarbon content (THC) is reported.

Exposed Mytilus and Littorina generally have the highest contents of both NPD and THC. Indications of seasonal variations are also found for these two species, but the data material is still too scarce.

In terms of trends a remarkable consistent picture emerges for NPD independent of species or reasonal variations. Highest concentrations are found in organisms from the HO basin and the concentrations measured in the LO basin are in between what is measured in the HO and control basins.

The same general trend, although not so consistent, is also found for THC. But due to relative high and variable amounts of biogenic hydrocarbons (which can not be distinguisted from petrogenic hydrocarbons) examples are found where the controls have higher concentrations than those sampled at the same date in the exposed basins.

Figure 5 and 6 shows typical examples of gas chromatograms of exposed organisms. Gas chromatograms of Mytilus and Littorina typical contain an unresolved complex matter with maximum at n-C_{20} alkane. The relative amount of n-alkanes versus the unresolved complex matter is low for these two species. Chromatograms of exposed Fucus and Asco also contian an unresolved complex matter, but substantial amounts of resolved components, including n-alkanes are also found.

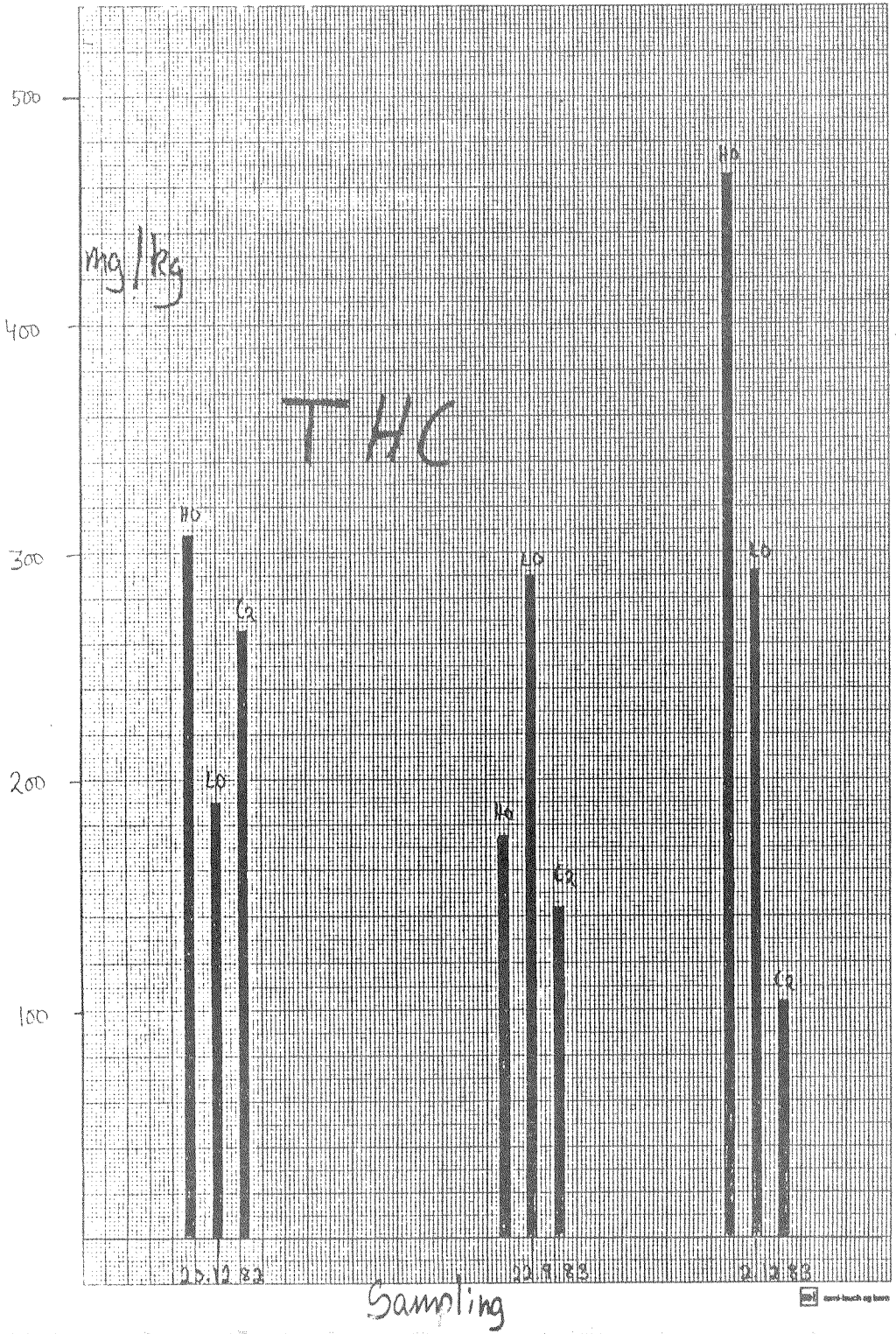
FUTURE WORK

The tissue samples analysed are less than desired to provide a picture of the hydrocarbon load and dynamics of the populations. In order to cover at least one season we will analyse 9 additional samples (3 Fucus collected 1.05.83, 3 Asco collected 15.07.83 and 3 Littorina collected March 84). Sediments from LO and C_2 as well as scrape-offs from the wave generators of the same basins collected after 14 months of exposure, will also be analysed. The additional samples will be analysed within the end of 1984.

TABLE 1. Total hydrocarbon content (THC) and amounts of selected aromatic hydrocarbons (NPD) (mg/kg dry material) in tissue samples from Fucus serratus, Ascophyllum nodosum, Mytilus edulis and Littorina littorea.

Basin	Date of sampling	THC	NPD
Fucus serratus:			
HO	20.12.82	307	2.42
HO	22.09.83	175	3.20
HO	02.12.83	467	3.46
LO	20.12.82	191	1.13
LO	22.09.83	292	1.02
LO	02.12.83	196	1.80
C_2	20.12.82	267	0.60
C_2	22.09.83	144	0.55
C_2	02.12.83	104	0.61
Ascophyllum nodosum:			
HO	20.12.82	57	2.07
HO	01.05.83	128	3.65
LO	20.12.82	152	0.86
LO	01.05.83	113	1.53
C_2	20.12.82	35	0.52
C_2	01.05.83	73	0.26
Mytilus edulis:			
HO	09.12.82	102	9.30
HO	25.03.83	186	12.5
HO	Nov. 83	602	25.4
LO	Nov. 83	854	20.4
C_2	25.08.83	97	1.65
C_4	09.12.82	59	1.15
C_4	Nov. 83	78	0.82
Fjord	25.03.83	183	2.83
Fjord	Nov. 83	93	1.26

Basin	Date of sampling	THC	NPD
Littorina littorea:			
HO	23.03.83	845	12.0
HO	20.07.83	526	3.29
HO	20.10.83	818	8.86
LO	23.03.83	330	4.20
LO	20.07.83	259	1.91
LO	20.10.83	343	2.59
C_2	23.03.83	155	1.10
C_2	20.07.83	130	0.92
C_2	20.10.83	125	0.92



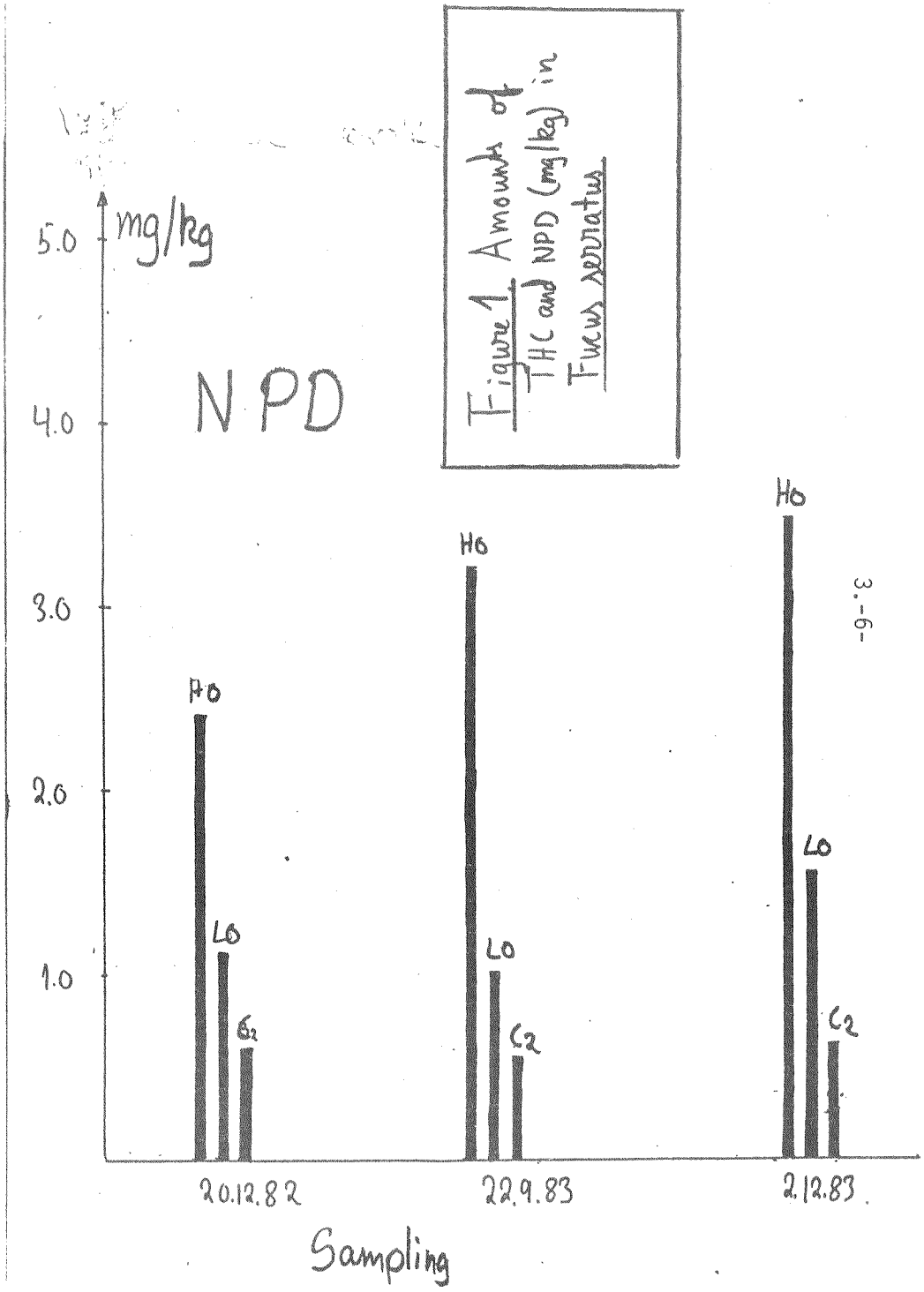


Figure 1 Amounts of THC and NPD (mg/kg) in Fucus serratus

Figure 2. Amounts of THC and NPD (mg/kg) in Amoophyllum nodosum

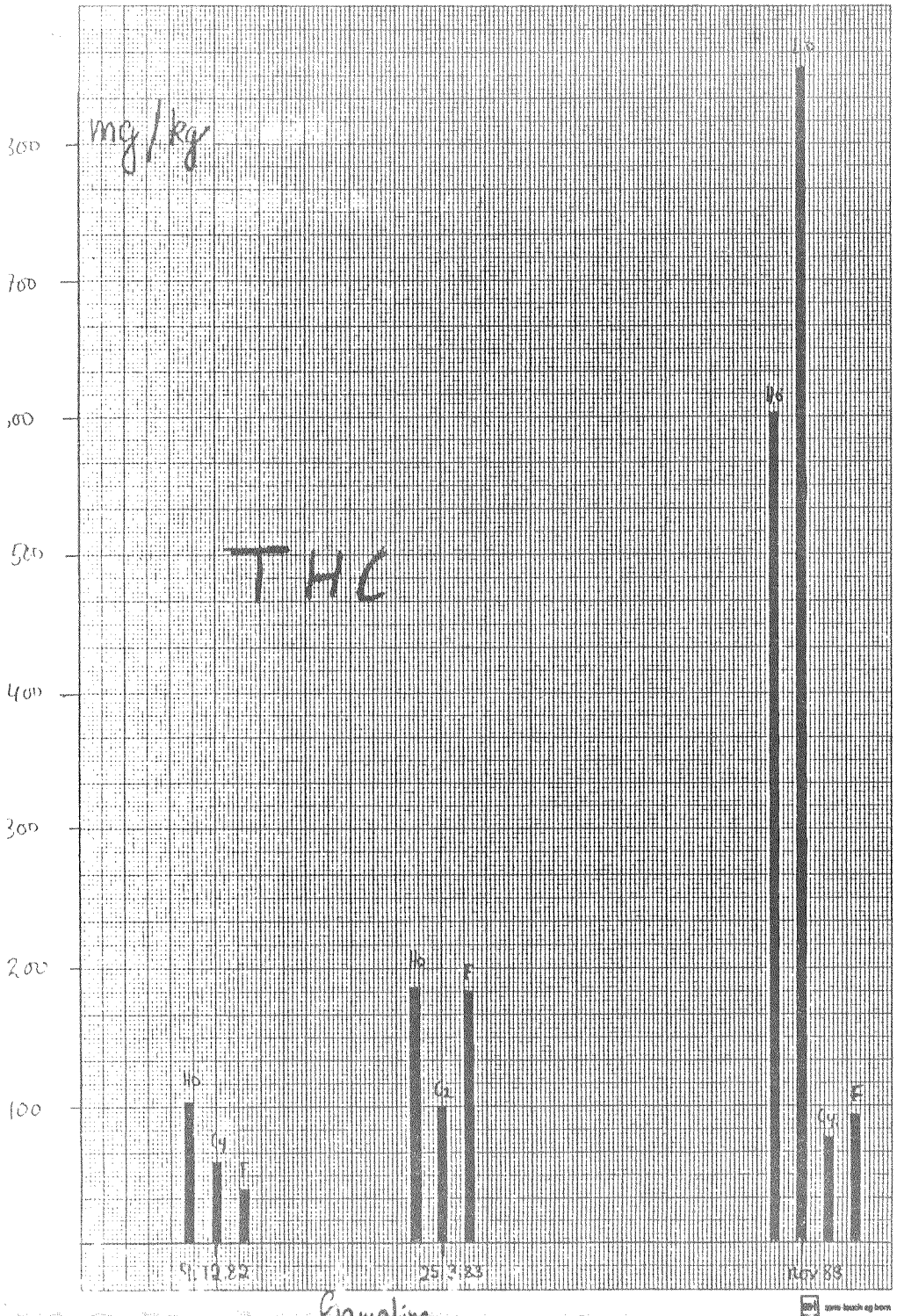
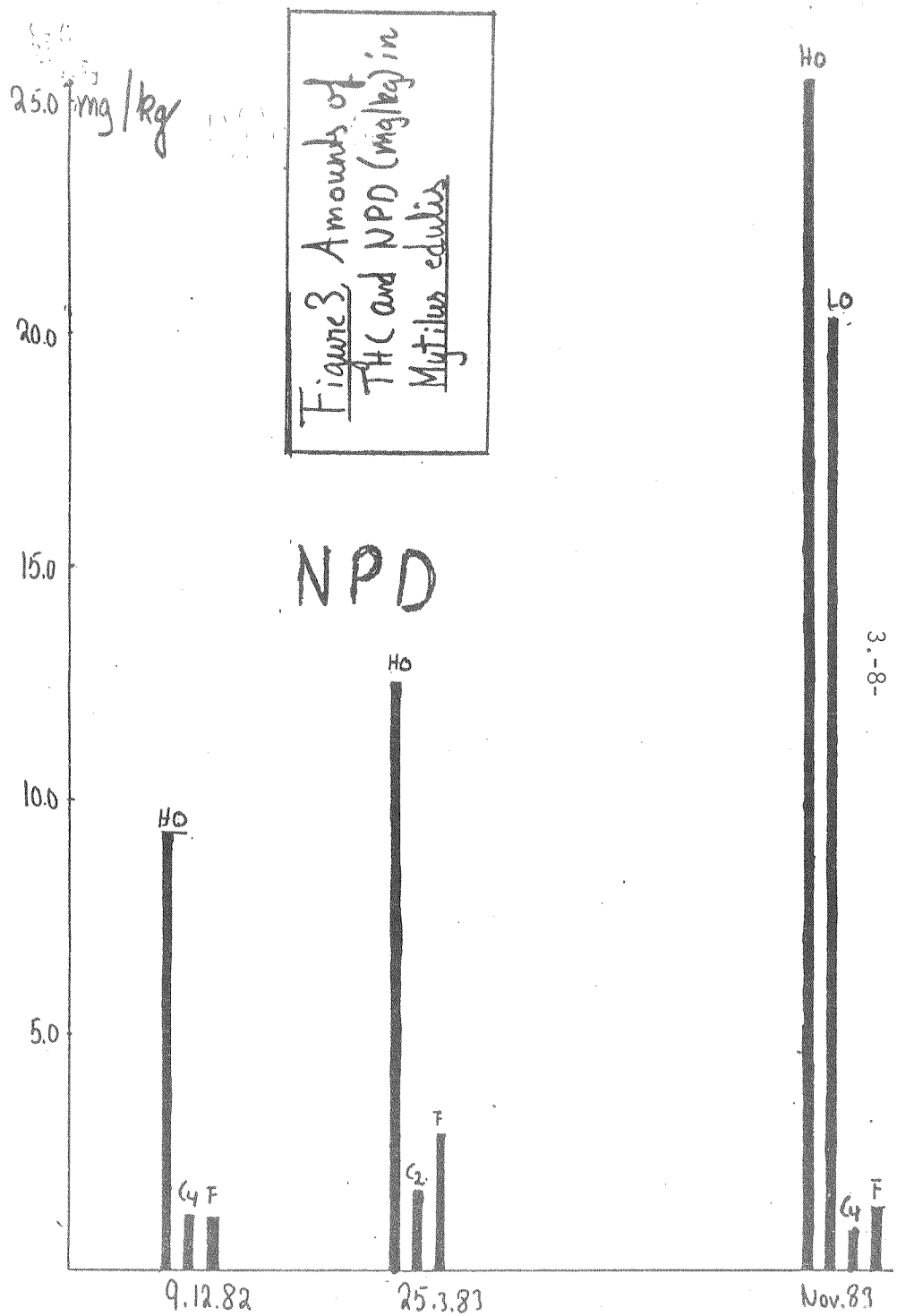
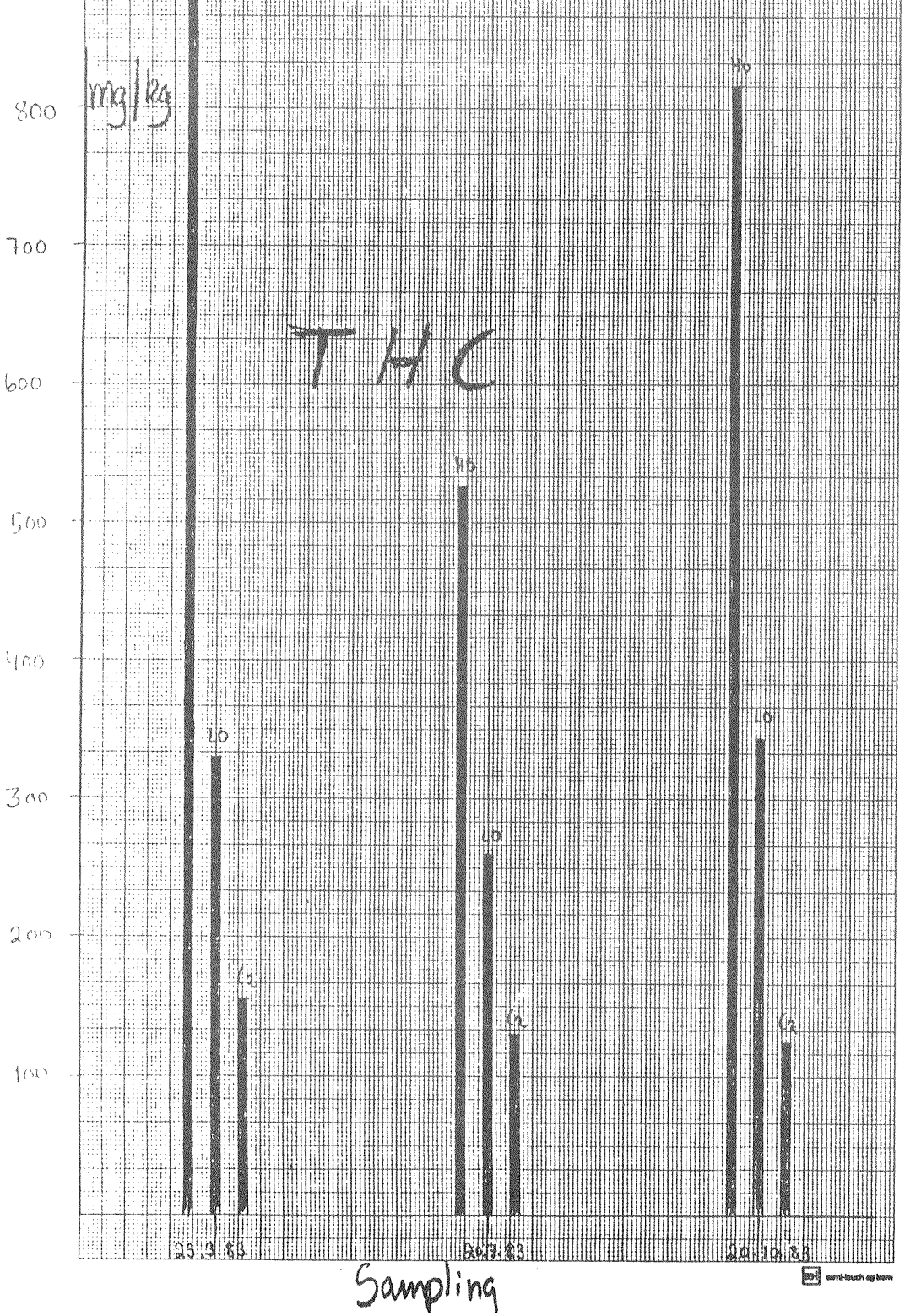


Figure 3. Amounts of THC and NPD (mg/kg) in Mytilus edulis





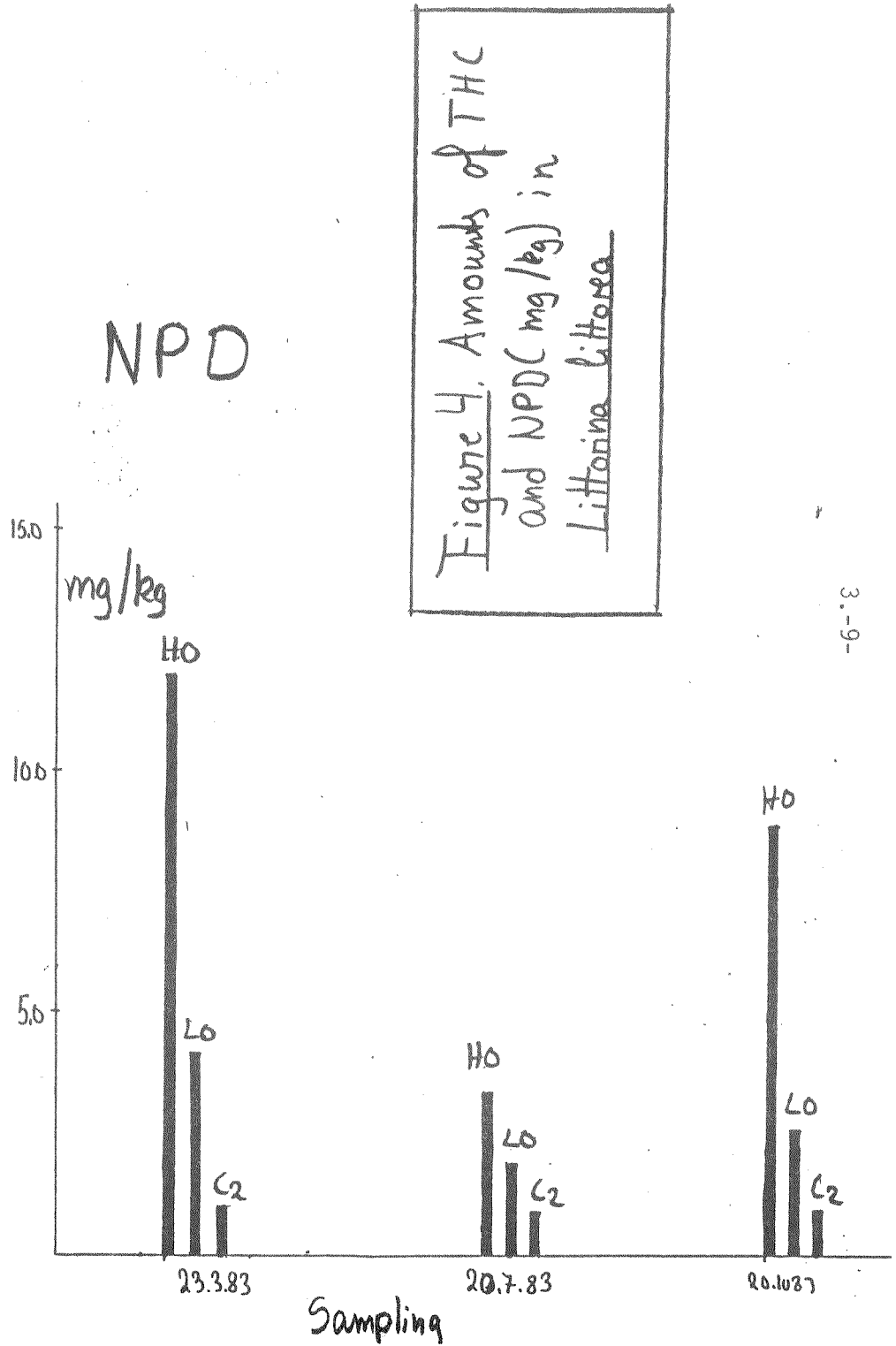


Figure 4. Amounts of THC and NPD (mg/kg) in Littorina littorea

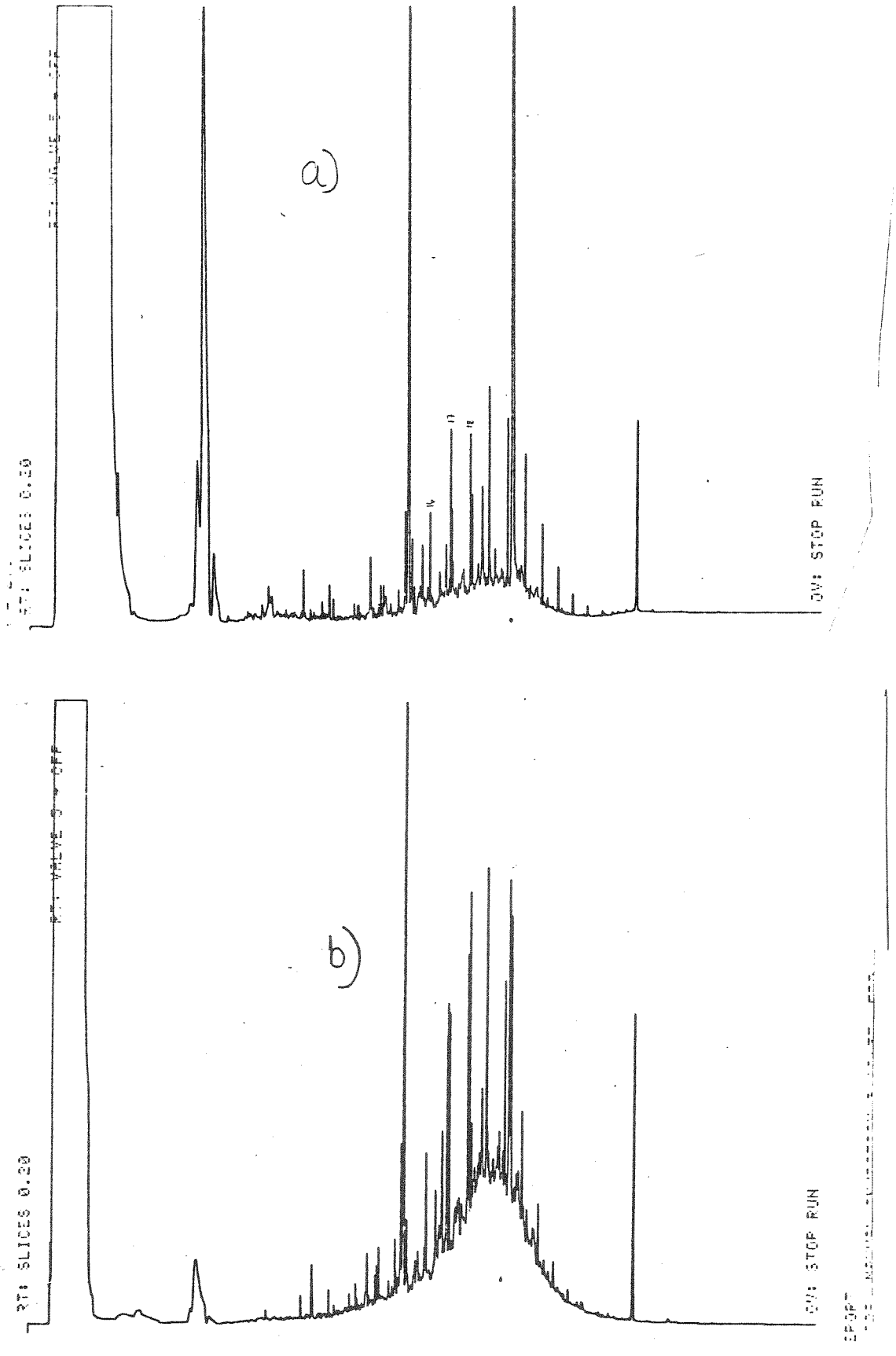


FIGURE 5. Typical gas chromatographic traces of exposed organisms.
a) Ascophyllum nodosum from HO (01.05.83)
b) Fucus serratus from HO (02.12.83)

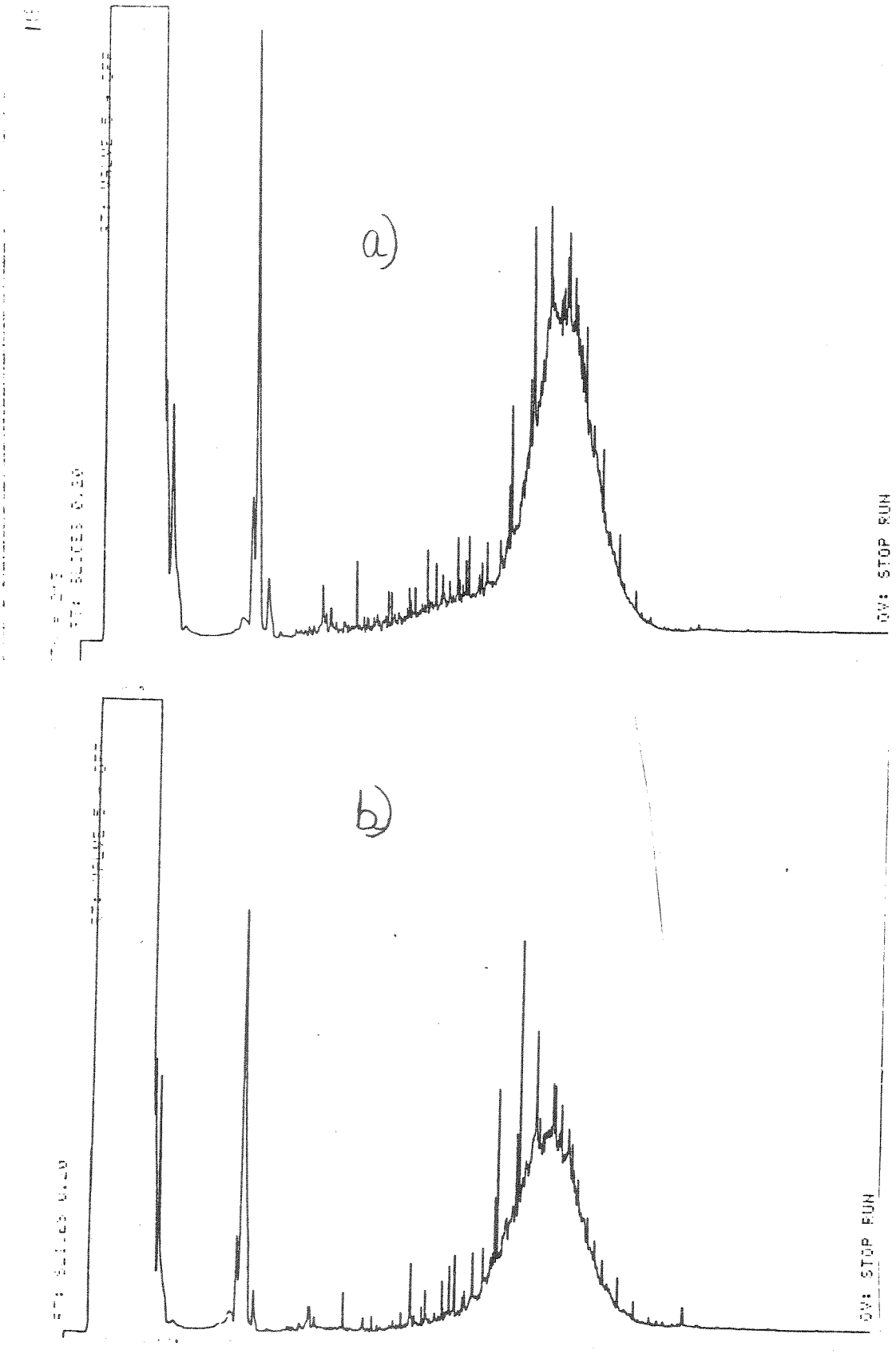


FIGURE 6. Typical gas chromatographic traces of exposed organisms
a) Mytilus edulis from LO (Nov. 83)
b) Littorina littorea from HO (20.10.83)

MARINE RESEARCH STATION SOLBERGSTRAND

4. COMMUNITY STRUCTURE

T. Bokn (NIVA) and F. Moy (UiO)

Introduction

The aim of the present subproject was to estimate the numbers of motile animals and covering degree of sessile plants and animals in set areas in every basin and in such a way detect any community changes and deviations between oil exposed basins and controls.

Since September 27th 1984 the diesel oil exposure during 26 months has been terminated, and the aim of this subproject is now to look for community and population recovery.

Material and methods

The composition of the littoral communities is characterized by monitoring percent cover of algae and sessile animals and number of motile animals. Cover degree is assumed to reflect the relative biomass of the organisms. Special frames adjusted to the dimension of the four basin steps and bottom are used for this (Figure 1). Six parallel quadrats from each step/bottom are investigated. The monitoring started in June 1982, and the periods of sampling each year have been:

15 January - 15 February
15 March - 15 April
15 May - 15 June
15 July - 15 August
15 October - 15 November

Thus 12 characterizations of the community structure have been performed during the period of investigation June 1982 - August 1984. The diesel oil exposed basins are: HO (∿100 µg/l oil), LO (∿25µg/l oil) and the controls are C2 and C4. Necessary equipment most of the year is SCUBA-diving gear with a full face mask connected by cable to a radio and a tape recorder.

The community structure is also documented by photograps in connection with the monitoring.

Data of the monitoring have been processed for about 20 species of the basins.

Graphs showing seasonal variation have been drawn.

Regression coefficient have been worked out based on: the regression equation $y = b_0 + b_1x$, with least squares criterion, and F-test for the significance of regression. With seasonal variation a linear equation is a poor assumption to biological data, and the significance of the calculated coeffisient, will be masked by a high variability. Only when the equation was found with a significance of $p<0.05$, the coeffisient will indicate an increase or decrease.

A paired-t-test was used to test the difference of the species' biomass. Differences are only referred to significant differences ($p<0.05$) in the following context.

To group the communities based on summer observations in 1982, -83 and -84, a cluster analysis was used. The data was sorted with Bray-Curtis index (Clifford & Stephenson 1975), and the Flexible fusing method recommended with Bray-Curtis index was used drawing the dendrogrammes. It should be emphasized that numerical superiority strongly direct the result of the cluster analysis. Transformation is therefore partly used.

Results

During 26 months about 50 different species of algae and benthic animals are registered (Bakke & al. 1984).

During the 12 monitoring periods some organisms have shown normal annual changes, while other species have been reduced or have increased in density. In the Oslofjord five species of fucoids are growing of which four were introduced in 1979. During the summer 1981 some specimens of the fifth fucoid Fucus spiralis were established. However, this species has never

become a common species in the four basins. The occurence of Ascophyllum nodosum has been very stable (Figure 2). Only the covering degree of LO was less than in the other three basins due to a smaller initial population.

F. vesiculosus has been stable in one of the controls - C4 (Figure 3). Reduced populations are observed in the other three basins, but a decrease was only found in LO and C2 (Table 1). However, F. vesiculosus had the highest covering degree in C2. Different initial populations have resulted in difference in covering degree, except for HO versus C4 (Table 2). When adjusting the initial populations to equal levels in all the four basins the covering degree of C4 would turn out to be greater than in the other three basins.

The graph showing covering degree of F. distichus ssp. edentatus in all four basins is presented in Figure 4. Except for C4 the covering degree has been reduced. From February 1984 the biomass in C2 has increased. Hence, the two oil exposed basins are the only basins to experience continual decrease (Table 1). The covering degree of C4 was greater than in the other three basins.

F.serratus (Figure 5) shows clear annual variations. HO had the least covering degree of the basins.

Population increase has been found for the fucoid germlings in all the four basins (Figure 6 and Table 1), but the growth was greater in the two controls than in HO (Table 2). During 1984 the increase of LO was highest. Monitoring was carried out during 6 periods in 1983/84.

As with F. serratus, Laminaria digitata showed annual variations (Figure 7). No difference was found between the four basins with respect to covering degree.

Chondrus crispus, Cladophora rupestris, Phymatolithon lenormandii and Ulva lactuca (Figures 8, 9, 10 and 11) have increased in all the four basins. The only exceptions are C. crispus in C2 and U. lactuca in C4 (Table 1).

All the four species showed greater covering degree in LO (Table 2). P. lenormandii showed less covering in HO than in the other three basins, and in C2 less corresponding data was stated for C. crispus.

Enteromorpha sp. showed annual variations. Especially C4 experienced great changes (Figure 12). In HO an increase was found despite the annual variations (Table 1), but a lower covering degree was calculated compared to LO and C2.

The development of the gastropod populations of Littorina littorea is shown in Figure 13. The huge population has been reduced in C4. However, the number of snails was higher than in the other basins, while C2 had the least number of the basins (Table 2).

The populations of Mytilus edulis (Figure 14) in HO and LO were reduced and disappeared during 1984. In C4 an increase was estimated (Table 1).

The development of covering degree for Balanus balanoides is presented in Figure 15. An increase was calculated for LO, C2 and C4 (Table 1), while a less covering degree was found in C2 compared with the other basins (Table 2).

Halichondria panicea (Figure 16) has grown in all the basins in a positive way (Table 1). The highest covering degree was found in C4 (Table 2).

The populations of the carnivores Asterias rubens (Figure 17) and Carcinus maenas (Figure 18) were reduced. An increase during summer 1984 in the population of C. maenas, however, has altered that reduction in HO and C2 (Table 1). Small differences are found between the basins with respect to numbers. A slight increase during summer 1984 was also found in the population of A. rubens, but only in the two controls.

Based on three summer seasons the clustering of 16 of the most common species (with no transformation) resulted in 2 main groups: not oil exposed communities and oil exposed communities (Figure 19). Furthermore C4 was clustered separately from the other basins mainly due to the high L. littorea populations.

The communities were separated into three ecological groups: 1) canopy species, 2) under storey species (incl. primary cover species), 3) mobile animals, of which all is log-transformed. Figure 20 shows high similarity between the canopy species in all the basins through three summer periods. The under storey species have developed in a more different way. The highest difference was found between a cluster group of HO and LO 1984 and all the other basins through the three summer periods (Figure 21). The corresponding values of mobile animals did create a dendrogramme (Figure 22) close to that.

DISCUSSION

The total covering degree of all the fucoid species is relatively equal in LO, C2 and C4, and somewhat reduced in HO. Mytilus edulis has disappeared from both diesel oil exposed basins, while the populations in the controls seem to be healthy. The open space in HO and LO is thus expanded and has given room for other benthic organisms to settle. In HO there has been a weaker development of Balanus balanoides than in the other three basins. Likewise the covering degree of fucoid germlings is significantly lower than in the controls. LO, however, has experienced a raise of both populations, which may lead to a conclusion that the oil concentration in HO can inhibit settlement and growth of B. balanoides and fucoid germlings. The controls show a similarity with respect to adult fucoids and M. edulis, but settlement of B. balanoides is very different. A significantly higher covering degree was found in C4, as well as more fucoid germlings. A huge Littorina littorea population is present in C4, and this may graze the filamentous algae on the steps. Thus the snails can create open areas for B. balanoides and fucoid germlings. In C2 the snail population is lower than the other basin populations (Table 2), thus little biomass of the carpet forming algae is grazed, which can inhibit new settlements.

To see if there is any relationship between the population size of L. littorea and the covering degree of the assumed snail preferred benthic algae, the green algae Enteromorpha spp. and Ulva lactuca and fucoid

germlings are assessed. A slight increase of the three species was found in HO, while U. lactuca and the germlings are growing more intensly in LO. No particular increase of the algae was stated in C2, even if the snail population was significantly lower than in the three other basins. The huge snail population in C4 did not seem to influence the covering degree of the fucoid germlings (Figure 6). The green algae, however, are grazed. Ulva lactuca, Figure 11, is registered with low covering degree, and the high peaks of Enteromorpha sp., Figure 12, during springtime are reduced to the levels of the other basins during summer time. The extreme peaks may be caused by high ammonia excretion of the large population of snails. Individual ammonia excretion rates were about equal in all the basins, T. Bakke (this report). On basis of the much higher snail population in C4 (Figure 13) one would assume the total ammonia input in C4 to be about four times that of the other basins.

Other under storey algae such as Chondrus crispus, Cladophora rupestris and Phymatolithon lenormandii together with U. lactuca were found to increase their covering degree. The growth of all the species was significantly higher in LO than calculated for the other three basins. The low oil concentration (mean 25 µg/l) of the sea water in LO may favour the growth of some algae. The lowest growth was estimated for HO and C2. The four algae did not seem to be much influenced by the huge Littorina littorea population in C4. The space available for establishment of sessile organisms and the level of diesel oil exposure have most likely been the important factors controlling the development for the communities of the basins.

The cluster analysis (Figure 19 - Figure 22) did indicate an evolution from 4 basins with minor differences to basins grouped as controls (C2, C4) and as oil-exposed basins (HO, LO).

The grouping of canopy species (Figure 20) reflects the stability of the perennial brown algae in the basins, thus the stated differences between the basins are due to the under storey species and the mobile animals, of which the populations in the controls experienced small changes from 1983 to 1984 compared to the oil exposed basins (Figures 21 and 22) mostly due to the fall of Mytilus edulis and Asterias rubens respectively.

References

Bakke, T. et al. 1984:
Long term effects of oil on marine benthic communities in enclosures. Littoral Rock Community Project. Progress Report No 4. Sublittoral Soft Bottom Project. Progress Report No 1. NIVA-report 0-82007. 189 pp.

Clifford, H.T. & Stephenson, W., 1975:
An Introduction to Numerical Classification Academic Press. 299 pp.

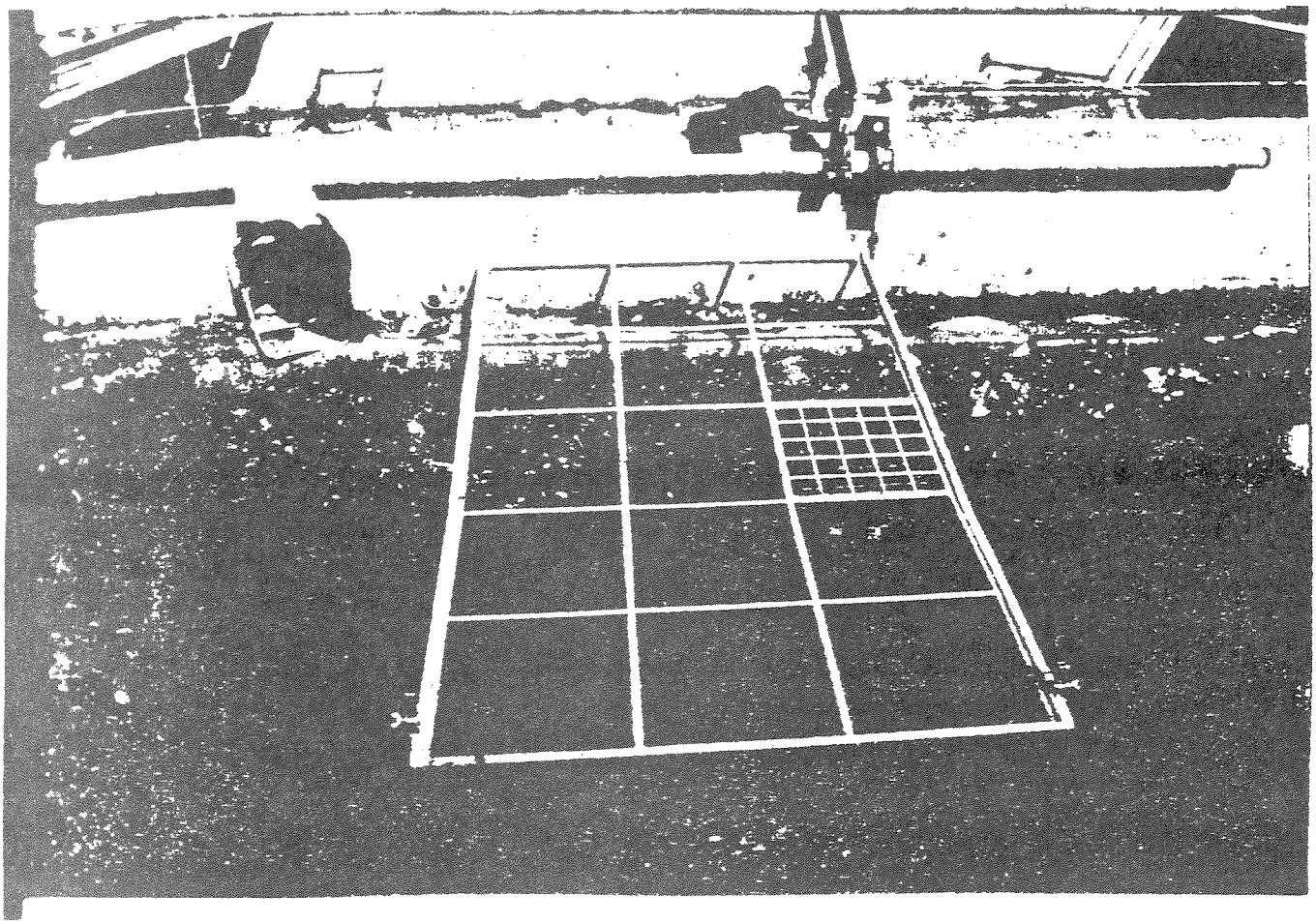


Figure 1. A special made frame to estimate covering degree and numbers of organisms, with subsquares to make it more easy to estimate covering degree

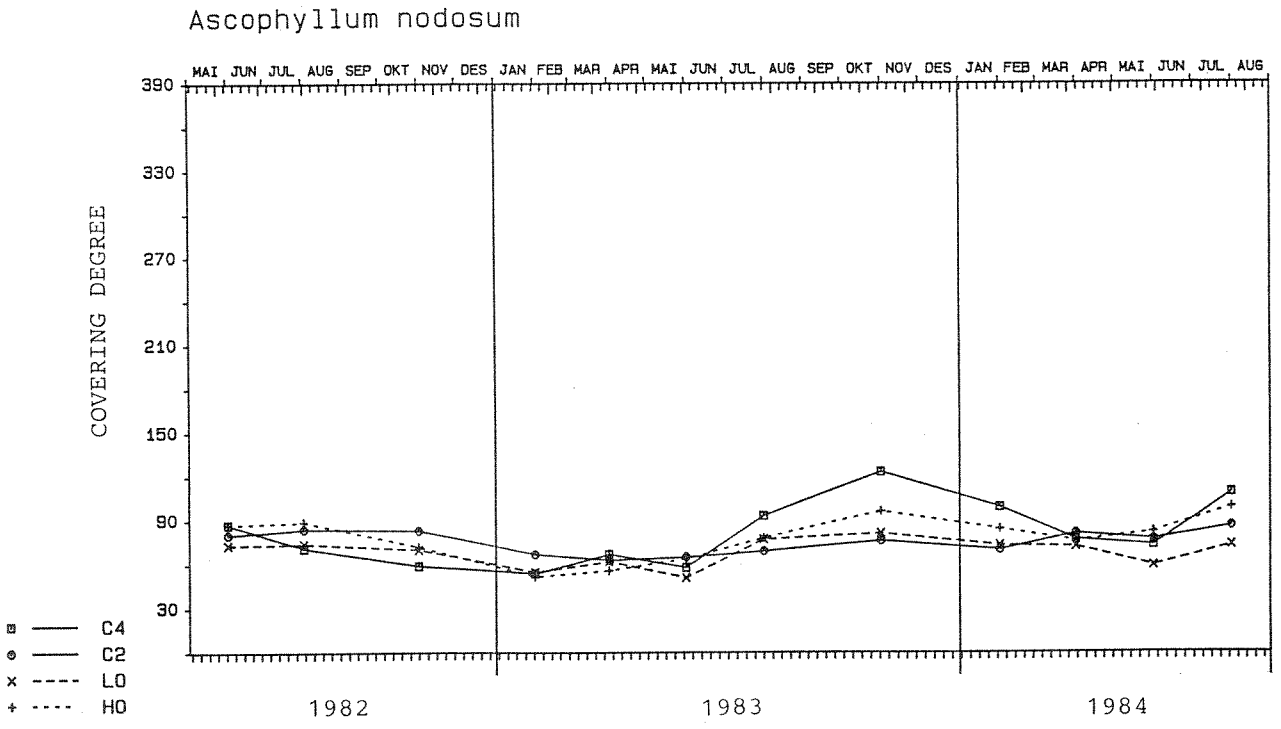


Figure 2. Covering degree of *Ascophyllum nodosum* in the four basins HO, LO (diesel oil exposed), C2 and C4 (controls) during 1982, 1983 and 1984

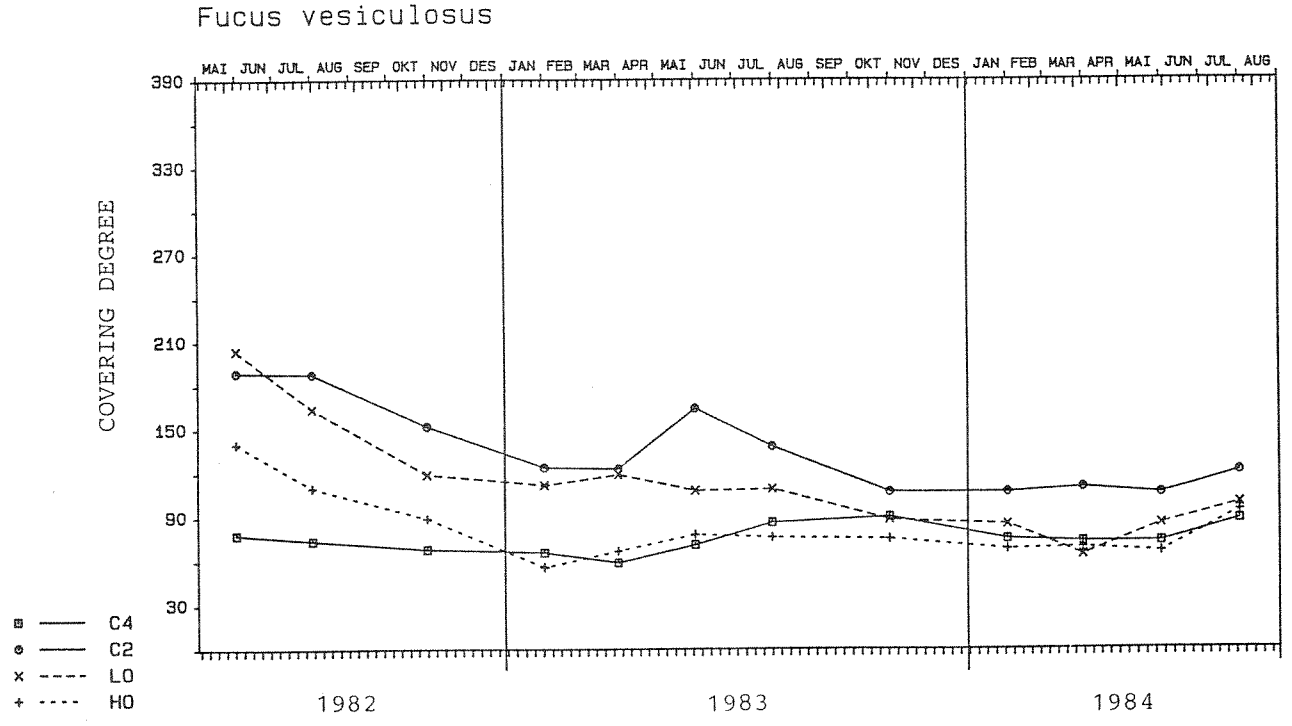


Figure 3. Covering degree of *Fucus vesiculosus* in the four basins HO, LO (diesel oil exposed), C2 and C4 (controls) during 1982, 1983 and 1984

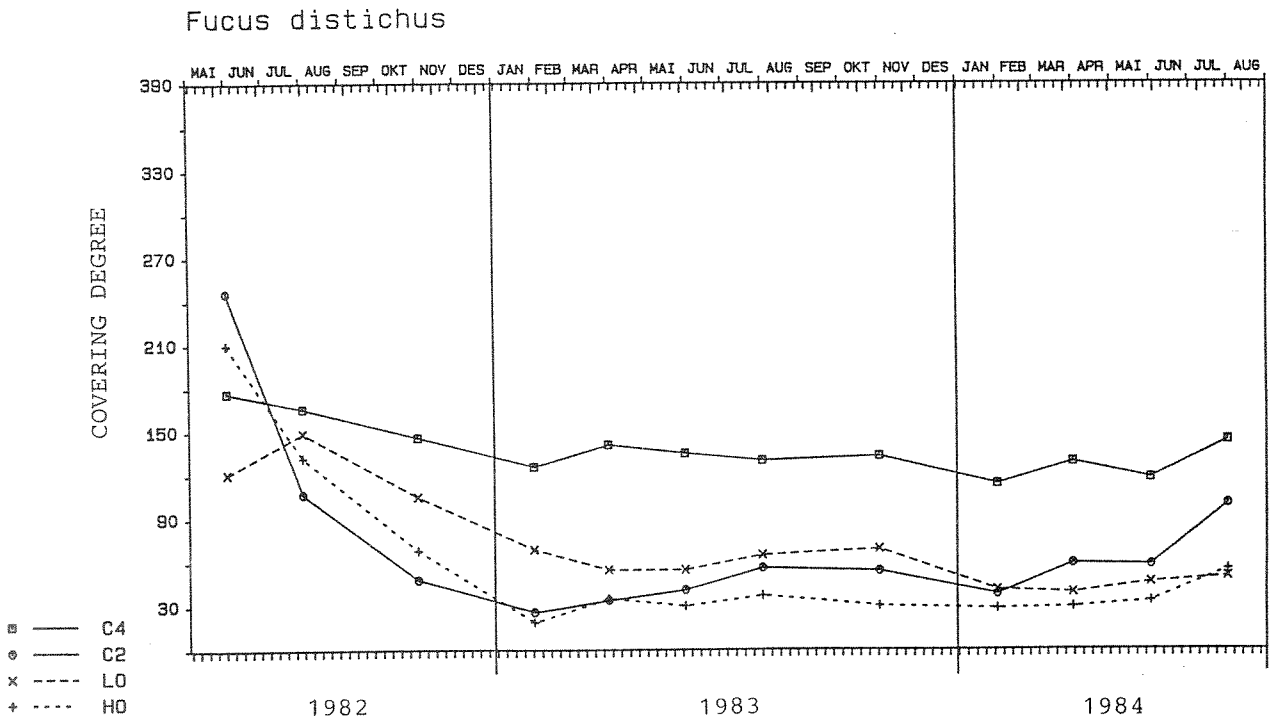


Figure 4. Covering degree of *Fucus distichus* in the four basins HO, LO (diesel oil exposed), C2 and C4 (controls) during 1982, 1983 and 1984

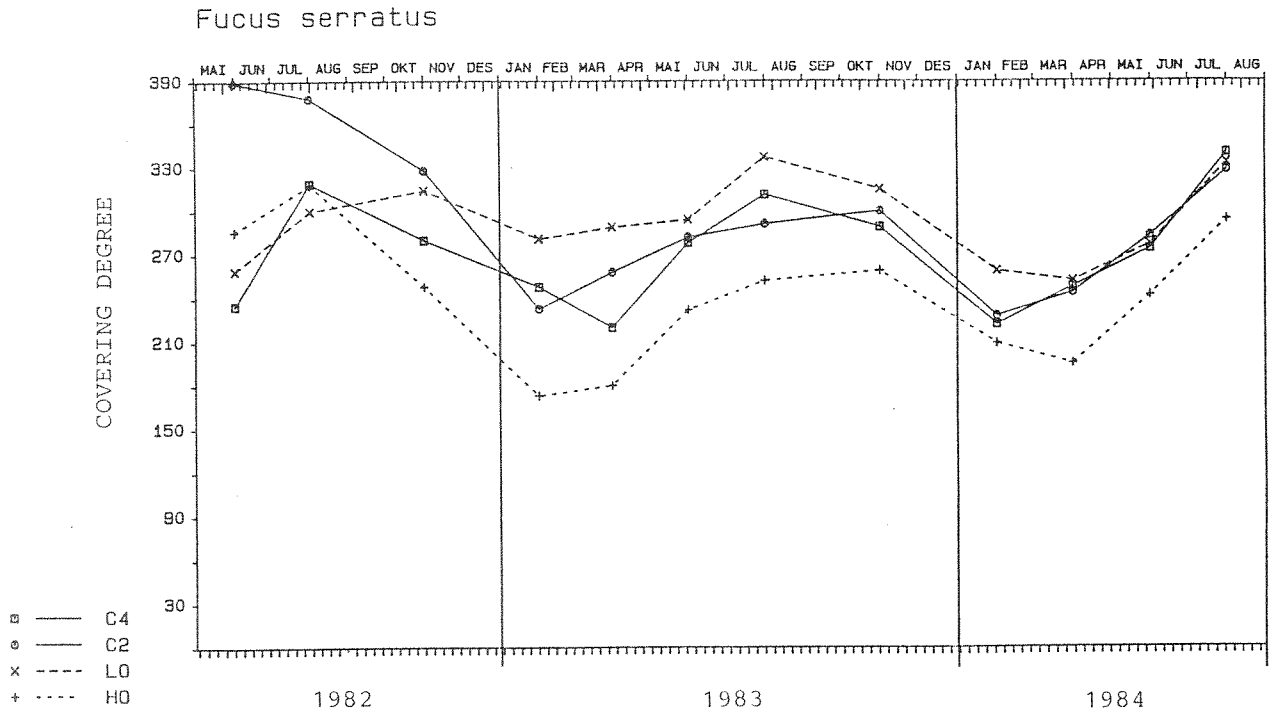


Figure 5. Covering degree of *Fucus serratus* in the four basins HO, LO (diesel oil exposed), C2 and C4 (controls) during 1982, 1983 and 1984

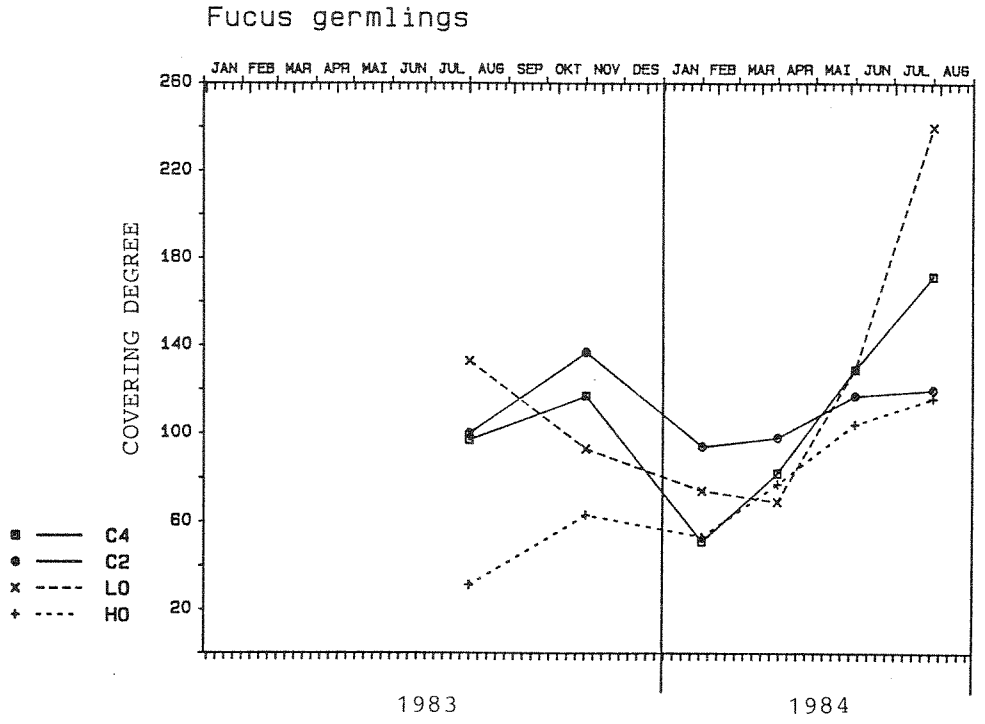


Figure 6. Covering degree of *Fucus germlings* in the four basins HO, LO (diesel oil exposed), C2 and C4 (controls) during 1982, 1983 and 1984

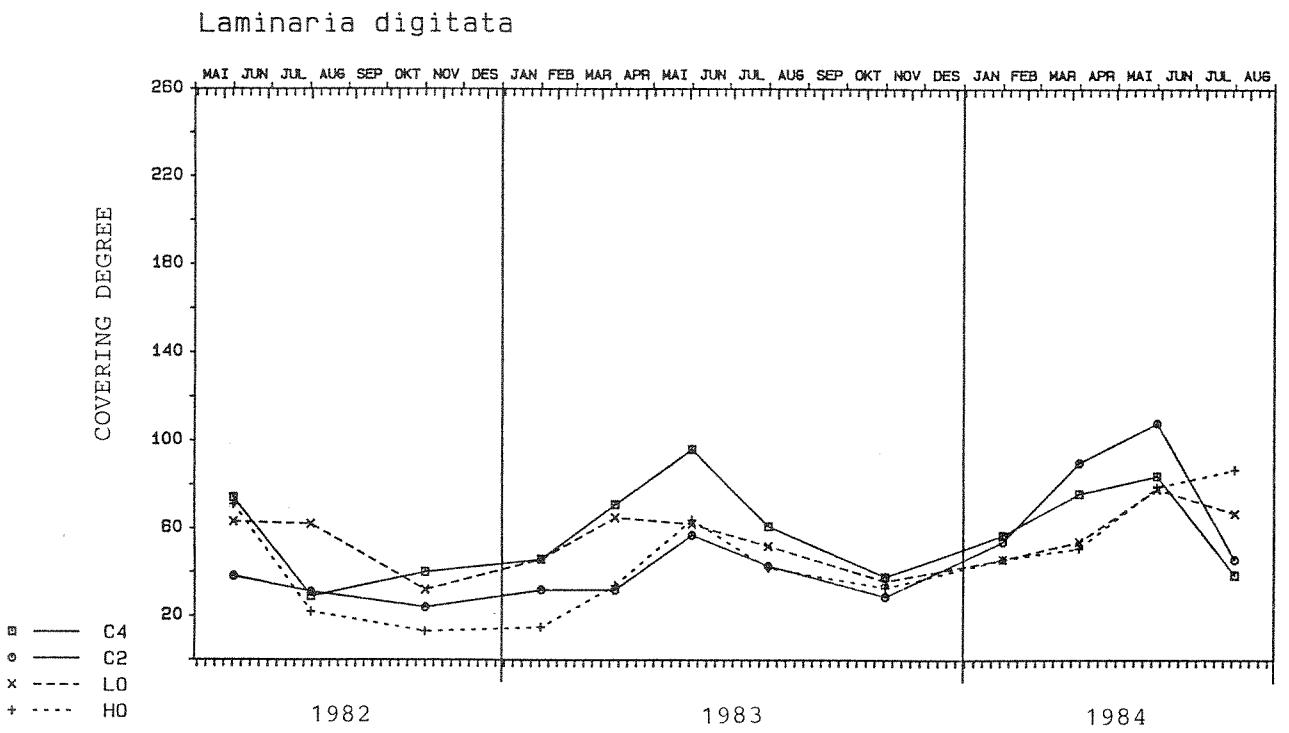


Figure 7. Covering degree of *Laminaria digitata* in four basins HO, LO (diesel oil exposed), C2 and C4 (controls) during 1982, 1983 and 1984

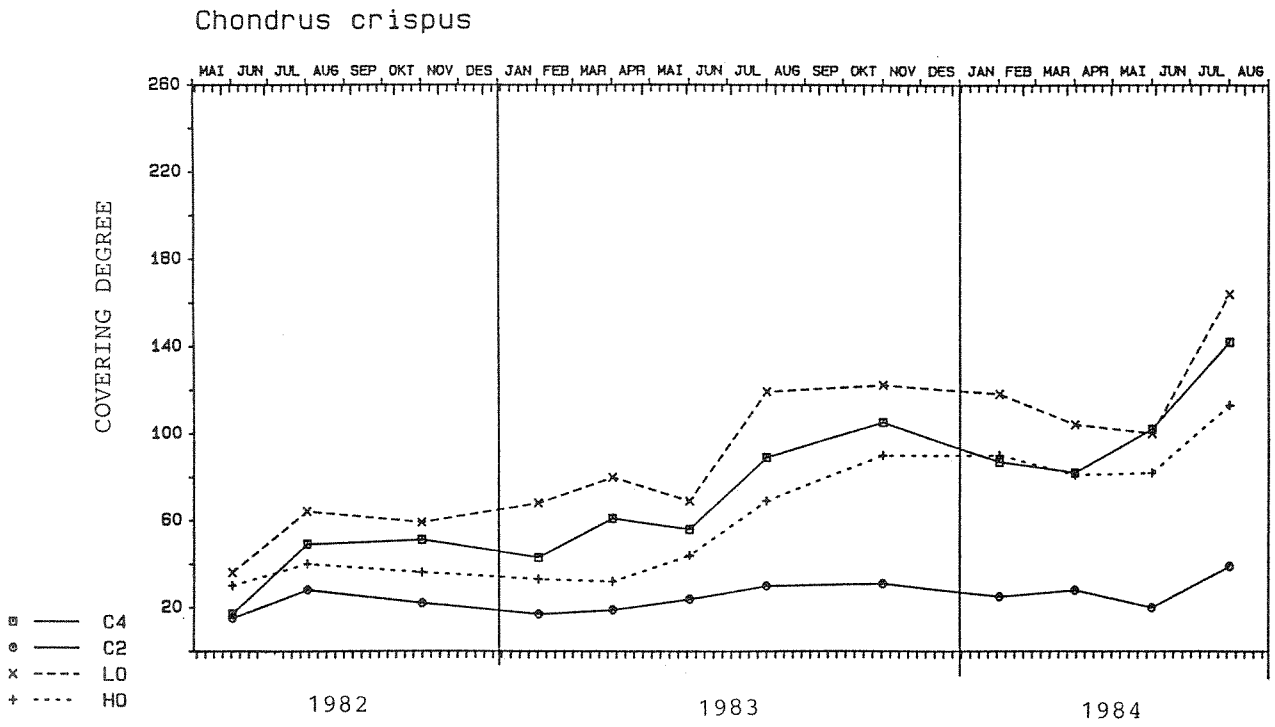


Figure 8. Covering degree of *Chondrus crispus* in the four basins HO, LO (diesel oil exposed), C2 and C4 (controls) during 1982, 1983 and 1984

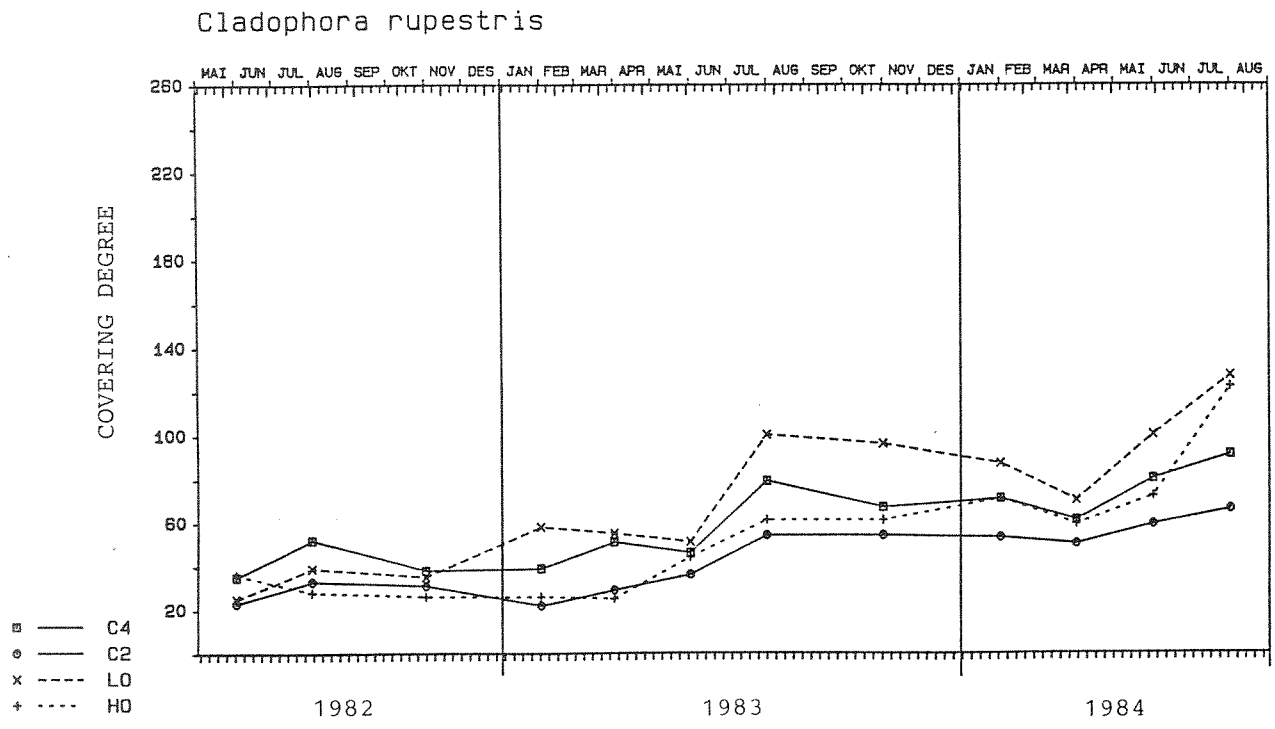


Figure 9. Covering degree of *Cladophora rupestris* in the four basins HO, LO (diesel oil exposed), C2 and C4 (controls) during 1982, 1983 and 1984

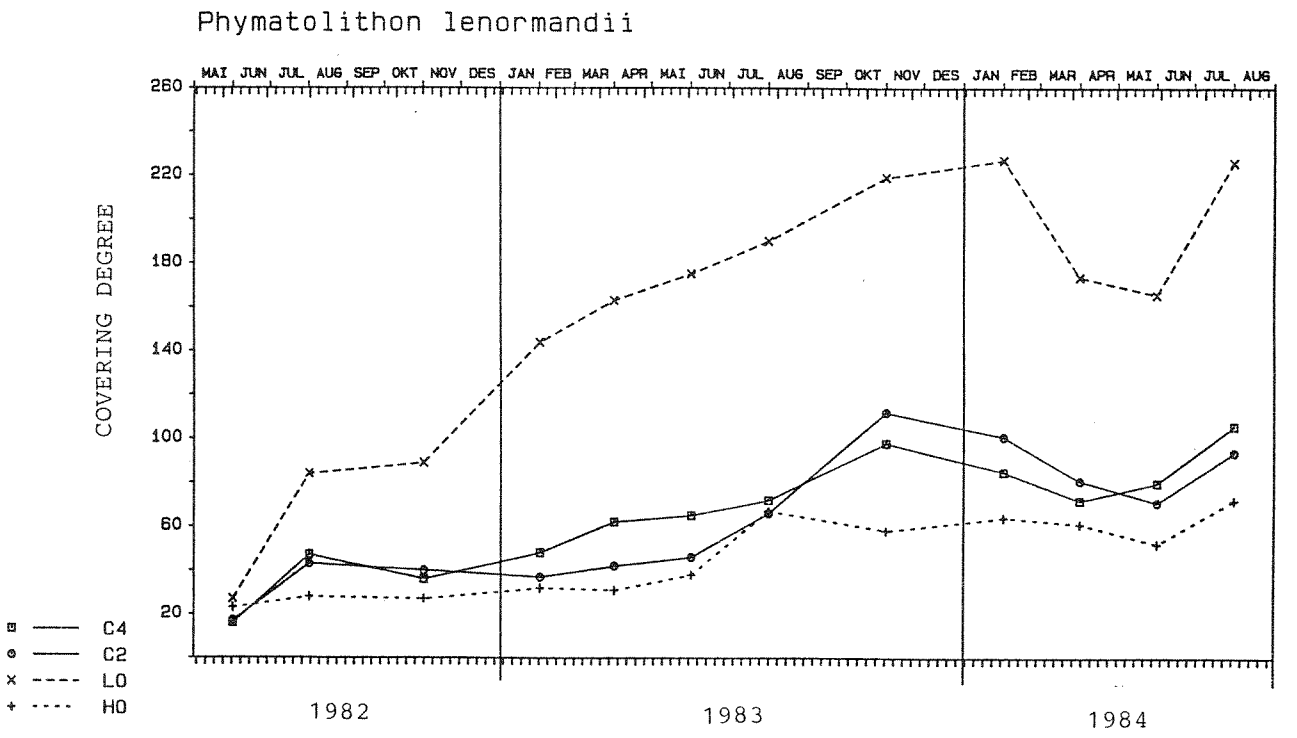


Figure 10. Covering degree of *Phymatolithon lenormandii* in the four basins HO, LO (diesel oil exposed), C2 and C4 (controls) during 1982, 1983 and 1984

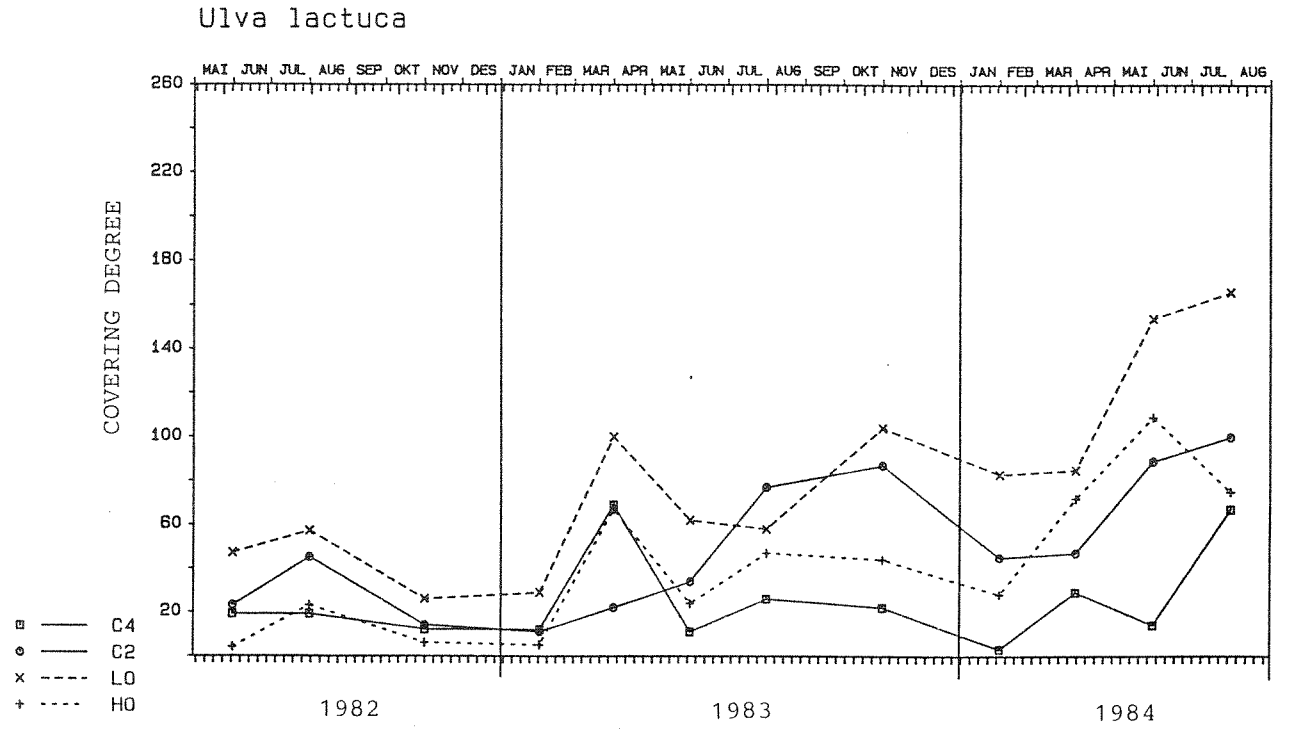


Figure 11. Covering degree of *Ulva lactuca* in the four basins HO, LO (diesel oil exposed), C2 and C4 (controls) during 1982, 1983 and 1984

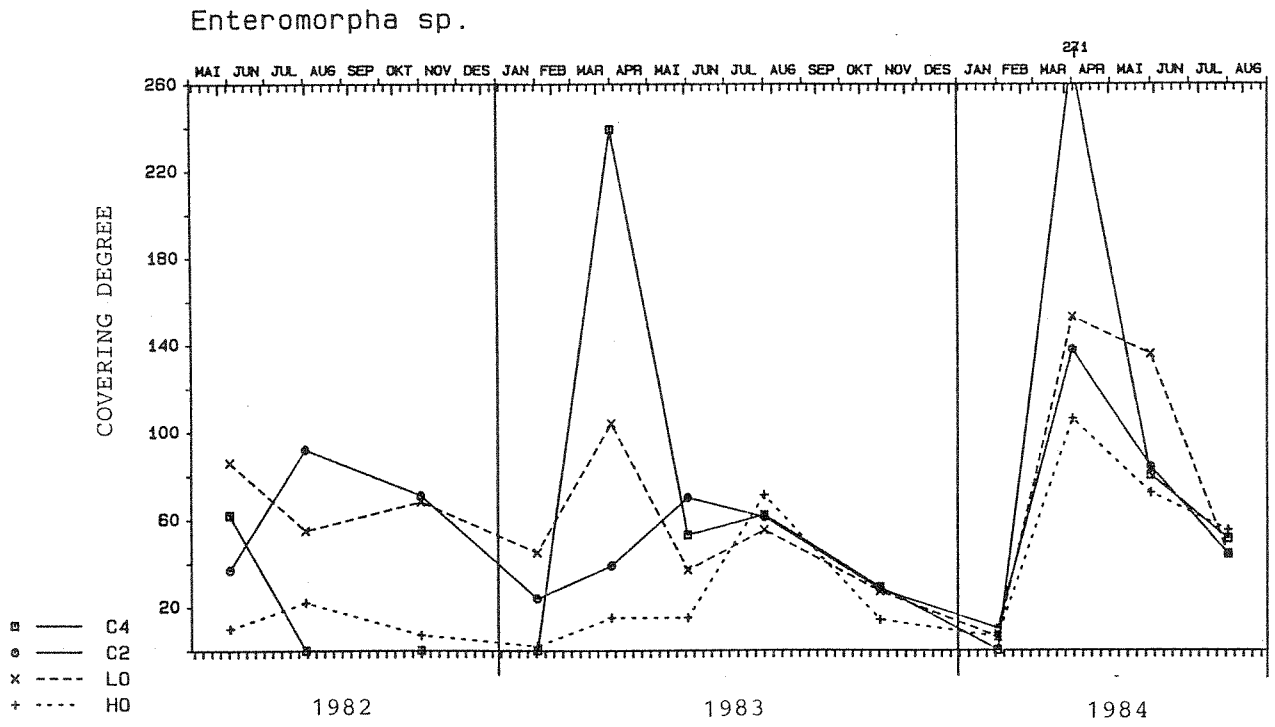


Figure 12. Covering degree of *Enteromorpha* sp. in the four basins HO, LO (diesel oil exposed), C2 and C4 (controls) during 1982, 1983 and 1984

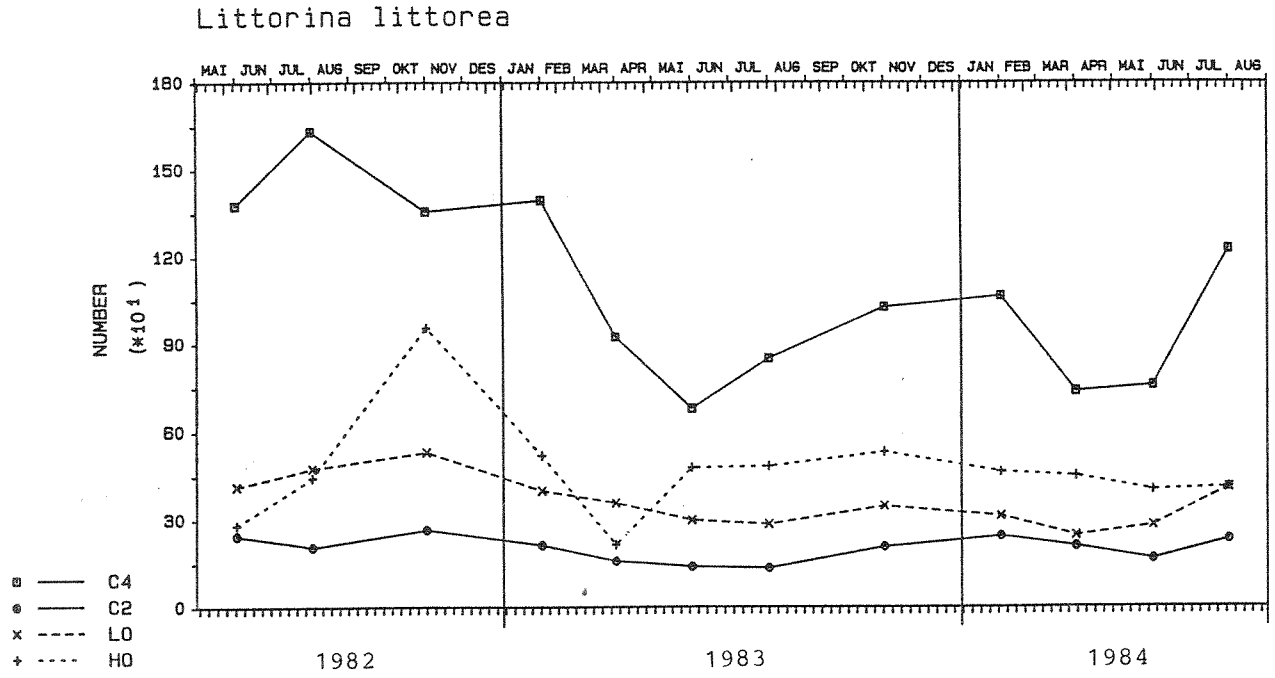


Figure 13. Number of *Littorina littorea* in the four basins HO, LO (diesel oil exposed), C2 and C4 (controls) during 1982, 1983 and 1984

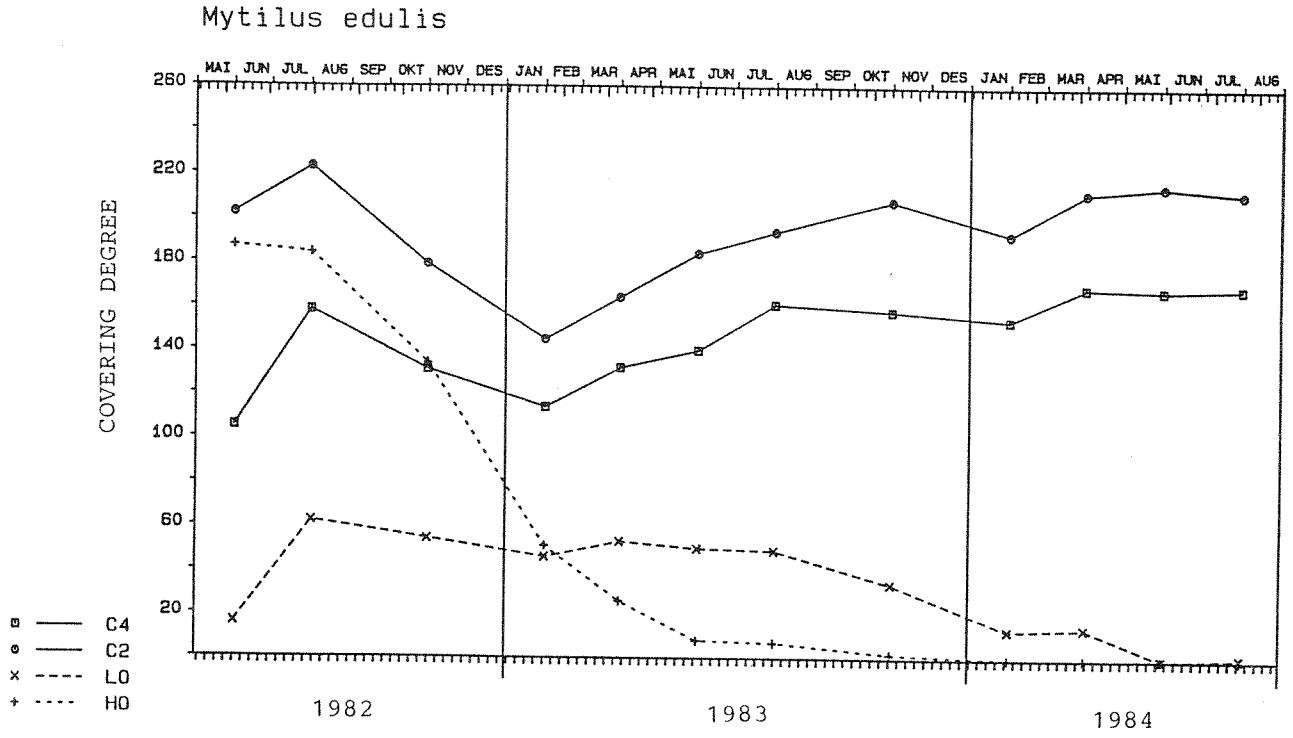


Figure 14. Covering degree of *Mytilus edulis* in the four basins HO, LO (diesel oil exposed), C2 and C4 (controls) during 1982, 1983 and 1984

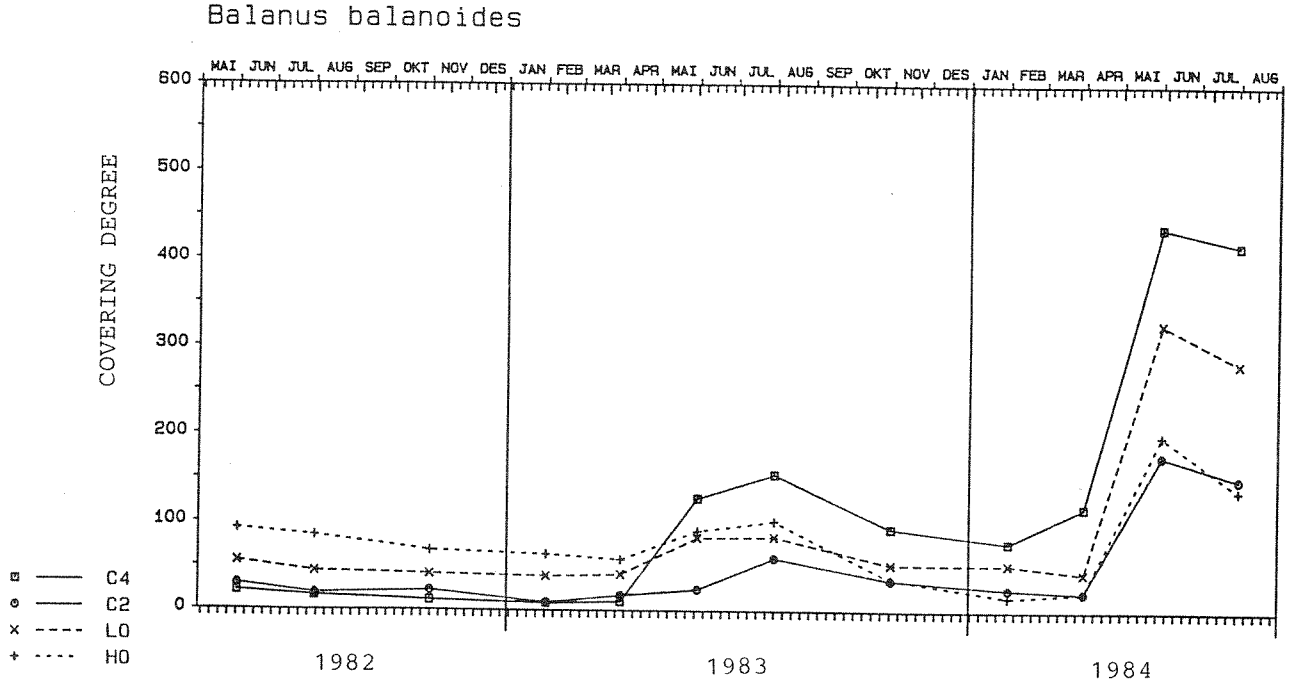


Figure 15. Covering degree of *Balanus balanoides* in the four basins HO, LO (diesel oil exposed), C2 and C4 (controls) during 1982, 1983 and 1984

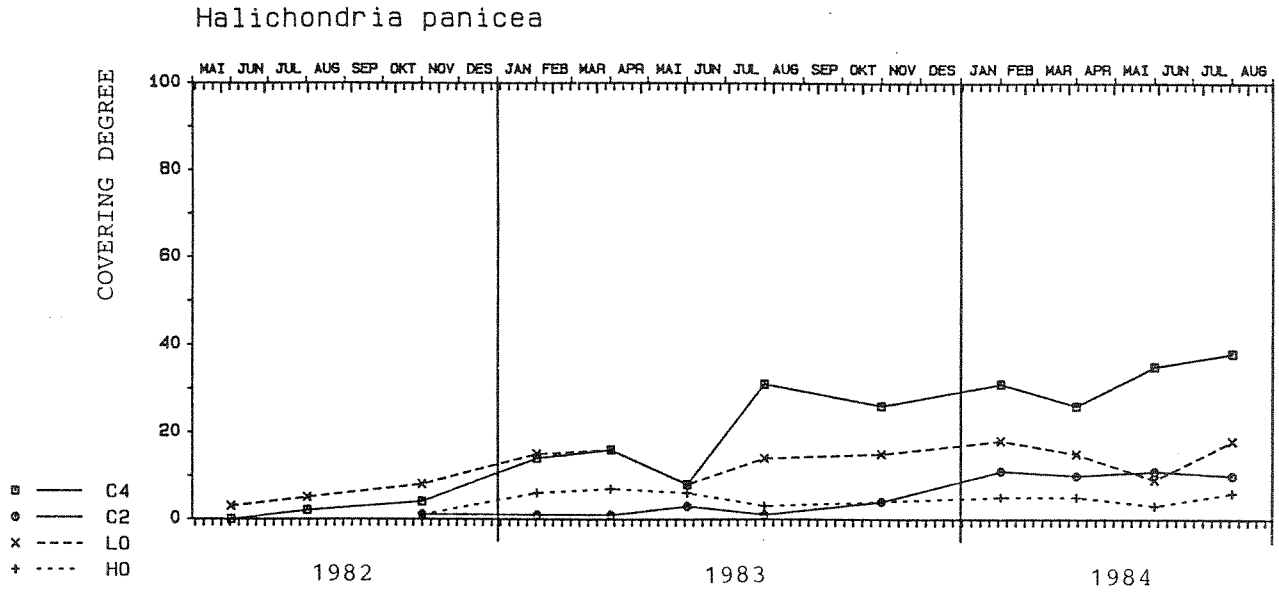


Figure 16. Covering degree of *Halichondria panicea* in the four basins HO, LO (diesel oil exposed), C2 and C4 (controls) during 1982, 1983 and 1984

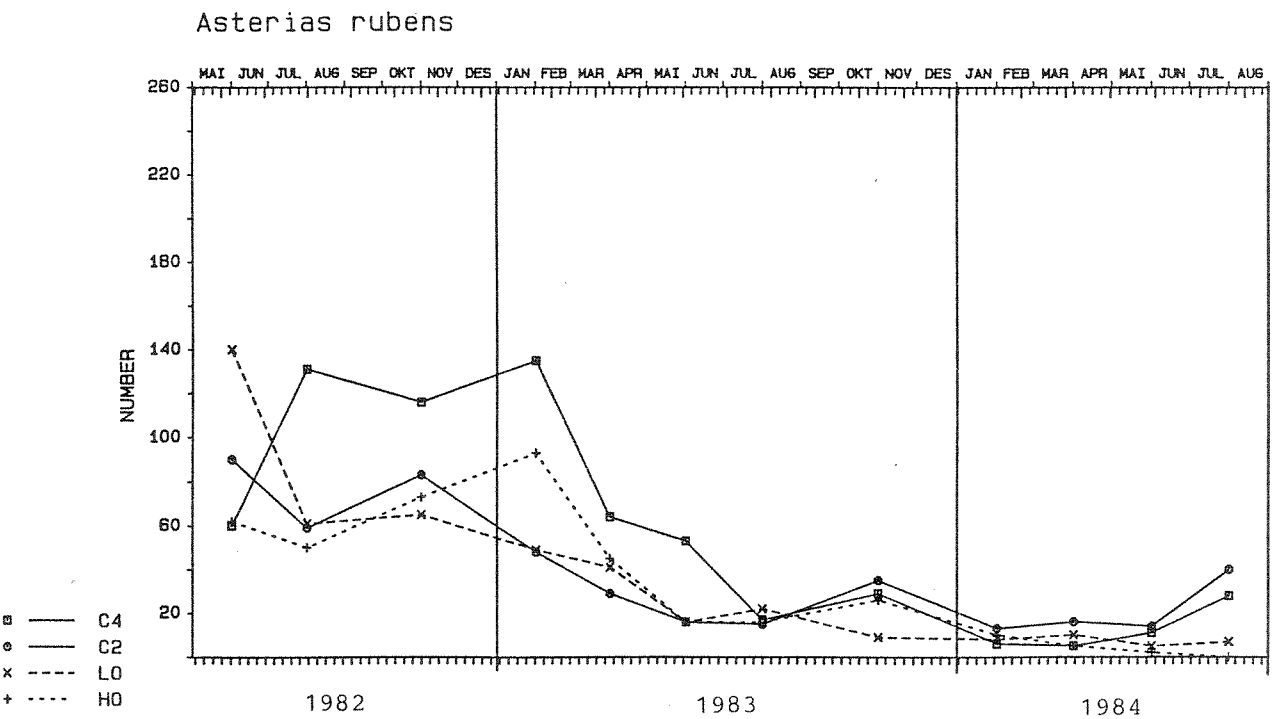


Figure 17. Number of *Asterias rubens* in the four basins HO, LO (diesel oil exposed), C2 and C4 (controls) during 1982, 1983 and 1984

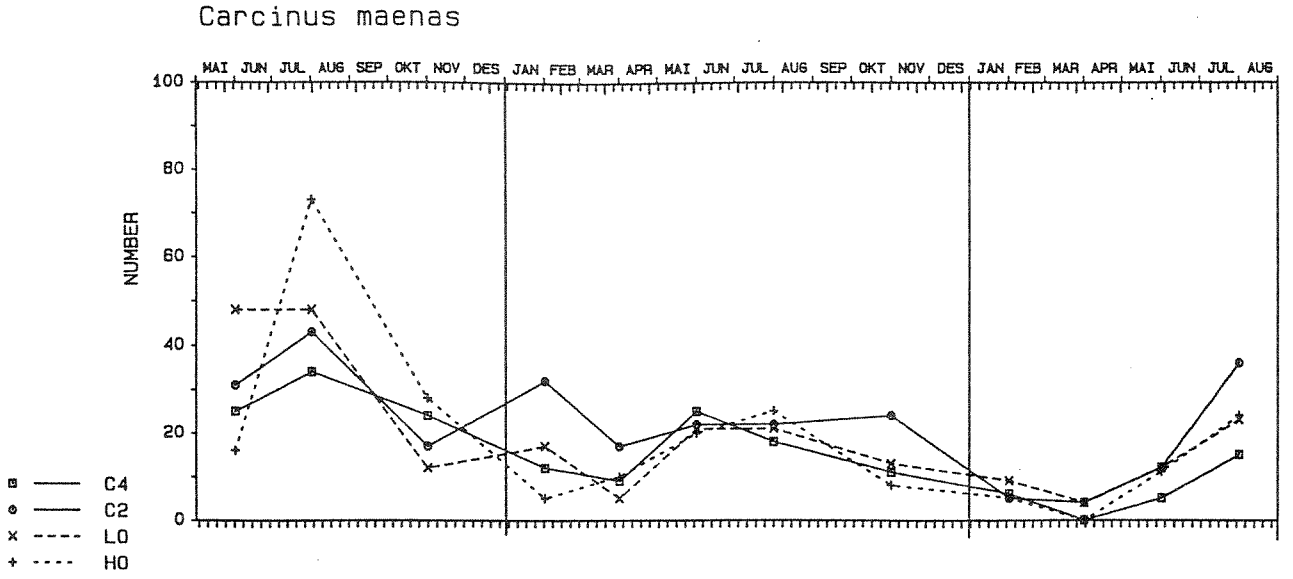


Figure 18. Number of *Carcinus maenas* in the four basins HO, LO (diesel oil exposed), C2 and C4 (controls) during 1982, 1983 and 1984

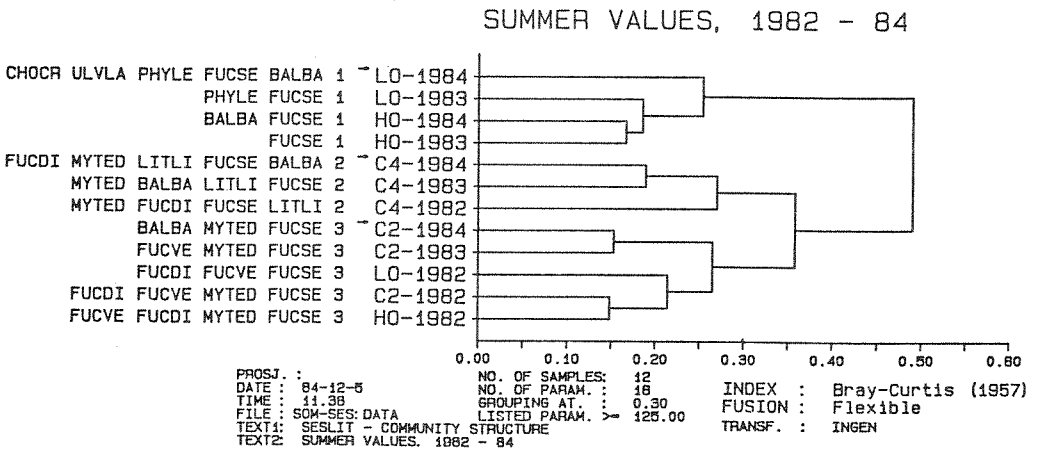


Figure 19. Dendrogramme of 16 of the most common species

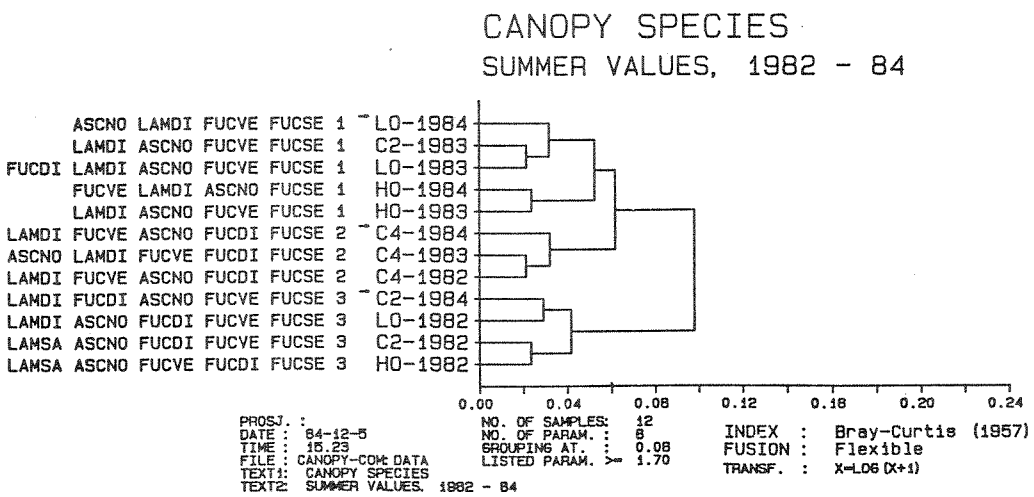


Figure 20. Dendrogramme of the canopy species

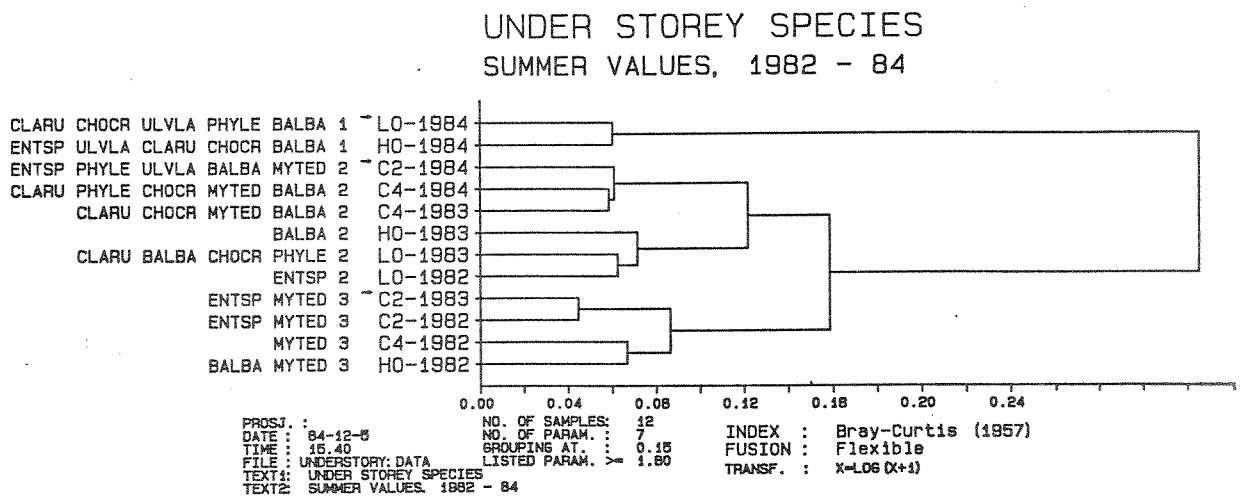


Figure 21. Dendrogramme of the under storey species (incl. primary cover species)

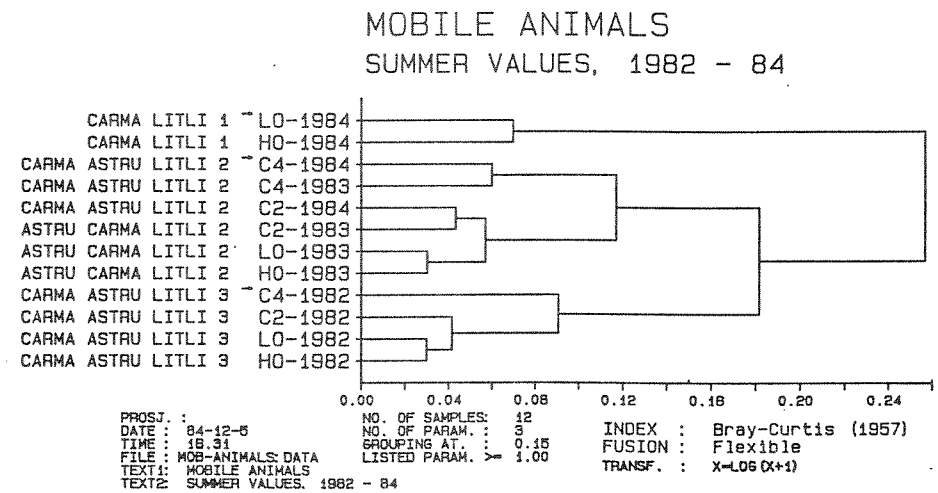


Figure 22. Dendrogramme of the mobile animals

Table 1. The significance of the regression coefficients of the organisms covering degree during 26 months

Species			
Fucus vesiculosus	H0	0.05	/
	L0	0.001	/
	C2	0.01	/
	C4	n.s.	
Ascophyllum nodosum	H0	n.s.	
	L0	n.n.	
	C2	n.s.	
	C4	n.s.	
Fucus distichus ssp. *edentatus*	H0	0.05	/
	L0	0.01	/
	C2	n.s.	
	C4	n.s.	
Fucus serratus	H0	n.s.	
	L0	n.s.	
	C2	0.05	/
	C4	n.s.	
Fucus germlings	H0		/
	L0		/
	C2		/
	C4		/
Laminaria digitata	H0	0.05	/
	L0	n.s.	
	C2	0.05	/
	C4	n.s.	
Chondrus crispus	H0	0.001	/
	L0	0.001	/
	C2	0.05	/
	C4	0.001	/
Cladophora rupestris	H0	0.001	/
	L0	0.001	/
	C2	0.001	/
	C4	0.001	/
Phymatolithon lenormandii	H0	0.001	/
	L0	0.001	/
	C2	0.001	/
	C4	0.001	/
Enteromorpha sp.	H0	0.05	/
	L0	n.s.	
	C2	n.s.	
	C4	n.s.	
Ulva lactuca	H0	0.01	/
	L0	0.01	/
	C2	0.01	/
	C4	n.s.	

Table 1. (cont.)

Species			
Littorina littorea	HO	n.s.	
	LO	0.05	/
	C2	n.s.	
	C4	0.05	/
Mytilus edulis	HO	0.001	/
	LO	0.05	/
	C2	n.s.	
	C4	0.05	-/-
Balanus balanoides	HO	n.s.	
	LO	0.05	/
	C2	0.05	/
	C4	0.01	/
Halichondria panicea	HO	0.05	/
	LO	0.01	/
	C2	0.001	/
	C4	0.001	/
Asterias rubens	HO	0.001	/
	LO	0.001	/
	C2	0.01	/
	C4	0.01	/
Carcinus maenas	HO	n.s	
	LO	0.05	/
	C2	n.s.	
	C4	0.01	/

HO: High oil exposed basin (~ 100 µg/l)

LO: Low oil exposed basin (~ 25 µg/l)

C2: Control basin

C4: Control basin

n.s.: Not significant

/ : Positive regression coefficient

\ : Negative regression coefficient

-/- : Significance rejected due to high variance

Table 2. Difference of the covering degree of the organisms in the four basins (paired t-test) with calculated significance

	Lowest covering degree	HO	LO	C2	C4
	Highest covering degree				
Fucus vesiculosus	HO				
	LO	0.001			0.01
	C2	0.01	0.01		0.001
	C4				
Ascophyllum nodusum	HO		0.01		
	LO				
	C2		0.05		
	C4		0.05		
Fucus distichus spp. *edentatus*	HO				
	LO				
	C2				
	C4	0.001	0.001	0.001	
Fucus serratus	HO				
	LO	0.01			0.05
	C2	0.001			
	C4	0.01			
Fucus germlings	HO				
	LO				
	C2				
	C4				
Laminaria digitata	HO				
	LO				
	C2				
	C4				
Chondrus crispus	HO			0.001	
	LO	0.001		0.001	0.001
	C2				
	C4	0.01		0.001	
Cladophora rupestris	HO				
	LO	0.01		0.001	0.05
	C2				
	C4			0.001	
Phymatolithon lenormandii	HO				
	LO				
	C2	0.05			
	C4	0.05			
Dumontia incrassata	HO				
	LO	0.05			
	C2				
	C4	0.01	0.05	0.01	

Table 2. (cont.)

		HO	LO	C2	C4
Enteromorpha sp.	HO				
	LO	0.01			
	C2	0.01			
	C4				
Ulva lactuca	HO				
	LO	0.001		0.01	0.001
	C2				0.05
	C4				
Littorina littorea	HO		0.05	0.001	
	LO			0.001	
	C2				
	C4	0.001	0.001	0.001	
Mytilus edulis	HO				
	LO				
	C2	0.001	0.001		0.001
	C4	0.01	0.001		
Balanus balanoides	HO			0.01	
	LO			0.01	
	C2				
	C4			0.05	
Halichondria panicea	HO				
	LO	0.001		0.001	
	C2				
	C4	0.01	0.05	0.001	
Asterias rubens	HO				
	LO				
	C2				
	C4	0.05			
Carcinus maenas	HO				
	LO				
	C2				0.05
	C4				

HO: High oil exposed basin (~ 100 µg/l)

LO: Low oil exposed basin (~ 25 µg/l)

C2: Control basin

C4: Control basin

MARINE RESEARCH STATION SOLBERGSTRAND

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5. EFFECTS OF DIESEL OIL ON COMMERCIAL BENTHIC ALGAE IN NORWAY [1]

T. Bokn (NIVA)

ABSTRACT

Length growth of the kelp *Laminaria digitata* and the fucoid *Ascophyllum nodosum* is studied to see if low continuous dosage of diesel oil has any effects on the growth during a two-year period of exposure. This is a project incorporated in a larger experiment of long-term effects from low concentration of oil on a simulated littoral rock community kept in four 50 m^3 concrete basins. During three years implanted associations of four fucoids with their associated flora and fauna have established luxuriant and relatively stable communities. The basins are equipped with artificially made waves and tide. The duration of dosage will be from September 1982 to autumn 1984. The diesel oil is mixed to the inlet seawater as a water accomodated fraction. The exposure level to the organisms averages about 100 µg/l total hydrocarbons in the highest diesel oil exposed basin and about 25 µg/l in the lowest. The other two basins act as controls. The work on *A. nodosum* started in June 1982. No significant growth difference was observed the first year. However, during 1983 and 1984, the new tips of the year in both oil exposed basins were significantly shorter compared to the tips of the control. The growth of *L. digitata* from March to July 1983 was found to be of no significant difference. However, during spring 1984, both oil exposed basins have become significantly different from the controls. Studies of recovery will start at the end of the diesel oil exposure and will continue until 1986.

1 Contribution No. 2 from the Marine Research Station, Solbergstrand.
To be presented at the "1985 Oil Spill Conference" in Los Angeles, February 25-28, 1985.

INTRODUCTION

The present situation within aquatic pollution biology is to a large extent characterized by two different approaches: descriptive work on specific recipients, and short-term experiments in the laboratory with one or a few species. As a compromise between field studies and laboratory experiments another type of experiment with simplified ecosystems has recently come into use. This type of studies is to a great extent concerned with planktonic systems (von Bodungen, *et al.*, 1976; Gamble, *et al.*, 1977; Mentzel, 1977; Steele, 1979) and soft bottom communities (Bakke, *et al.*, 1982. Oviatt, *et al.*, 1982).

However, the intertidal zone is potentially the most vulnerable to pollution impact at the surface of the sea, such as oil spills. Besides large spills the littoral community is also exposed to chronic pollution stress, especially by oil hydrocarbons and degradation products thereof, in the vicinity of harbors and other areas of heavy boat traffic and around petrochemical refineries and other coastal industrial plants.

A marine research station at Solbergstrand in the Oslofjord on the east coast of Norway has become a tool for such pollution studies. The present experimental study is a project incorporated in a more extended experiment on long-term effects of low concentrations of diesel oil on communities kept in four 50 m^3 concrete basins, Figure 1. The experiment includes littoral communities established in October 1979 by transplanting stones from the outside shore together with their natural cover of algae and animals. The stones were positioned on steps along one side of each basin, with the same vertical distribution as on the shore. Later recruitment has occurred both internally and by dispersion stages brought in with the water. The communities experience the same seasonal and climatic fluctuations as the shore outside except damaging ice movements in winter.

About 30 species of intertidal flora and fauna have been recorded in the basins, Figure 2.

The aim of the study presented here is to see if low continuous dosage of diesel oil has any effects on the length growth of two commercial species of seaweeds.

A considerable crop of seaweeds (fucoids and kelps), about 150.000 metric tons, is at present harvested in Norway. An estimate of the standing crop of *Ascophyllum nodosum* along the Norwegian coast is about 1.8 million metric tons. A yearly yield of the kelps is estimated to 20 kilogram/meter^2 (2 kg/m^2). Along the Norwegian coast kelp will cover an area of about 8.000 km^2 (Rueness, 1980).

Other subprojects of this research program are described in Bokn and Kirkerud, 1981, Bakke, *et al.*, 1983 and Bok, in press.

MATERIAL AND METHODS

The length growth of two species of seaweeds, the fucoid *Ascophyllum nodosum* (knobbed wrack) and the kelp *Laminaria digitata* is measured in four different concrete basins during June 1982 - August 1984 and March - July 1983/84 respectively. The basins are equipped with wave generators and tidal simulation. Sea water is supplied from 1 meter (m) depth, and the present basin turnover rate is 2½ h. (Water volume 25 m^3 per basin) (Figure 3). Two of the basins are continuously exposed for diesel oil from September 1982. The mean exposure levels of hydrocarbons in the water during the first 12 months were 101 and 26 µg/l respectively for the two basins. Similarly the mean exposure levels during the second year were 112 and 24 µg/l, Bakke and Sørensen, (this volume). The basin exposed for highest concentrations of diesel oil is called HO, the corresponding basin with lowest concentrations for LO and the two basins acting as controls for C2 and C4.

During June 1982, July 1983 and June 1984 25 tips of *A. nodosum* were tagged in HO, LO, C2, C4 (23), in HO, LO, C2, and in HO (23), LO, C2, C4 respectively. Mostly, different tips of identical plants were tagged each year. The plants were tagged with numbered spaghetti tags (Topinka and Tucker, 1981). Growth was followed as their rate of elongation by measuring from the upper end of the youngest vesicle to the very top of the tip by using a slide caliper. Parallel to these measurements corresponding tips were cut and measured *in situ* and then brought to the laboratory for drying and weighing. 33 tips each time were taken from three localities in the Oslofjord in the beginning of June and August 1982. In that way it is possible to compare length growth with increase in weight.

The tagging system was changed during March before a new set of specimens was tagged in July 1983. The new tagging system consists of a soft 1 millimeter (mm) thick nylon line tied with adhesive tape in a decided succession of five different colours. This system was kept for the third year.

The start of the *Laminaria digitata* tagging was in March 1983, where 25 individuals were tagged in each of HO, LO and C2 (C4 had too few specimens). In March 1984 were 25 new individuals tagged in each of HO, LO, C2, while 22 specimens could be marked in C4. The tagging system used was identical with the second tagging of *A. nodosum*. During this period the kelps were measured at 9 different times. The measurements have been carried out according to Sundene, 1964, Figure 4. The analyses of data is done by computerized paired t-test.

RESULTS

Ascophyllum nodosum (L.) LeJolis

During the period June - September 1982 *A. nodosum* was growing significantly in all four basins. The highest growth was found from June to July (Figure 5a).

During the first 9 months the mortality was large. About 67 per cent of the specimens has been lost in different ways. Especially was the tagging system suspected. The spaghetti tag could possibly be too rigid for the branches of *A. nodosum*. The loss was most heavy in C4, uncertain why, and this basin was taken out of the measuring program for another 1½ years. Measurements during the second tagging period showed significantly growth in LO and C2, but not in HO during July - September 1983. During the second period the loss was 63 per cent. Eventually the third and last tagging period appeared similar to the second period, the plants in C2, C4 and LO were growing significantly during June - August 1984, while HO failed, Figures 5b and c. During the three tagging periods the number of individual shoots tagged and the remaining shoots

	1982/83	1983/84	June-Aug. 84
HO	25/13	25/9	23/21
LO	25/9	25/11	25/22
C2	25/7	25/7	25/25
C4	23/0	-	25/22

From June to September 16th 1982 all four basins acted as controls. The diesel oil was exposed to basins HO and LO at September 16th. The first measurements in June 1982 showed significantly higher length growth in C2 and C4 than in HO. During the remaining growth period (July, Sept., Oct., Dec., Feb., Mar., and Apr.) no significant difference in length growth was found between the four basins, Figure 5a and Table 1. During the period July 1983 - May 1984 the growth in C2 was significantly higher than in HO and LO. Eventually during the third

period from June to August 1984 significant difference was only measured in August between C2/HO and C4/HO, Figure 5c and Table 1. There was never found significant difference between the two controls.

In Figure 6 a correlation plot between length growth and weight of *Ascophyllum nodosum* is shown. The unbroken regression line is calculated from the data collected in June, the dotted line is corresponding regression line from August.

Laminaria digitata (Huds.) Lamour.

The highest growth rate of *L. digitata* was recorded in April - May both investigation periods, March - July 1983/84. Figure 7 shows significantly reduction in lenght growth during June and especially during July. In the Figure 8 daily mean length increase during the different intervals is indicated.

During the two tagging periods the number of individual plants tagged and the remaining plants was:

	1983	1984
HO	25/20	25/18
LO	25/18	25/19
C2	25/20	25/22
C4	-	22/16

The first tagging period of the kelp (1983) no significant difference between oil exposed basins and the controls was found. However, the second tagging period (1984) significant difference was found between C2 and HO during the entire period. Except for July, the same difference was stated for C4 and HO, Table 2.

LO seemed to be unstable in growth. In some periods there was no significant difference between the controls and LO, but in the following periods the significant difference could be very high, Table 2. No significant difference was found between the two controls at any time.

DISCUSSION

Ascophyllum nodosum

According to Figure 5 *A. nodosum* in HO (high oil basin) was growing significantly during the summer period 1982 as were the other three basins. MacFarlane, 1932 has also found that the most rapid growth takes place between early May and August/September in Halifax, Nova Scotia. She stated that the growth is continuous and found the annual average linear growth to be about 9 centimeter (cm). The corresponding growth in the inner Oslofjord is indicated by Rueness, 1973 to be about 7 cm. The average growth of *A. nodosum* in C2 at Solbergstrand for 1982/83 and 1983/84 was 6.8 and 7.4 cm respectively, which could indicate natural conditions for the wrack in the control basin. During the next two summer periods, however, HO did not grow significantly compared with the other basins. At September 16th 1982 the diesel oil was exposed to basins HO and LO.

Except for the first month in the first tagging period (June 1982 - April 1983) no significant difference in length growth was found between any of the basins. However, significantly higher growth was found in C2 compared with HO and LO during the whole second tagging period (July 1983 - May 1984), Figure 5 and Table 1. Surgical experiments (Moss, 1970) strongly suggest that the apical cell excerts the controlling within the apex, the growing point. The duration of desiccation and exposure to air temperature are also important factors when calculating the length increase (Strømgren, 1977). Desiccation up to one to two hours enhences length growth but desiccation exceeding this time decreases the growth rate.

Sudden temperature increases may have a very strong positive effect on growth rate for wracks. The specimens in the basins may have experienced all the possibilities described above to a great extent, when breaks in the waves and tide cycle have occurred due to different work.

A. nodosum has difficulties to re-establish when removed from an area, and when settled the germlings grow remarkably slow (Sundene, 1973). But an adult wrack can be resistant, and the entire maximal life-span of shoots from Trondheimsfjord, Norway, and Galway, Ireland, appears to be 11-13 years (Baardseth, 1970). In the basins loss of whole specimens is not observed. When the branches cease or reduce its length increase it can be due to mechanical or chemical factors. However, one should bear in mind that new apical meristems can be regenerated from wound-healing tissue (Bold and Wynne, 1978). The reason why there is a significant higher growth of *A. nodosum* in the control than in the two diesel oil exposed basins the second year but not the first, can be explained by the fact that the apical meristems of the plants may be highly developed before the oil exposure in September 1982. However, in the second year the meristems are developed in oil exposed water and thus may be inhibited to some degree. During the third tagging period no significant difference between the controls and HO was found until August. The reason for this lag period and that LO is growing in the same way as the controls is not fully understood. One reason can be the cold water temperature during July 1984 (max. 20.0, mean 17.65 ^{o}Celsius (C), St. dev. 1.37) compared with corresponding temperature data during summer 1983 (max. 24.8, mean 20.36 ^{o}C, St. dev. 2.07).

There was never found significant difference between the two controls (only one control basin during second period), underlining the possibility of length growth inhibition of *A. nodosum* by low concentration of diesel oil.

The weight of the commercial seaweed *A. nodosum* correlated to length increase is indicated in Figure 6. The two regression lines show an exponential curve. Reduction in linear growth of longer tips will thus cause a higher loss of weight than shorter tips.

Laminaria digitata

The laminarians have shown a typical growth curve for the Oslofjord populations during spring and early summer of 1983 and 1984 in all the basins (only three in 1983) according to Sundene, 1962, Figures 7 and 8. To measure the validity of the length growth in the basins compared with populations from the Oslofjord and the west coast of Norway, the mean growth per cm per day in the period of most rapid growth is calculated according to Sundene, 1964. The measurements from C2 in April 1983 and 1984 were 0.027 and 0.030 cm respectively compared with the natural populations of about 0.030 cm (Sundene, 1964), which should emphasize the natural conditions the laminarians experience in the control basins.

Only a slight significant difference between LO and HO has been stated during 1983. A considerable individual variability within each basin weakens the significance of this difference. Opposed to the first tagging period in 1983 the length increase of *L. digitata* was significantly higher in the control basins . than in the oil exposed basins in the second tagging period in 1984, Figure 7 and Table 2. The lamina (blade) of *L. digitata* is annual, but the stipe (stalk) and intercalary meristem or transition zone are perennial. No significant difference in length growth between HO, LO and C2 was stated in 1983, presumably due to too short diesel oil exposure time for the intercalary meristem (growing point). The clear significance found in 1984 between HO and the controls could be a long time affect of the oil on the laminarians' transition zone. The great reduction of length increase in June and especially in July is in accordance with that of populations from Southern Norway which is induced of temperatures exceeding 18 ^{o}C

(Sundene, 1964). The more unstable length increase of *L. digitata* in LO is more unpredictable. The very low concentration of diesel oil in the water (~ 25 µg/l) may lead to periodical inhibition of growth.

According to Sundene, 1964, "... the greatest increase in width of the lamina coincides with maximum growth in length, ...". The length increase could thus be a direct measure for weight increase of *L. digitata*. No significant difference was found between the two controls, which lends weight to the assertion that growth of *L. digitata* is inhibited by low concentration of diesel oil.

In conclusion, low concentrations (about 25-100 µg/l) of diesel oil during several years may inhibit growth of the two commercial benthic algae *Ascophyllum nodosum* (knobbed wrack) and the kelp *Laminaria digitata* from the second growth season.

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REFERENCES

1. Baardseth. E., 1970. Synopsis of biological data on knobbed wrack, *Ascophyllum nodosum* (Linnaeus) Le Jolis. - *F.A.O. Fisheries. Synopsis*, 38.
2. Bakke, T., Bokn, T. and Gray, J.S., 1983. Solbergstrand Experimental Station - a facility for ecosystem research (english summary) *NIVA in the 80s*, pp27-37.
3. Bakke, T., T. Dale and T.F. Thingstad, 1982. Structural and metabolic responses of a subtidal sediment community to water extracts of oil. *Netherlands Journal of Sea Research*, 16, pp524-537.
4. Bakke, T. and Sørensen, K., in press. Experimental long term oil exposure on rocky shore mesocosms. (This volume).
5. Bokn, T., in press: Effects of diesel oil on recolonization of benthic algae. *Hydrobiologia*, pp6.
6. Bokn, T. and Kirkerud, L., 1981. Oil Monitoring Experiments on Marine Littoral Communities Kept in Basins. *Water Science and Technology*, 13, pp625-629.
7. Bold, H.C. and Wynne, M.J., 1978. *Introduction to the algae. Structure and reproduction*. Prentice-Hall, Inc. New Jersey. 706pp.
8. Gamble, J.C., J.M. Davis and J.M. Steele, 1977. Loch Ewe bay experiment 1974. *Bulletin of marine Science*, 27, pp146-174.
9. MacFarlane, C., 1932. Observations on the annual growth of *Ascophyllum nodosum*. *Nova Scotian Institute of sciences Proceedings & Transactions*, pp27-31.
10. Mentzel, D.W., 1977. Summary of results; controlled ecosystem pollution experiment. *Bulletin of marine Science*, 27, pp142-145.

11. Moss, B., 1970. Meristems and Growth Control in *Ascophyllum nodosum* (L.) Le Jol. *New Phytologist*, 69, pp253-260.

12. Oviatt, C., J. Frithsen, J. Gearing and P. Gearing. 1982. Low chronic additions of No 2 fuel oil: chemical behaviour, biological impact and recovery in a simulated estuarine environment. *Marine Ecology Progress Series*, 9, pp121-136.

13. Rueness, J., 1973. Pollution effects on littoral algal communities in the inner Oslofjord, with special reference to *Ascophyllum nodosum*. - *Helgoländer wissenschaftliges Meeresuntersuchungen*, 24, pp446-454.

14. Rueness, J., 1980. Praktisk betydning av marine makroalger. *Naturen*, 5, pp195-210.

15. Steele, J.M. 1979. The uses of experimental ecosystems. *Philosophical Transactions of the royal Society of London*, 286, pp583-595.

16. Strømgren, T., 1977. Length growth rates of three species of intertidal Fucales during exposure to air. *Oikos*, 29. pp245-249.

17. Sundene, O., 1962. Growth in the sea of Laminaria digitata. Sporophytes from culture. *Nytt Magasin for Botanikk*, 9, pp5-24.

18. Sundene, O., 1964. The ecology of Laminaria digitata in Norway in view of transplant experiments. *Nytt Magasin for Botanikk*, 11, pp83-107.

19. Sundene, O., 1973. Growth and reproduction in Ascophyllum nodosum (Phaeophyceae). *Norwegian Journal of Botany*, 20, pp249-255.

20. Topinka, J.A. and Tucker, L.R., 1981. Long-term oil contamination of fucoid macroalgae following the *Amoco Cadiz* oil spill. *in Amoco Cadiz, Conséquences d'une pollution accidentelle par les hydrocarbures* (ed. G. Piketty) CNEXO, pp393-403. Paris. 881pp.

21. *von* Bodungen, B,. K. von Brockel, V. Smetacek and B. Zeitzschel, 1976. The plankton tower, 1.A Structure to study water/sediment interactions in enclosed water columns. *Marine Biology*, 34, pp369-372.

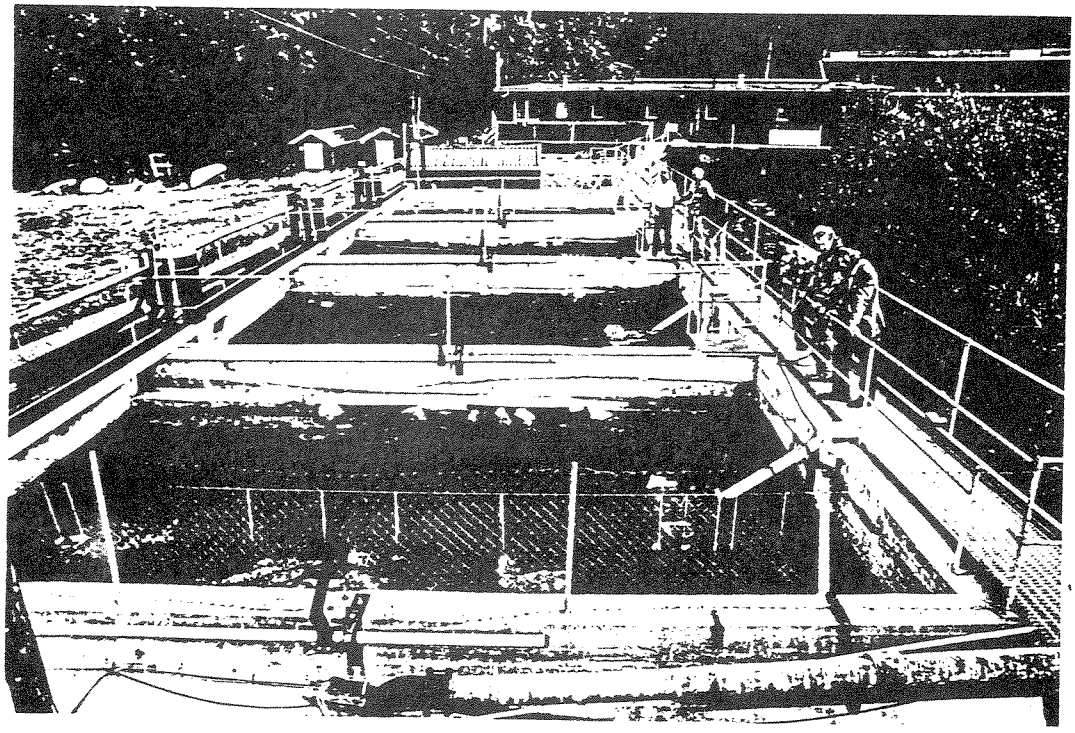


Figure 1. The laboratory building at Solbergstrand and the rocky shore basins.

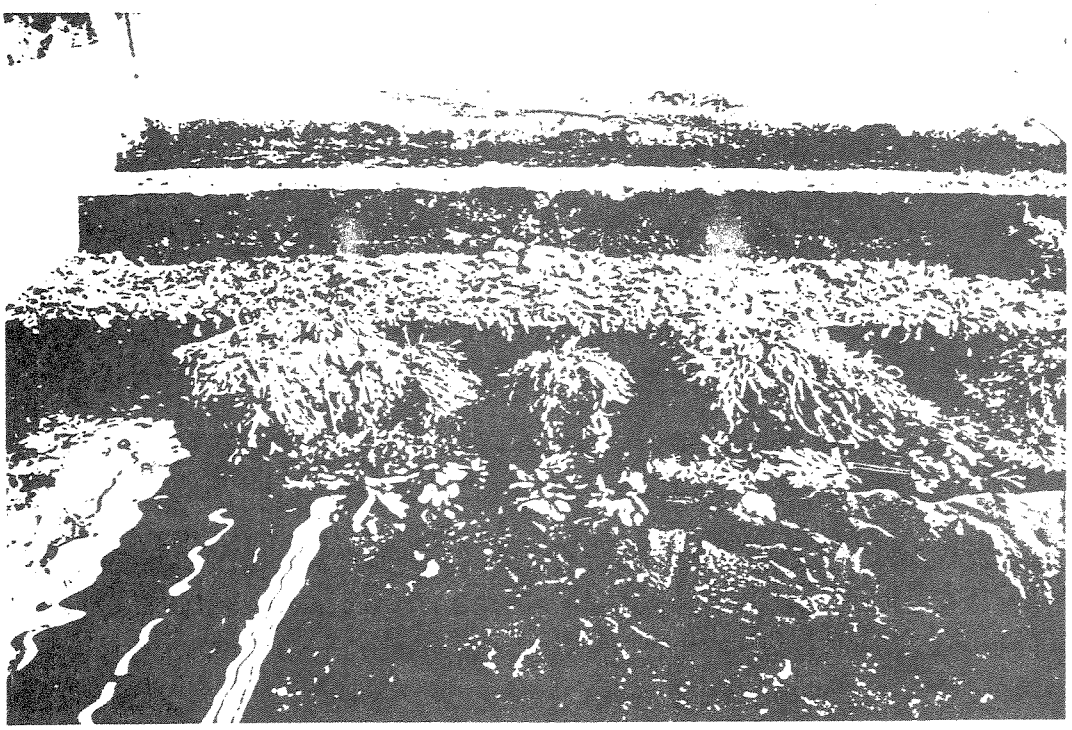


Figure 2. Established littoral communities on different steps in a rocky shore basin.

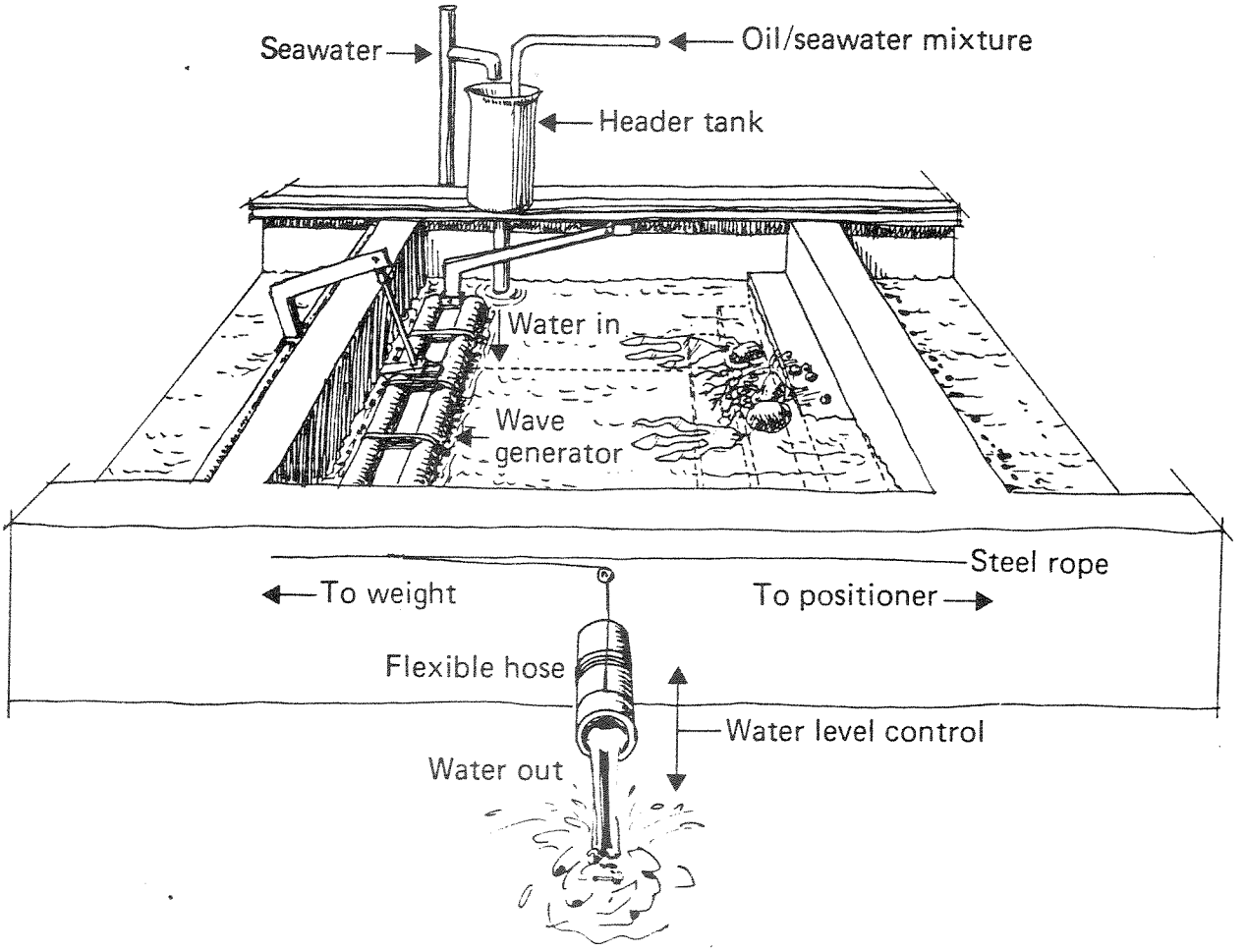


Figure 3. Sketch of a rocky shore basin.

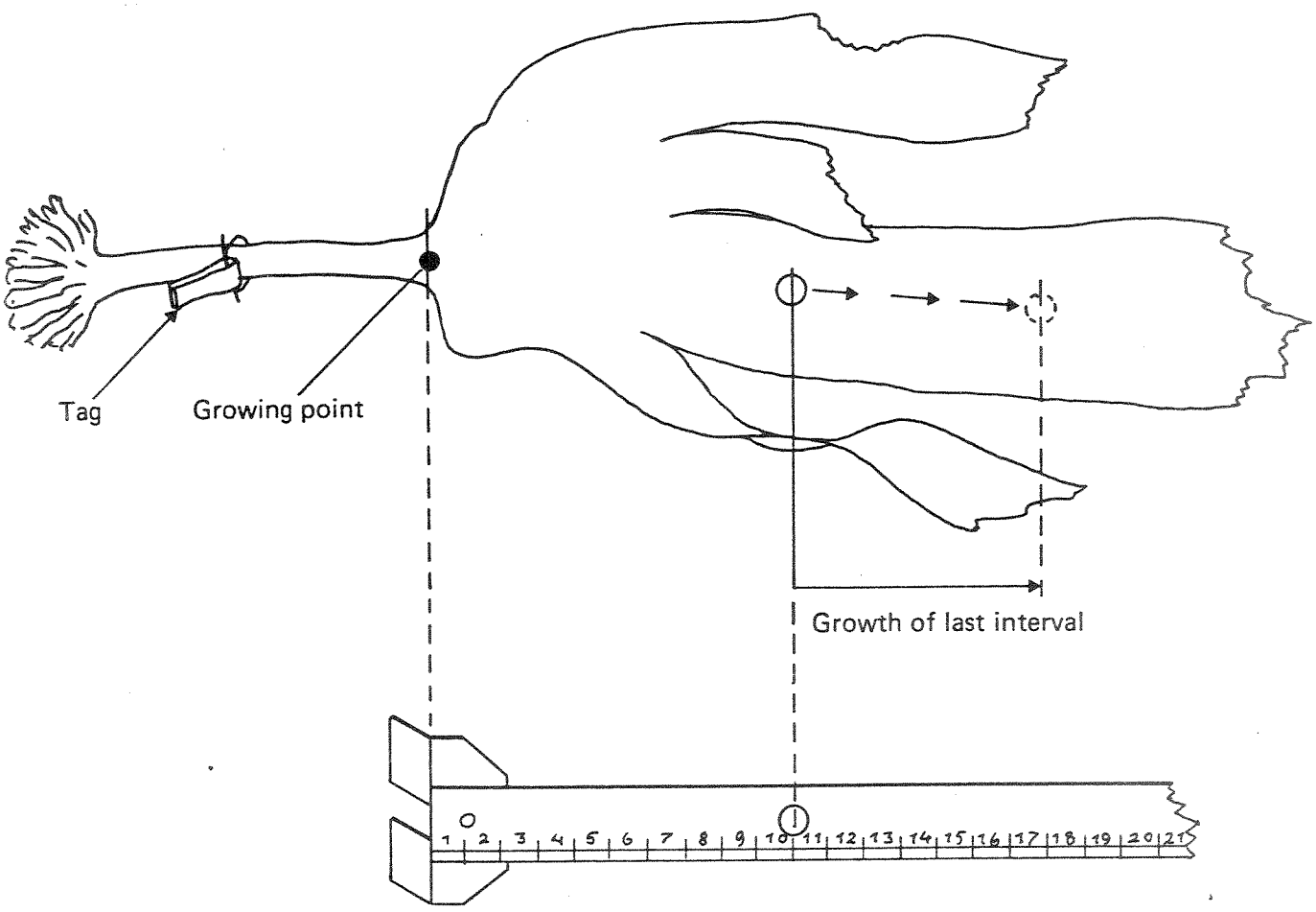


Figure 4. Method for growth measurements of the lowermost portion of the lamina (after Sundene, 1964).

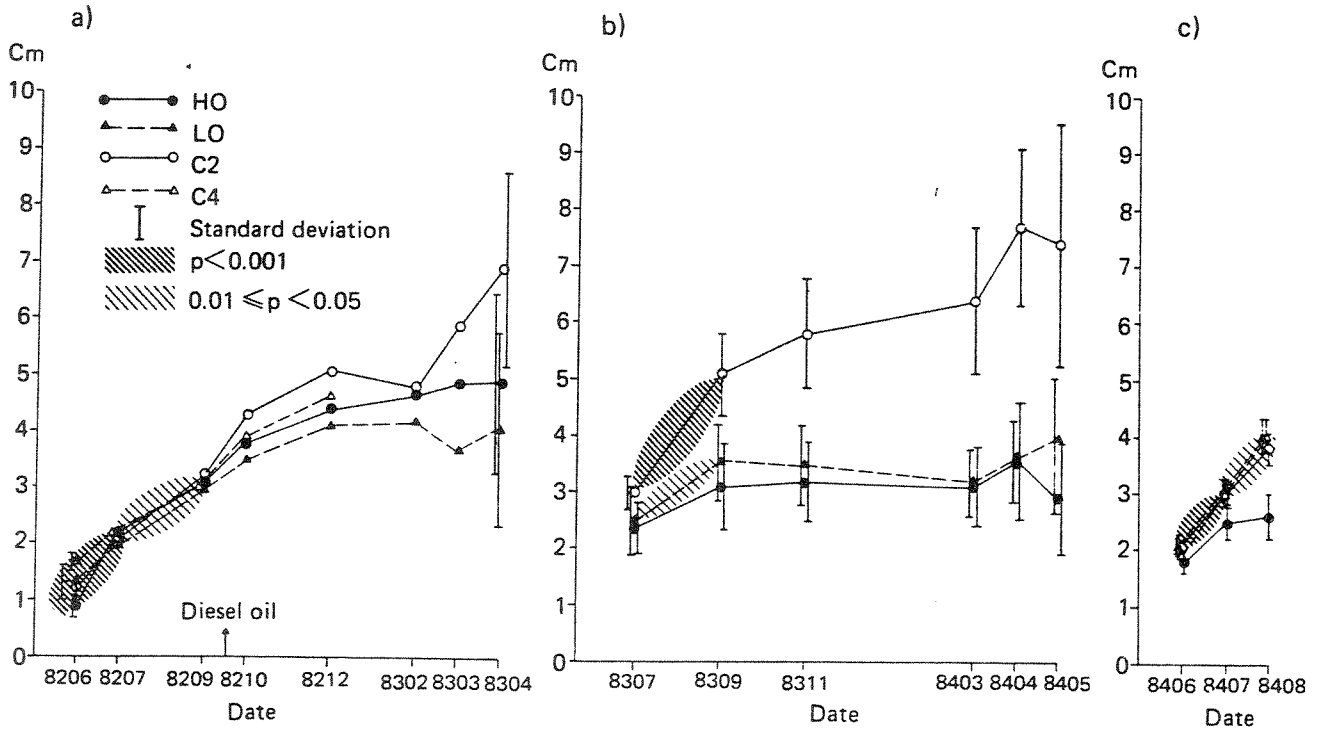


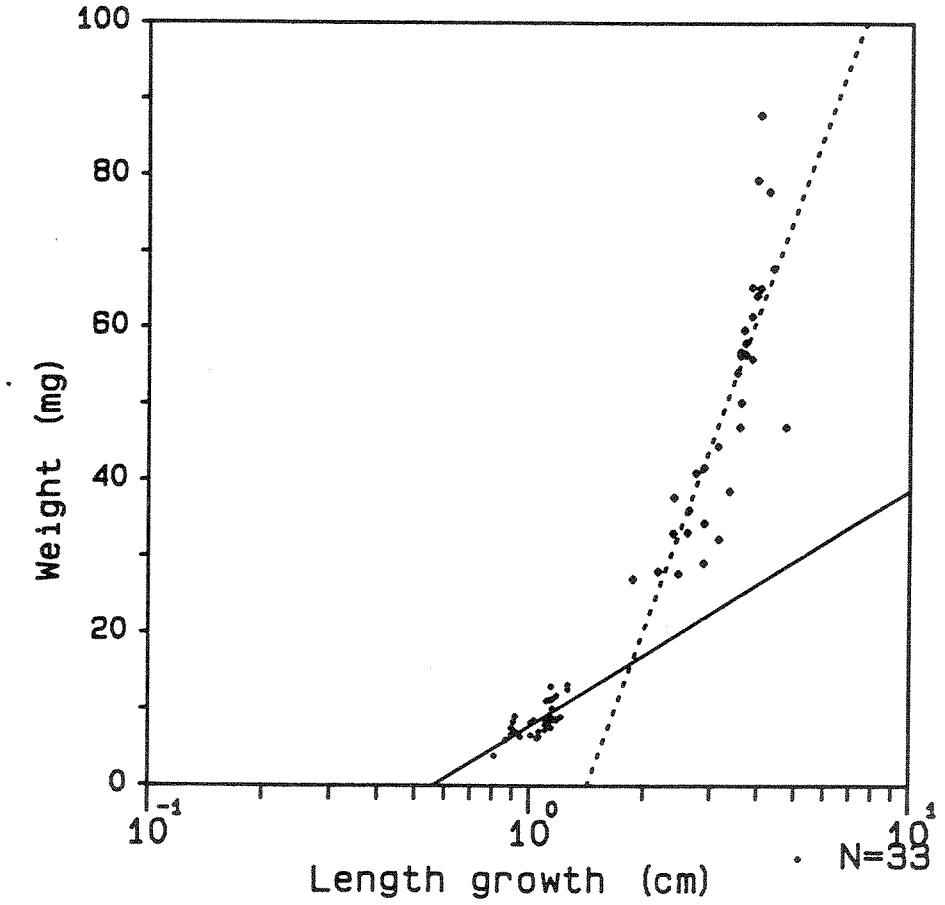
Figure 5. Length growth of *Ascophyllum nodosum* tips.

a) 1. tagging period, 8206-8304.

b) 2. " " , 8307-8405.

c) 3. " " , 8406-8408

Significant growth (p) during these intervals is indicated.



N=32

Y =13.41 LN(X) +7.80 R=0.70 P≤ 0.001 SD=9.03

Y =59.44 LN(X) +20.78 R=0.81 P≤ 0.001 SD=28.32

Figure 6. Correlation between length growth and weight of *Ascophyllum nodosum* tips from the Oslofjord summer 1982.

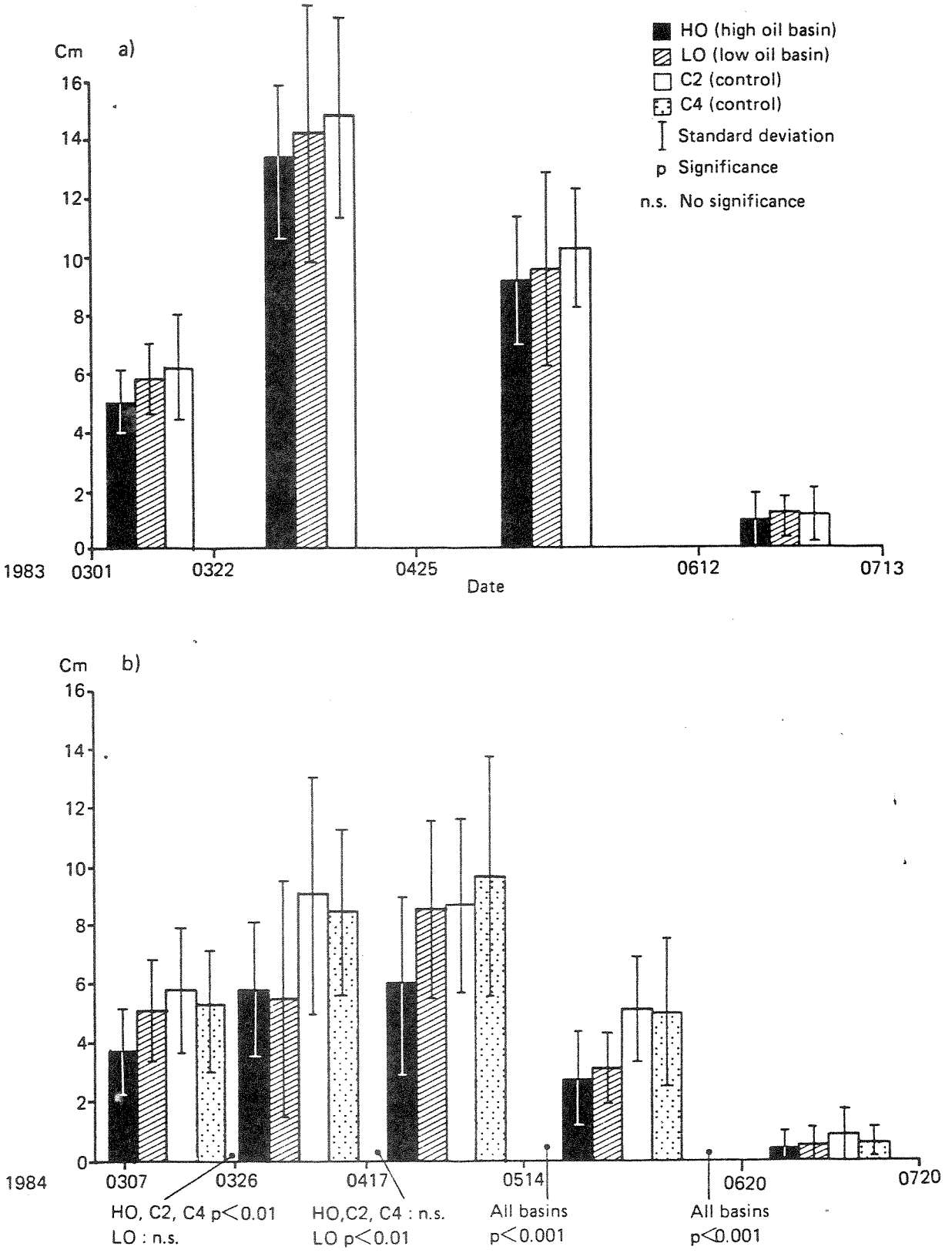


Figure 7. Length growth of *Laminaria digitata*

a) 1983. Significant growth in all basins during the three intervals, $p < 0.001$ (0425-0612, LO: $p < 0.01$)

b) 1984. Significance indicated on the figure.

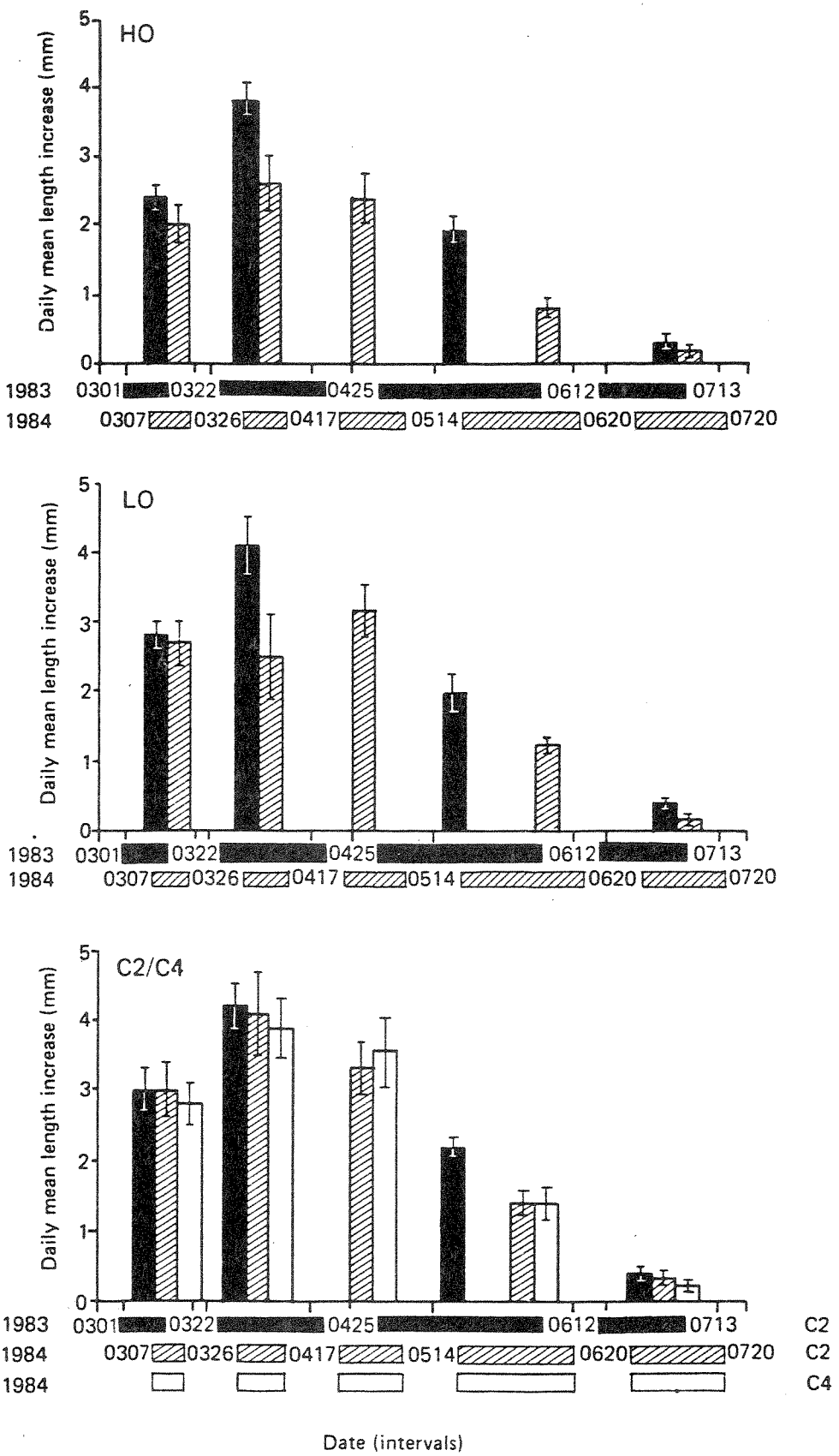


Figure 8. Daily mean length increase of *Laminaria digitata* during the different intervals of 1983 and 1984.

HO (high oil basin), LO (low oil basin), C2 and C4 (controls).

Table 1. Test of difference (significance:p) between basins in mean tip length of *Ascophyllum nodosum* at various dates. HO (high oil), LO (low oil), C2 and C4 (controls) with three tagging periods.

	1. tagging period		2. tagging period						3. tagging period		
Date	8206	8207-8304	8307	8309	8311	8403	8404	8405	8406	8407	8408
C2/HO	p<0.01	none	p<0.01	p<0.01	p<0.01	p<0.05	p<0.05	p<0.05	none	none	p<0.01
C2/LO	none	none	p<0.05	p<0.01	p<0.01	p<0.05	p<0.05	none	none	none	none
C2/C4	none	none	-	-	-	-	-	-	none	none	none
C4/HO	p<0.05	none*	-	-	-	-	-	-	none	none	p<0.01
C4/LO	none	none*	-	-	-	-	-	-	none	none	none
LO/HO	none	none	none	none	none	none	none	none	none	p<0.05	p<0.01

* no data from 02, 03 and 04

- no data

Table 2. Test of difference (significance:p) between basins in mean length increase of *Laminaria digitata* at various dates. HO (high oil), LO (low oil), C2 and C4 (controls) with two tagging periods.

	1. tagging period		2. tagging period				
Date	8303	8304-8307	8403	8404	8505	8406	8407
C2/HO	none	none	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.001$	$p<0.05$
C2/LO	none	none	none	$p<0.01$	none	$p<0.001$	none
C2/C4	-	-	none	none	none	none	none
C4/HO	-	-	$p<0.001$	$p<0.01$	$p<0.01$	$p<0.01$	none
C4/LO	-	-	none	$p<0.01$	none	$p<0.01$	none
LO/HO	$p<0.05$	none	$p<0.01$	none	$p<0.05$	none	none

- no data

6. Settlement, growth and community structure on granite chips. A comparison between four basins at S. E. S and a sheltered locality in the Oslofjord.

Odd - Arne Follum (UiO)

Introduction

Last progress report (no. 4 in "Littoral Rock Community Project") showed differences in settlement, growth and community structure between the four rocky shore basins, and between one of the control basins and a natural, exposed locality. Further discussion also showed dissimilarities between the two control basins, so in many occasions there were uncertainty if the observed events here were caused by the oil contamination or by "built-in" differences in the basins.

The aim of this project was to see how littoral rock communities developed in each basin, comparing them with each other and with a natural locality. When also comparing settlement and availibility of organisms in the water, this will, hopefully, tell something about what kind og communities one could expect, and how we can separate oil effects from natural events.

I would like to thank the Fæste family at Emmerstadbukta who kindly let me use theire private pier and who allways showed greate interest for my investigation and concern about the living sea shore.

Material and methods

Four granite chips (15x15 cm) were placed in aluminium frames, vertically, in each basin and in a natural locality (Emmerstadbukta, approx. 3 km south of Solbergstrand). They were hanging about 25 cm below the water surface at low tide and out of reach of predators. The two sides of the chips were facing north-south.

At the same time, four vertically standing chips, also in frames, were placed at the bottom (stairs) of all localities. These chips were set out at the same depth as the "pelagic" ones, but they were predated naturally.

Two chips from the "pelagic" and two from the "benthic" treatment at each locality were allowed to stay out in the sea from March to December 1984. The other two in each treatment were changed with new substrate every month in the same periode. Every chip were analyzed once a month on both sides by counting percent cover for all species under 100 evenly distributed points.

The long-time chips were analyzed non-destructivly and placed back in the frames in their fixed position together with the new short-time chips.

All data which have been used in ANOVA are transformed with arcsin $\sqrt{p}$.

The natural locality, Emmerstadbukta, was suggested by the meeting at Tømte in February 1984 as a sheltered control locality. This is a bay south of Solbergstrand which is very sheltered in southerly weather, and probably more alike the basin environment than the locality just outside the station. The sea water here is influenced by two small rivers flowing out at each side of the bay, following the same fluctuation with precipitation and snow melting as the river flowing out at Solbergstrand.

In the same periode, plankton hauls were taken from the inlet of each basin, from the overflow of the pipelines and from the surface of the fjord outside Solbergstrand. This, to see if planktonic organisms with different sizes and weights (and then different species) were distributed to the basins in different amounts.

Preliminary results and discussion

The first organisms to appear on the chips were benthic diatoms. All sides on the pelagic chips in basin 1 (HO) were completely covered with diatoms (Fig. 1). This dense cover has been characteristic for the high-oil basin since the chips were set out. Also basin 3 (LO) and basin 4 (C4) have been densely covered with diatoms except for September. Only chips in basin 2 (C2) have not had very much of this kind of primary growth. All the chips in the natural locality have been free from diatom cover until October.

The chips from the benthic treatment had less cover of benthic diatoms and algal canopy than the pelagic treatment, probably because of Littorina grazing. The difference of growth between the basins for the benthic chips is believed to be caused by the interaction of Littorina grazing and predation from Carcinus maenas. Basin 2 had the most dense population of shore crabs (see Moe, Follum and Lystad some other place in this report) and had also very few, but big specimens of Littorina littorea. The algal cover in this basin was more dense than in basin 3 and 4 (Fig. 4 and 6), probably because of less grazing. Basin 4 had the most dense population of periwincles, and the chips here were also free from algae when exposed to grazing (Fig. 6).

The barnacle, Balanus balanoides, settled down on the chips in April, and dominated the primary growth (when not considering the diatoms) from May to September. The natural locality was most densely covered with barnacles in May and June until the shells began to fall off the chips because of heavy wave action (from strong northerly winds).

The barnacles settled and grew as singular individuals in the low oil basin and the two control basins. Basin 1 missed almost the whole barnacle settlement. The high oil basin lacked any primary growth until September because of the dense cover of benthic diatoms in the pelagic treatment. Only some Bryozoa covered the substrate from August (Fig. 2).

Basin 3 and 4 were very similar in respect to barnacle settlement, in fact more similar than basin 2 and 4 which was expected. It is difficult to offer an explanation to this

pattern. It seemed that the low oil contamination played a minor part than the natural differences between the two basins, because basin 3 and 4 were very similar also in respect of algal cover (Fig. 3 and 6). On the long-time chips, Polysiphonia spp. dominated from June in all the basins, but on the short-time chips, basin 3 and 4 had settlement of Blidingia/Enteromorpha from July to September while basin 2 had a strong growth of Polysiphonia spp. and Laomedea spp. in August, and not until September, any kind of opportunistic green algae.

The grazing has probably been greater in the fjord than in any of the basins, because the periwincles were more abundant (Table 1). The chips which were exposed to herbivore predation at Emmerstadbukta lacked any algal cover (Fig. 6).

Table 1. The number of Littorina littorea found on the benthic chips on each analysis from April to October on each locality.

	A	M	J	J	A	S	O	$\bar{x}$
B1	0	4	3	0	7	6	2	3.1
B2	0	7	2	3	7	6	7	4.6
B3	6	10	10	0	3	6	7	6
B4	10	8	9	10	10	2	12	8.7
E	10	10	40	32	6	18	9	17.9

Because of the denuding of the pelagic substrate in June and July at the natural locality, the barnacle Balanus improvisus could settle here. Very few of this barnacle was observed on the basin chips. The benthic chips at Emmerstadbukta also lacked settlement of this species, which is believed to be due to heavy Littorina grazing in June and July (about 40 L. littorina were found on the chips in June).

As a preliminary conclusion, the following are proposed: Benthic diatoms were more abundant in basin 1 because of the oil contamination. This gave less primary growth here than in the other basins, either because a great amount of diatoms

prevent other organisms or because other organisms dont settle down in heavy oil pollution and therefor the diatoms can dominate the substrate with little or no competition.

Some sollution of the problems might be found in the microscopical field. The oil could possible create a bacteria/ mucus layer on the substrate on which the diatoms prefer to settle, or which prevents settling of other organisms.

The sparse settlement in basin 1 (HO), the less grazing and crab predation here and the deviation in growth and community structure compared with the other localities were probably, and as expected, due to the oil pollution. If some of the differences were created by some other causes than the oil, these were difficult to observe and probably eclipsed by oil effects.

The differences between the two control basins are probably due to "basin effects" which are difficult to explain (basin 2 is bigger than 4, the wave generators are not creating equal exposure in the two basins, and a wall has been build in basin 2). It seemed that basin 3 and 4 had a similar environment and that the oil pollution was too weak to make any effects on settlement, growth and community structure for the species involved in this research.

The plankton hauls are at the present still not analyzed. The general view seems nevertheless to be a very strong density of phytoplankton in the fjord compared to the basin hauls which are apparently free from phytoplankton. In some occasions, the hauls from basin 1, which is the first in the row of four basins, contain a lot of sand and great fragments of algae. The elements of sand and heavier particles seem less evident as one moves to the end of the pipelines. This, I think, will suggest that the different basins not always are offered the same amount of organisms, and this can influence settlement and growth between the basins.

Any further discussion of this will be done after all the hauls are analyzed and compared with the settlement and growth in each basin.

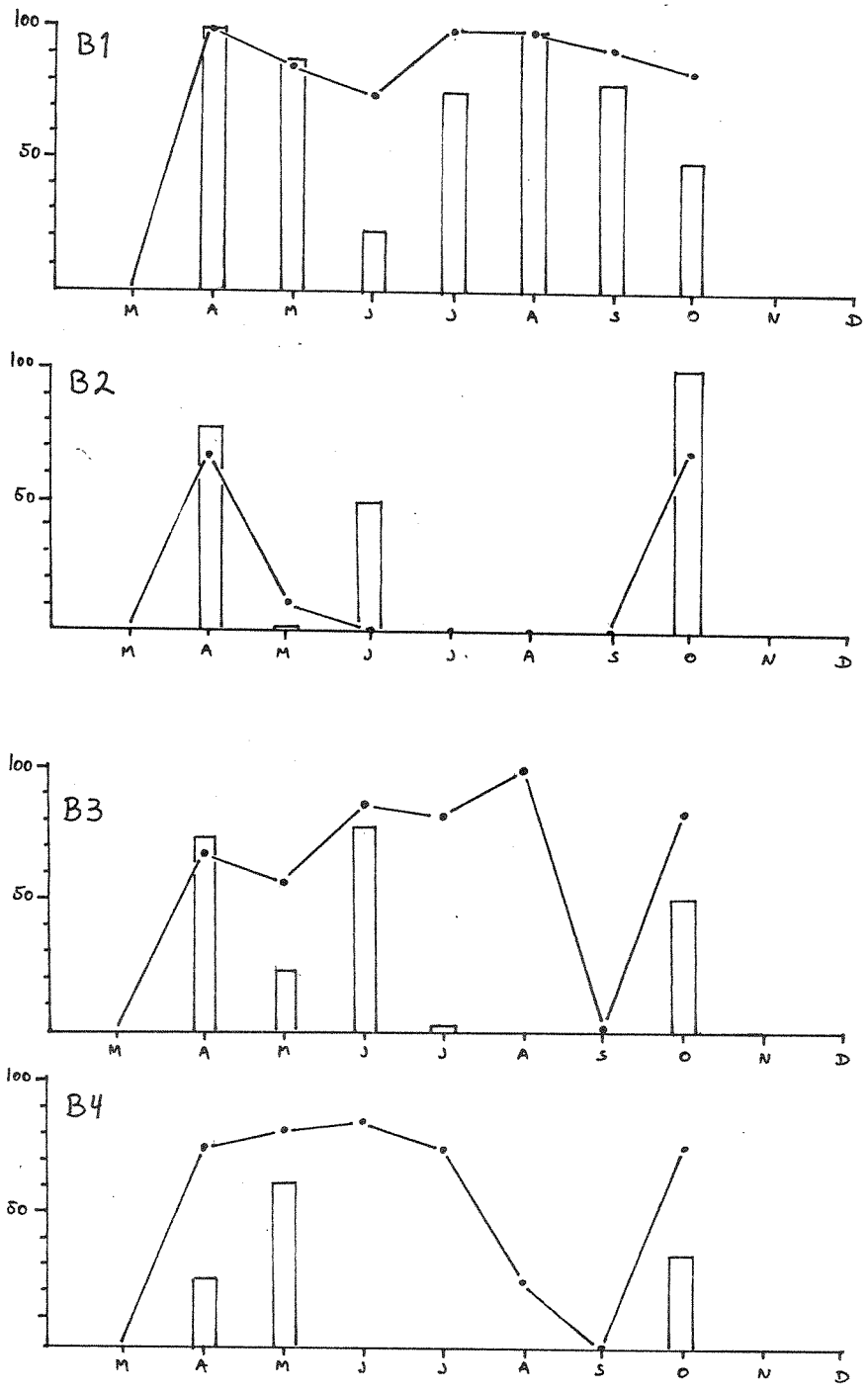


Fig. 1. Percent cover of benthic diatoms at all localities from March to October for the pelagic treatment. South and north sides of two granite chips in each series. The natural locality, Emmerstadbukta, had no diatom cover.

——•—— long-time chips

short-time chips

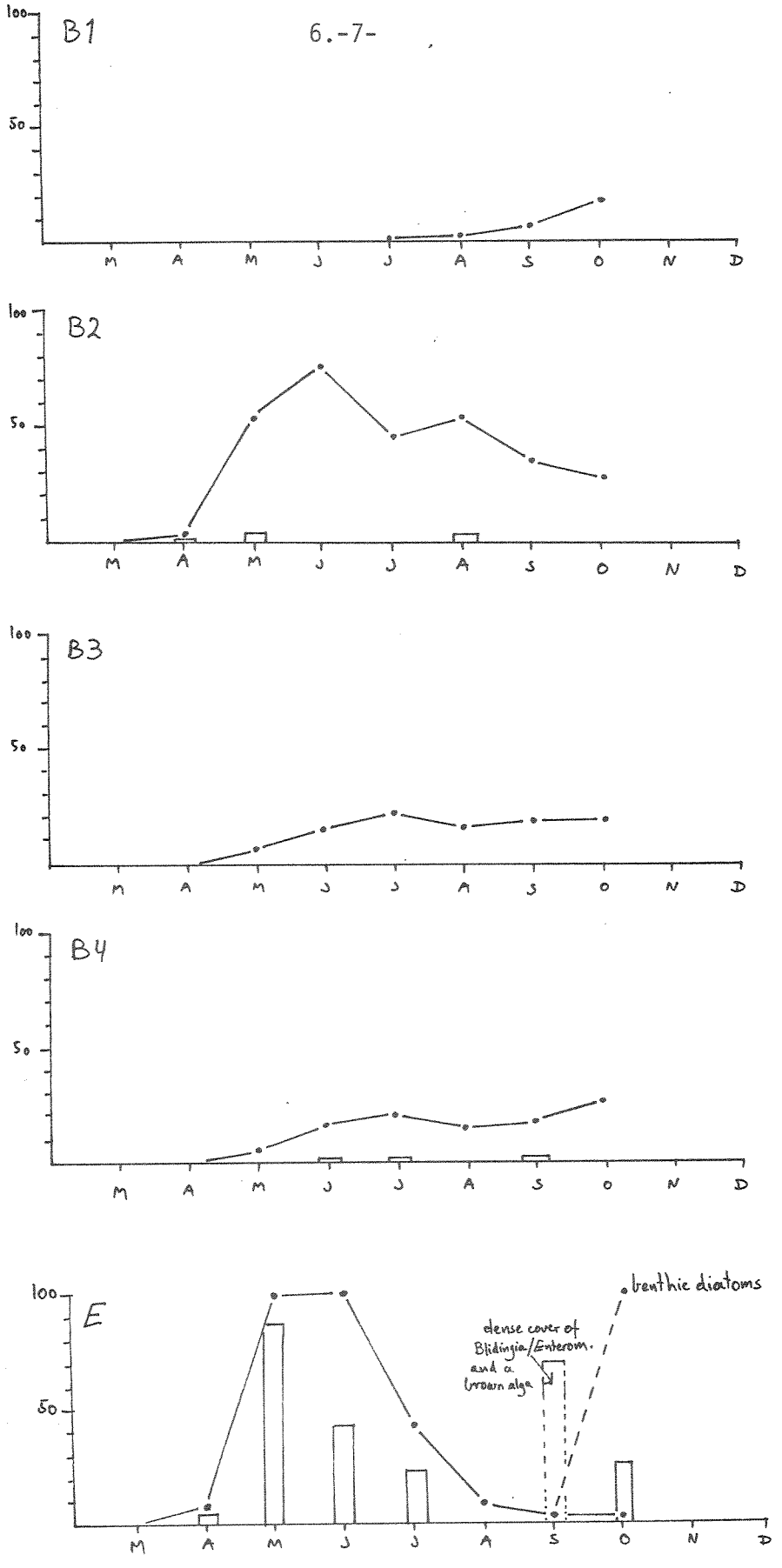


Fig. 2. Percent cover of primary growth (not diatoms) at all localities from March to October for the pelagic treatment. South and north sides of two granite chips in each series.

long-time chips

short-time chips

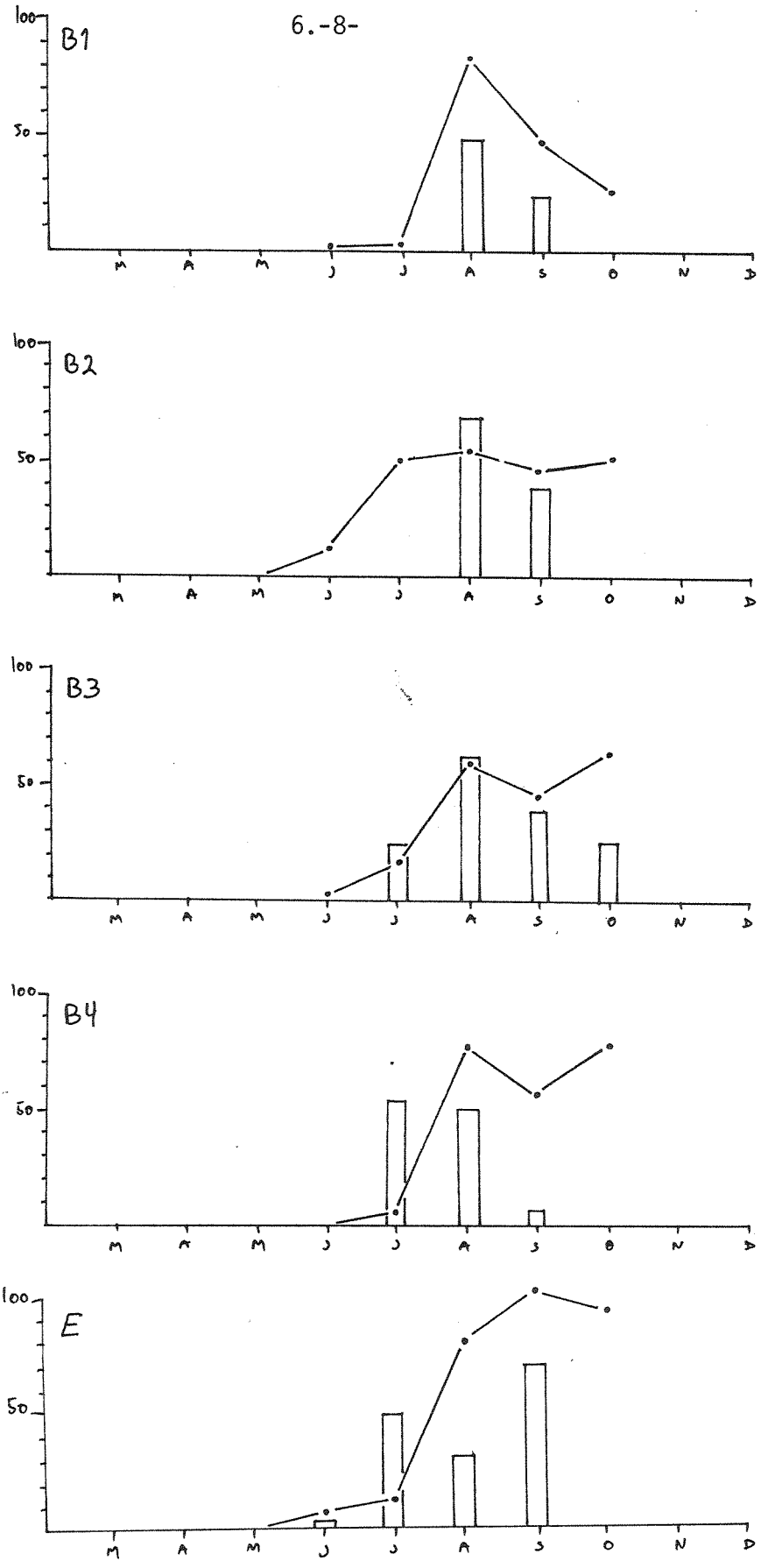


Fig. 3. Percent cover of algal and hydroide canopy at all localities from March to October for the pelagic treatment. South and north sides of two granite chips in each series.

——•—— long-time chips

short-time chips

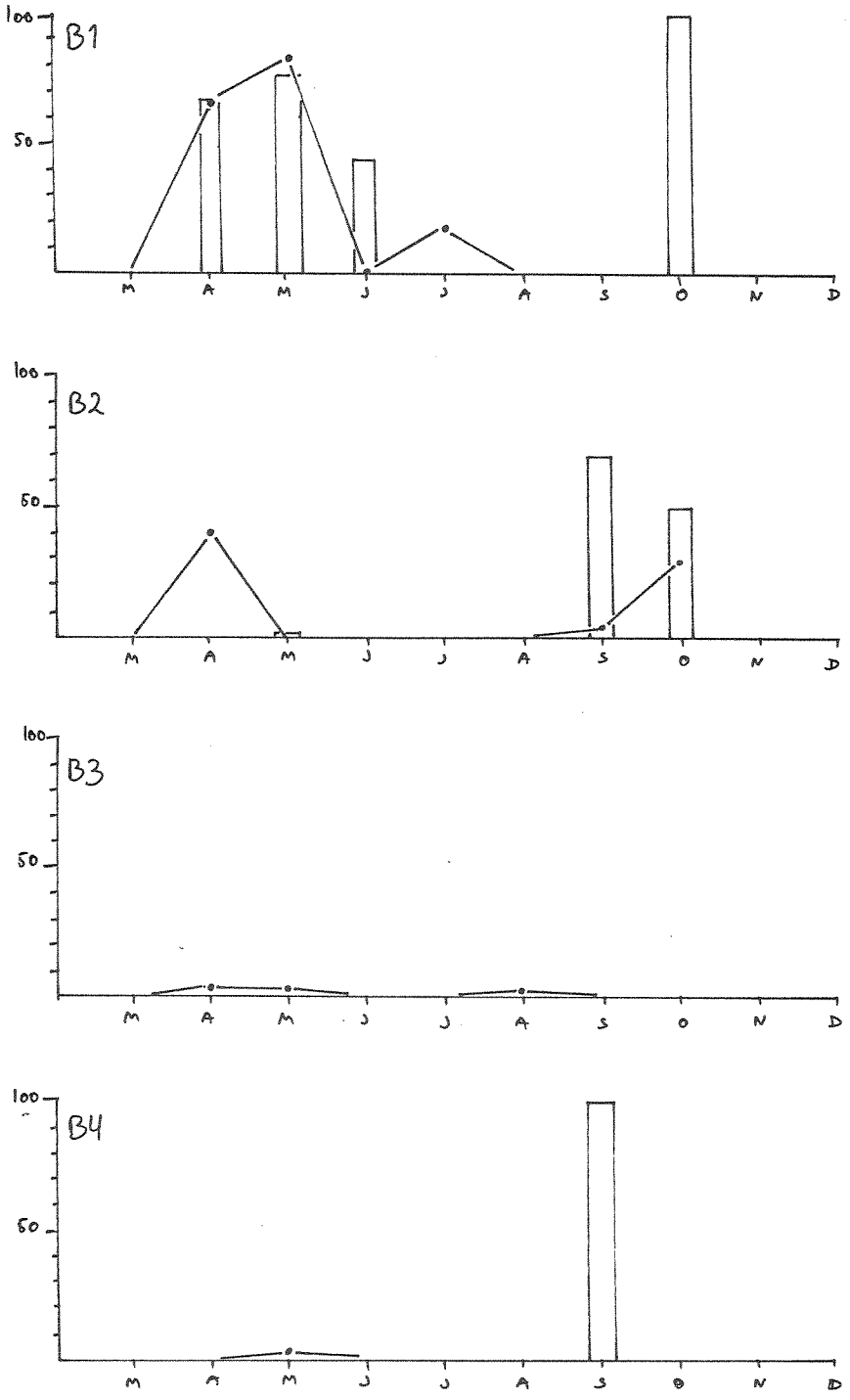


Fig. 4. Percent cover of benthic diatoms at all localities from March to October for the benthic treatment. South and north sides of two granite chips in each series. The natural locality, Emmerstadbukta, had no diatom cover.

——•—— long-time chips

short-time chips

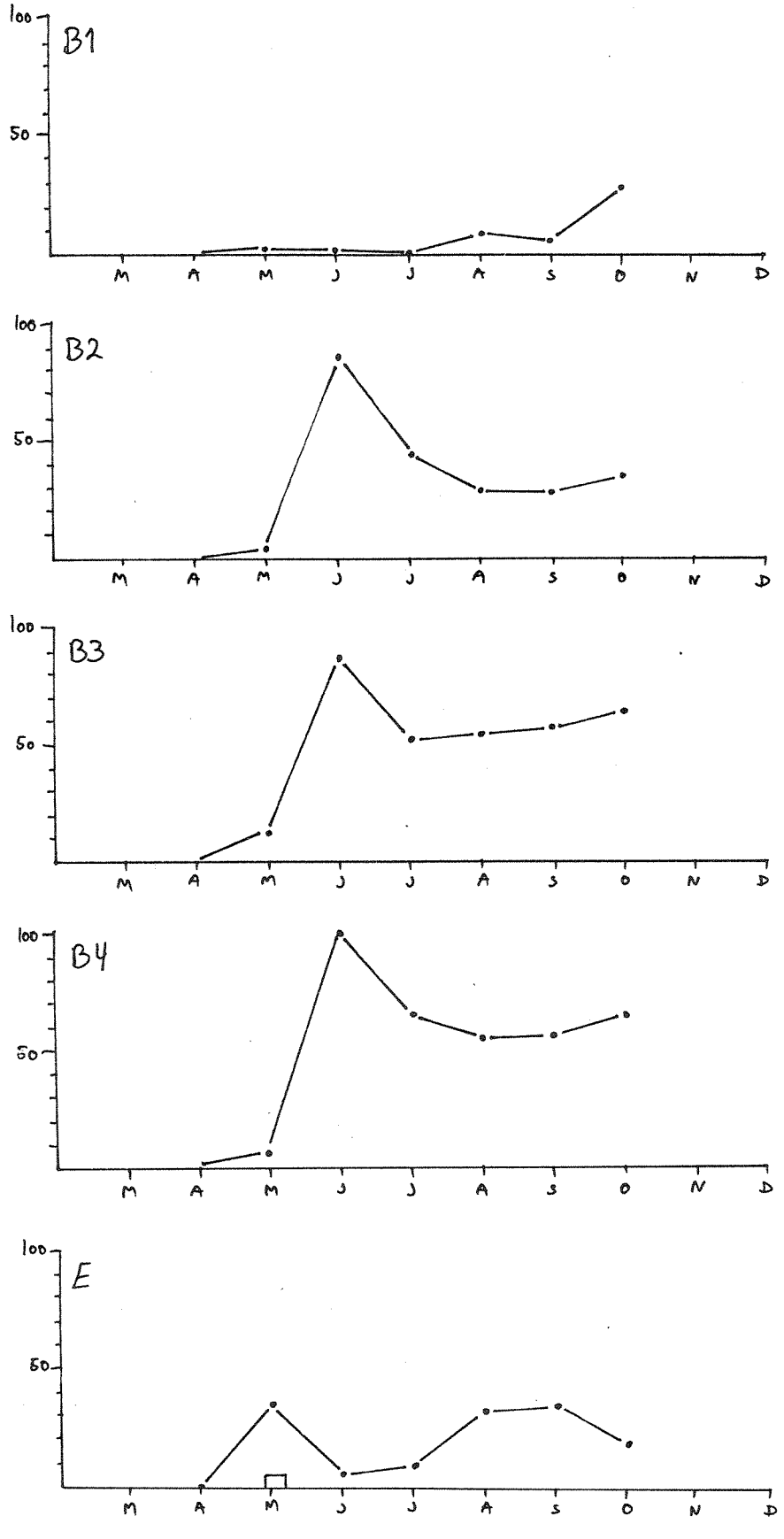


Fig. 5. Percent cover of primary growth (not diatoms) at all localities from March to October for the benthic treatment. South and north sides of two granite chips in each series.

——•—— long-time chips

short-time chips

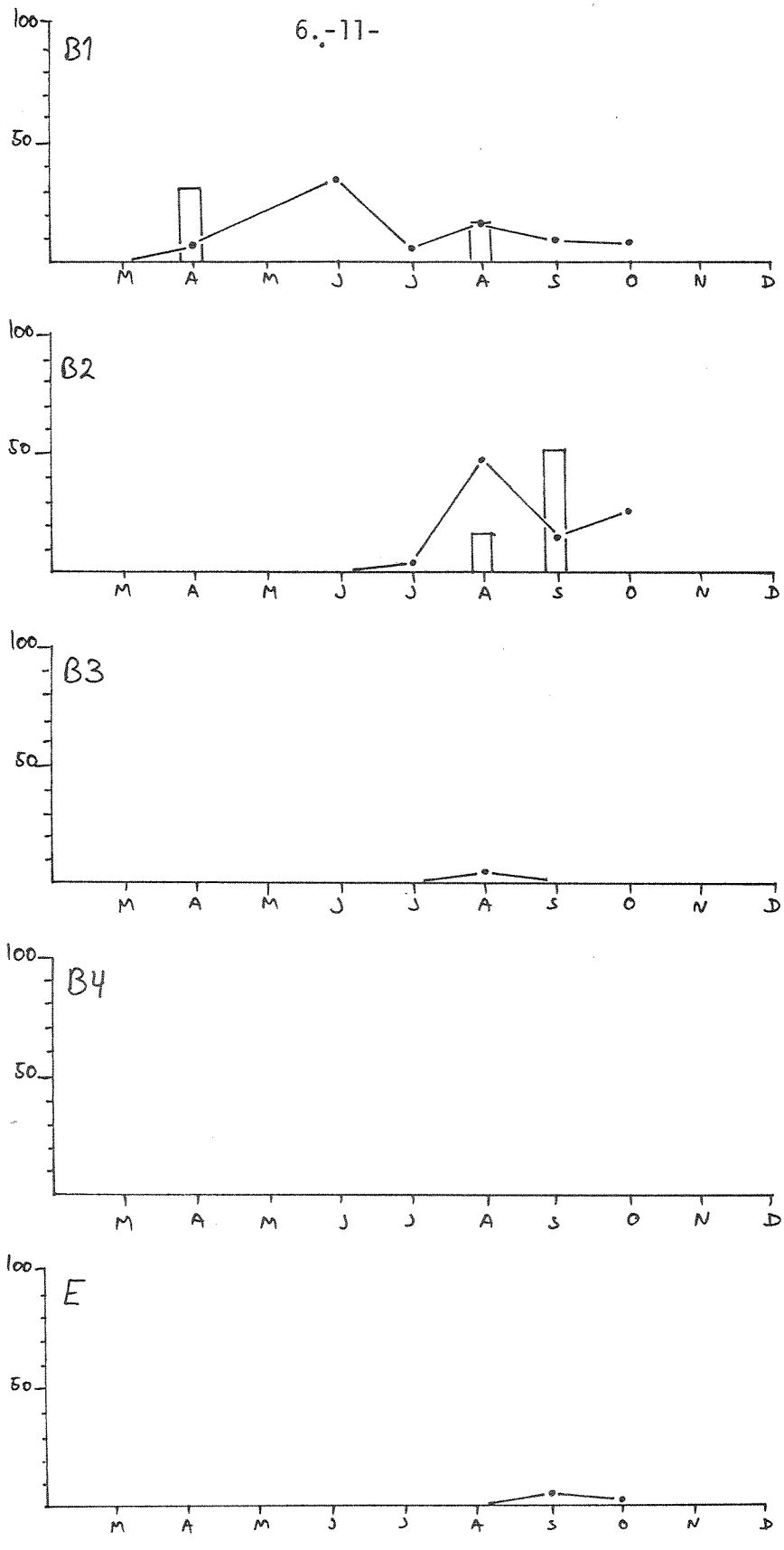


Fig. 6. Percent cover of algal and hydroide canopy at all localities from March to October for the benthic treatment. South and north sides of two granite chips in each series.

long-time chips

short-time chips

7. POPULATION DYNAMICS OF Littorina littorea IN AN OIL CONTAMINATED ENVIRONMENT AT SOLBERGSTRAND EXPERIMENTAL STATION.

Participants: Kjell Moe & Einar Lystad

Students at the University of Oslo.

Aim and purpose

Detect effects of low oil contamination on populations of Littorina littorea (L.) at both individual and population level. The five populations examined are designated as follows; HO - high oil , C2 - control 2 , LO - low oil , C4 - control 4 , CF - control fjord (previously CP).

Description of the work.

A continuation of the work described in Progress Report 1-2-3-4. All 5 populations have been examined in the period March 84 - November 84. Minor changes in sampling techniques have been performed and new ones are added.

Establishment of a new fjord control was necessary after the demolishment of the old one during reconstruction of the pier. The new CF is localized near the pump house , fig 1.

Description of the techniques used in our work is under each section. In addition to the things presented here there are analysis of dry weight, population estimates and gonad histology in preparation.

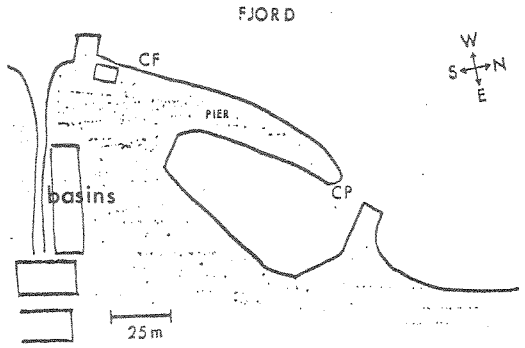


FIG 1: LOCALIZATION OF FJORD CONTROLS.

SIZE FREQUENCY DISTRIBUTION

To find an overall change in size -frequency we have used transect sampling.All individuals from a specific area are measured and let back into the basin at the place they were found.This sampling technique has been used over a period of 28 months. In the enclosures the transects are placed on the stairs and downwards from stair A to the bottom one ,E.During the sampling period the same transect was used from July 82 to September 83 and new ones from March 84 to November 84. There has not been any major change in composition in the two transects from different areas so the homogenety seems to be satisfactory.In C4 stair A has got a good habitat for juveniles due to eruption in the concrete suface.
The fjord population has a difficult habitat to examine with respect to juveniles due to sand and small cavities.
The results are presented in Figure II-A and II-B.

Spawning occurs mainly in April-May and hatching of the eggs and metamorphosis goes on in the summer.The juveniles(F_0) are found in our samples by September - November.It is interesting to observe the absence of this group in HO and LO by September 83 and the reoccurence in the November 84 transect.This while the other non-contaminated enclosures all show juveniles. This suggests that the eggs and/or larvae are susceptable to contamination of the kind.The occurence of juveniles by November 84 can indicate a late metamorphosis this year.
What happens specificly we dont know ,but a change in number of eggs in each capsule is recorded in some articles about the subject.

Overall growth can be detected by dislocation of the size-frequency columns from one period to the next.Figure II-A and II-B clearly show this. An accumulation of big winkles takes place in all populations except CF from July 82 until July 83.After this there was a change of location for CF due to recontruction of the pier and thereby major changes in the habitat.The new CF population is situated 50 m southwards with respect to the original one.

The accumulation of bigger winkles expresses itself by making some columns dominant in the histograms.The comparison of the July 82 to the July 84 show this.The trend in the CF population does not go the same direction.Can this be an enclosure-feature ? When the total data from the period March 84 to November 84 have been examined we may be able to give better explanations.

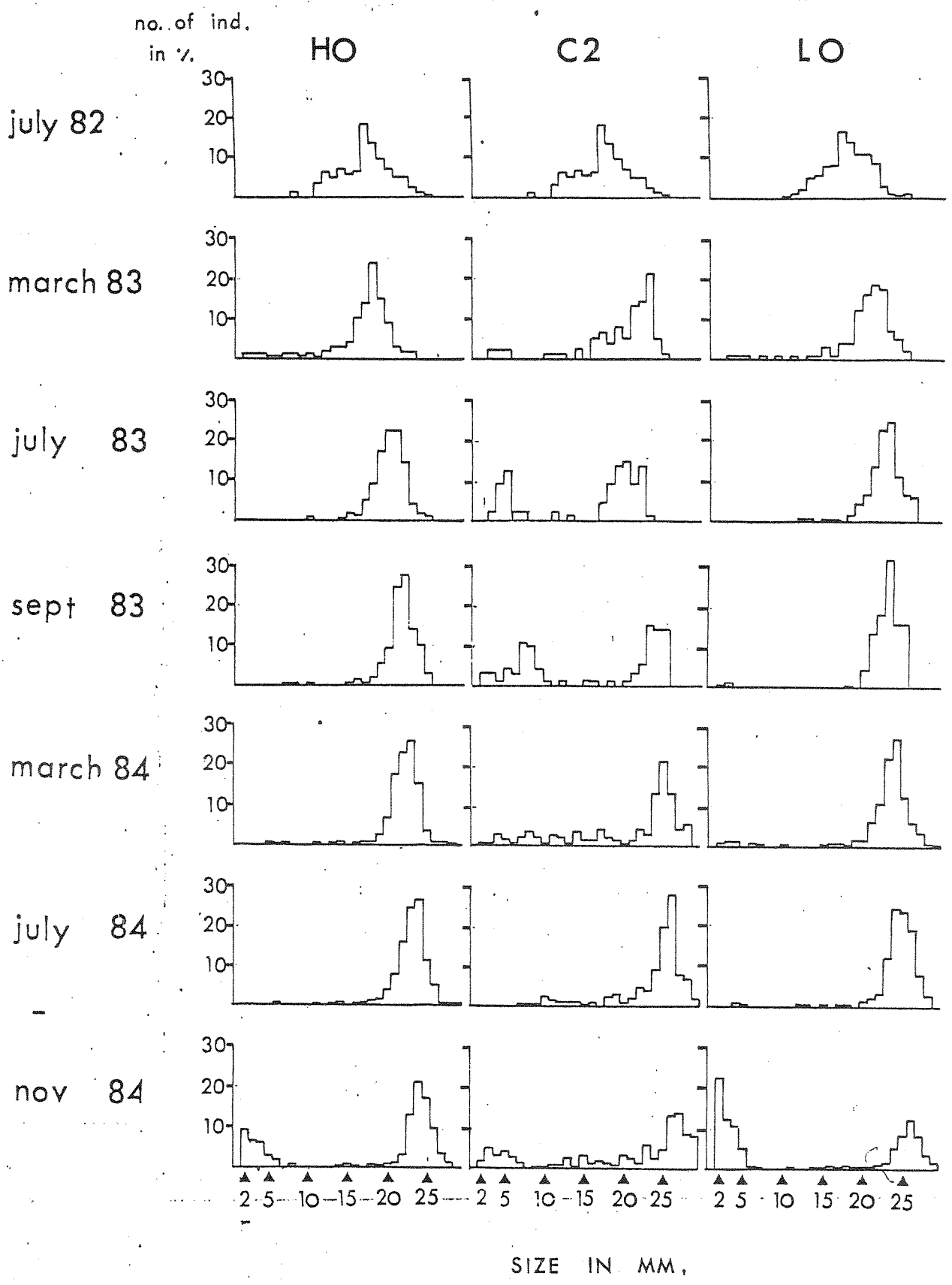


FIG 2-A

SIZE FREQUENCY DISTRIBUTION FOR THE LITTORINA LITTOREA POPULATIONS IN HO , C2 AND LO FROM JULY 82 UP TO NOVEMBER 84.

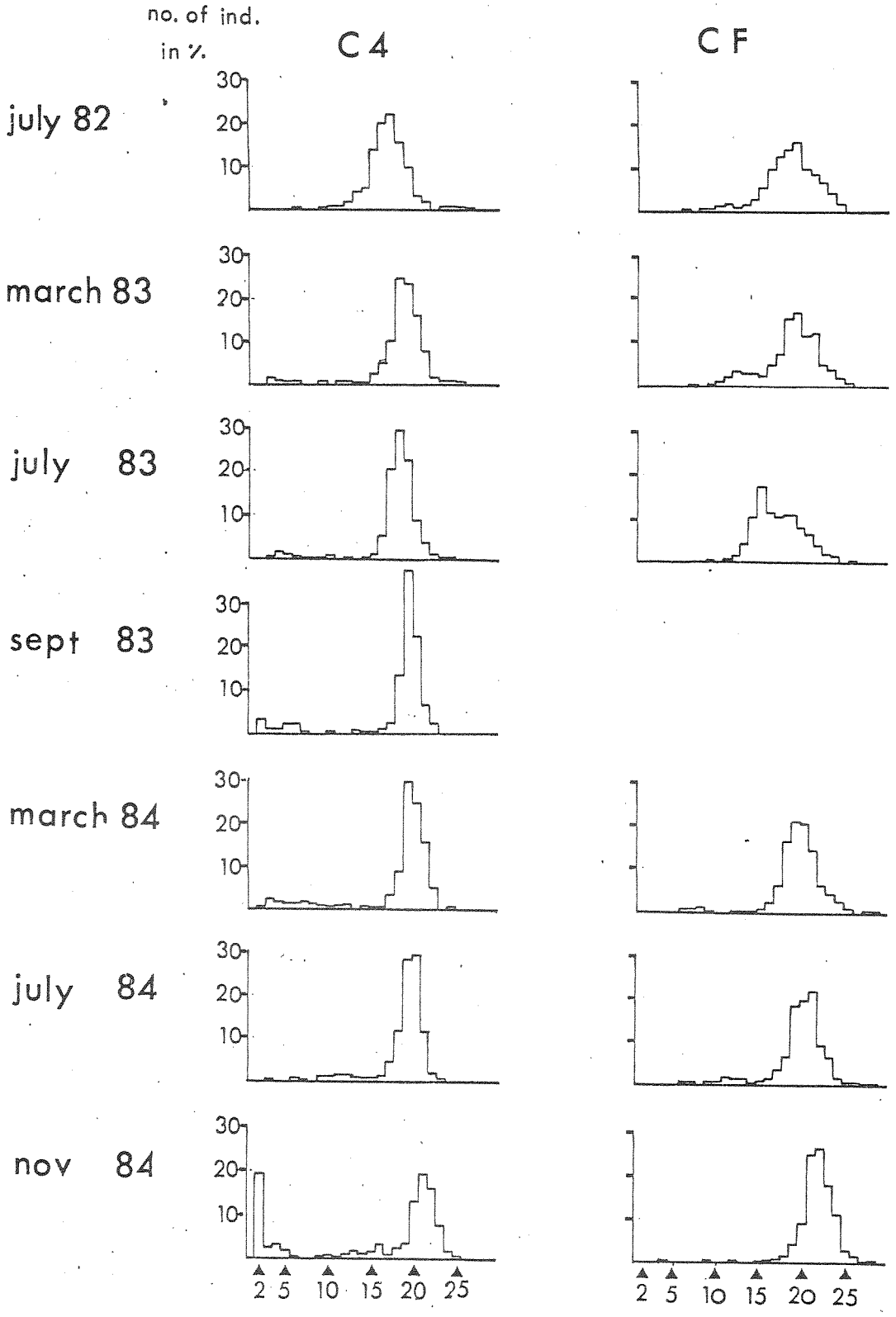


FIG 2-B

SIZE FREQUENCY DISTRIBUTION FOR THE LITTORINA LITTOREA POPULATIONS IN C4 AND CP (CF). FROM JULY 82 UP TO NOVEMBER 84.

Individual growth.

In July 1982 Littorina litorea from all basins and fiord (CF) were marked individually and measured for growth every other month until Sept. 1983 (Lystad and Moe in prep.) This work started again in March 1984 and carried out in the same prosedure until Nov. 1984. This is the period were the Littorinas show their largest growth.

Growth for 3 size-intervals (15-18mm, 19-21mm and 21-24mm) were published in Progress Report no. 4. A propriate way to compare these curves with the data for growth obtained in 84, was first to find the length values for each interval and basin at March 83. Then among the individuals marked in March 84 assemble groups (of approximately 10 randomly chosen individuals as in the 83 size intervals), with mean length as close as possible to each March 83 value. The growth of all these groups are outlined in Table 1 and Fig. 3.

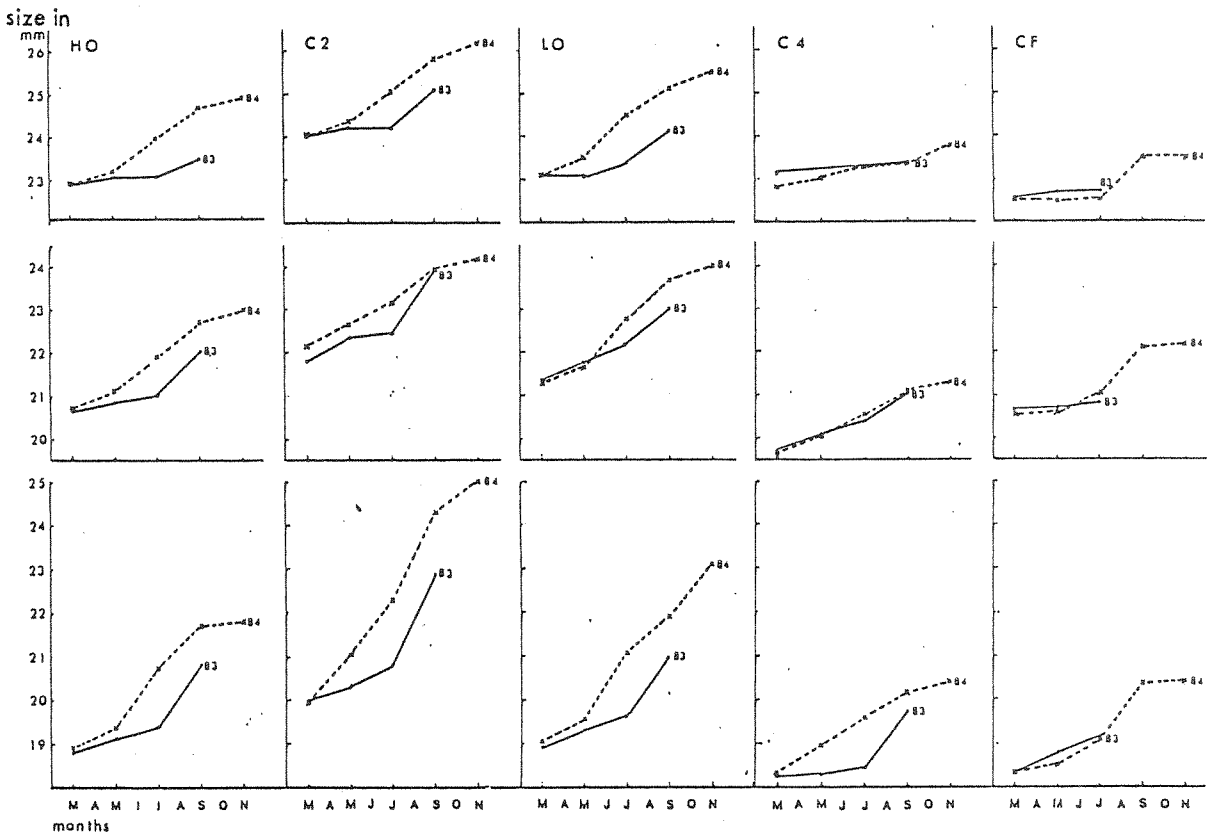


Fig. 3. The growth curves of 3 size-intervals in all basins and CF in the periods March 83 to Sept. 83 and March 84 to Nov. 84 (broken lines). Note the different starting values (March) for the growth curves in each basin. The curves are based on the growth from July 82 when the interval means were much alike for each basin (Tab.) and hence indicate the different growth also for the period July 82 to March 83.

Table 1. Mean length values of 3 different size-intervals (15-18mm, 19-21mm and 21-24mm) for all basins and CF in the period from July -82 to Nov. -84. Note the individuals who represent the 82-83 data, are not identical to those individuals marked and measured through 84.

Size-interval 21-24mm:

	HO	C2	LO	C4	CF	
	mean length	mean length	mean length	mean length	mean length	(mm)
July 82	22.45	21.95	22.24	23.20	22.12	
March 83	22.90	24.00	23.10	23.30	22.60	
Sept. 83	23.55	25.11	24.15	23.40	22.65 +	
March 84	22.94	24.12	23.11	22.69	22.50	
Sept. 84	24.69	25.80	25.15	23.35	23.54	
Nov. 84	24.96	26.20	25.51	23.78	23.58	

Size-interval 19-21mm:

	HO	C2	LO	C4	CF	
	mean length	mean length	mean length	mean length	mean length	(mm)
July 82	19.05	19.22	19.70	18.82	19.58	
March 83	20.65	21.80	21.35	19.72	20.65	
Sept. 83	22.05	23.95	23.02	20.53	20.81 +	
March 84	20.72	22.17	21.27	19.64	20.53	
Sept. 84	22.73	23.97	23.63	21.01	22.11	
Nov. 84	23.00	24.17	24.01	21.28	22.18	

Size-interval 15-18mm:

	HO	C2	LO	C4	CF	
	mean length	mean length	mean length	mean length	mean length	(mm)
July 82	16.15	16.18	16.20	16.08	16.68	
March 83	18.78	20.01	18.90	18.21	18.29	
Sept. 83	20.95	22.85	21.01	19.74	19.15 +	
March 84	18.94	19.96	19.10	18.33	18.34	
Sept. 84	21.69	24.28	21.92	21.21	20.43	
Nov. 84	21.76	24.99	23.09	20.43	20.50	

\+ July 83.

HO: Values in Table 1 indicate higher growth in all size intervals for the period March to Sept. 84 than for the same period in 83.

C2: The very high growth values and especially for individuals in the size interval 15-18mm in 84 draw the picture of the Littorinas in C2. With exception of the individuals in size interval 19-21mm, the growth values in 84 are higher than in 83.

LO: As in HO and C2, the growth values of Littorina littorea in LO, unspecified changes in 84 compared to 83. Fig. 3 showes

higher growth values for all intervals in 84.
C4: Low growth values for all size intervals characterize the Littorinas in C4. It is only a small, if any, variation between the values in 84 and 83.
CF: Incidently, due to mechanical damage of the CF station in Aug. 83, there are no growth data obtained after that date in 83. For this reason it is hard to compare the 83 and 84 periods of growth. Though both data for 83 and 84 indicate slow growth for individuals in all size intervals.

A comparison of the growth data from March 83 to Nov. 84 for all basins and C4 stress some of the previous assumptions. Littorina littorea in C4 and CF have slower growth rates for all sizes beeing represented compared to the other basins. Growth rates for 83 are in order of high to low growth: C2, LO, HO and C4/CF. The growth in 84 makes this picture more turbid. The situation in C4 CF, as mentioned, seem to be unchanged. Littorinas in HO still have somewhat slower growth than those in C2 and LO (but higher than those habiting C4 and CF). The differentiation, especially between C2 and LO, has been more balanced in 84.Only in the size group of the smallest individuals, Littorinas in C2 show higher growth than in LO. In addition to the earlier mentioned higher growth rate in 84, Fig. 3 points out an earlier start of the growth period in 84, compared to 83. This is the fact for HO, C2 and LO, but not for C4 and CF. This earlier start may be one of many explanations of the higher 84 growth rates. To draw more conclusions based on the growth curves (Fig. 3), without take into account the energy budget of Littorina littorea (Bakke 1984), changes in the microhabitat of each basin (Bokn 1984 and Follum 1984b), the physical data for the period (Sørensen 1984), in addition to more data of the population sizes of Littorina l. etc., would be a waste.

Recruitment.

To elucidate some of the factors which presumably determine the recruitment of Littorina littorea in the basins, plancton hauls from both inlet and outlet water were taken in the period 19 -22/4 1983. In this period spawning and capsules were registred in previous years (Follum 1984 and Eidnes 1983). The studies of gonads development stressed this fact (Lystad and Moe in prep.).
The hauls were taken at tideturning point (H.T.) each day to ensure inlet water would be taken from the same level and that the volume of water running through the net was approximately like for both inlet and outlet samples. Samples were taken from the end of the tubes into the headertanks (inlet) and from the tideregulating tubes (outlet). The sampling period was 5 min. The samples were fractioned by plancton-divider and from one half to one tenth of each sample were analysed. Littorina capsules, Bivalve larvaes and Cyprid larvaes were counted. The number of Littorina capsules from both inlet and outlet samples are shown in Table 2.

Table 2. Number of Littorina capsules taken in plancton hauls in the period 19 to 22/4 1983. In HO number of egg capsules containing more than 2 eggs pr. capsule were counted (3→).

	HO				LO		C4	
	Inlet		Outlet		Inlet	Outlet	Inlet	Outlet
egg pr. capsule	→2	3→	→2	3→				
Date								
19/4	176	10	2396	124	702	4664	184	5528
	tot. 186		tot. 2520					
20/4	245	19	885	67	600	648	1062	1592
	tot. 264		tot. 952					
21/4	115	11	1324	140	126	772	352	3216
	tot. 128		tot. 1468					
22/4	1210	90	1452	132	672	864	180	822
	tot. 1304		tot. 1596					

Numbers of Littorina capsules show as expected daily variation. In addition to this fact, we make three assumptions based on the data in Table 2.

I. Numbers of Littorina capsules running out of each basin are much higher for all basins and dates than numbers coming in (Fig. 4). It seems that the Littorinas in the basins are highly productive and compensate for more than the capsules beeing destroyed by the pumps. Another reason for this fact is, that it may be some sort of accumulation of capsules in the basins during tide coming in (and water volume running out is less than the input).

II. The differentiations between the outlet values of the basins are visualized in Fig. 4. In 3 of the four of the sampling period C4 has the highest number of capsules in the outlet samples. According to the size of the Littorina population in C4, which is the highest among the basins, this is no surprise.

III. Number Of eggcapsules in the inlet samples for each basin varies during the period. The data in Table 2 and Fig. 4 do not support the hypothesis that C4, the end station for inlet water, recieve more larvaes etc. than the other basins for this period.

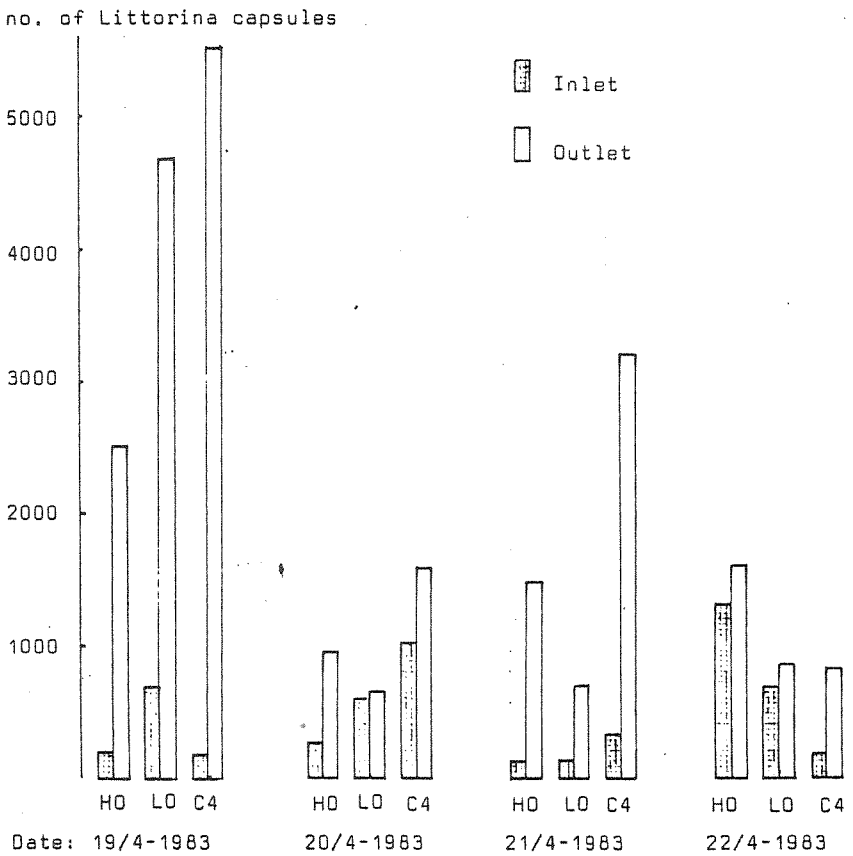


Fig. 4. Total number of Littorina capsules taken in plancton hauls in the period 19-22/4 1983. The columns are based on the values given in Table .

Staveland (1978) concluded that 13 months after an oil spill on the Norwegian West Coast, the part of Littorina littorea capsules with more than 2 eggs was higher than for Littorinas (from the same population) hatching in the laboratory. Littorina capsules with more than 2 eggs were counted (Table) for HO, both inlet and outlet samples. The values were not significant The proportions of 1 and 2 eggs pr. capsule were half and half in all basins, both inlet and outlet samples. 200 ppb continously oil dozing seem not to have the same effect on Littorina littorea's eggnumber pr. capsule as living 13 months in an area once polluted by 2000 t Iranian crude oil.

Growth of females and males.

In connection to the sampling of slow and fast-growing Littorinas in HO for genetic analysis by S. Fevolden, the sex of each individual was determined and their growth were measured. 49 individuals with growth less than 4 mm in the period July 82 to Oct. 84 were randomly selected. The sex ratio in the group was 25 males and 24 females. 34 individuals with growth more than 7 mm in the same period were selected in the same way as the slow-growing ones. In this group the sex ratio was 16 males and 18 females. The question stated was then if males or females within same group showed significant growth. A simple Student's t-test gave the following values for each group and sex:

	Slow growing ind.		Fast growing ind.	
	♂	♀	♂	♀
n =	25	26	16	18
mean growth =	2.69mm	2.30mm	9.18mm	8.43
S.D. =	0.81	1.08	1.85	1.35
t =	1.48 (DF = 49)		1.36 (DF = 32)	

Though it seems to be a slight differentiation between the growth of each sex (within the same group), the calculated t-values gave no significance at the level of $p = 0.05$. (At the level of $p = 0.2$ the growth of each sex within the same group was significant.)

LITERATURE

Bakke, T. (1984) SES Progress Report no. 5.

Bokn, T. (1984) SES Progress Report no. 5.

Eidnes, T. (1983) En sammenligning av kjønns-syklus, populasjons-struktur og vekst hos ulike populasjoner av vanlig strandsnegl, Littorina littorea (L.) i Oslofjorden. Hovedfagsoppgave i marin zoologi ved Universitetet i Oslo.

Follum, O.A. (1984) a. Thesis in preparation.

Follum, O.A. (1984) b. SES Progress Report no. 5.

Lystad, E. & Moe, K. (1985) Thesis in preparation.

Staveland, J.T. (1979) Effects on hatching in Littorina littorea after an oil spill. Mar. Poll. Bull. Vol 10, pp.255-258.

Sørensen, K. (1984) Physical parameters. SES Progress Report no. 5.

8. POPULATION GENETICS OF LITTORINA LITTOREA AT SOLBERGSTRAND

Svein E. Fevolden

INTRODUCTION

An organism, which in addition to Mytilus edulis was found in large numbers in the Solbergstrand basins prior to oil dosing, was Littorina littorea. With the same aim and purposes as for Mytilus edulis (see previous report) Littorina littorea was chosen as the second initial organism to be studied for potential effects of oil pollution on elctrophoretic variants. The survey design and methods applied were identical to those outlined in the Mytilus report.

MATERIAL

14 individual samples of Littorina littorea have so far been electrophoretically analyzed. They are as follows:

B1, B2, B3, B4: Basins prior to oil dosing. Sampled in September 1982.

FJRD: Adult controls from the shore immediately outside Solbergstrand. Sampled in September 1982.

HOSG: Adult individuals from the high oil basin which had shown a slow growth (<2 mm) one year after oil dosing started. Collected in September 1983.

HOFG: Correspondingly fast growers (>4 mm).

NRHO, NRLO, NRC2, NRC4: New recruits in the different basins one year after oil exposure started. Collected in September 1983.

NRFJ: New recruits from the fjord outside the basins. Collected in September 1983.

FG84: Adult individuals from HO which had shown fast growth (>7 mm) over the last two years. Collected in October 1984.

SG84: Correspondingly slow growers (growth <4 mm).

The number of new recruits in the basins in the fall of '83 was very low. The number of individuals that could be scored was further reduced due to the difficulties in distinguishing between such small individuals of L. littorea and Littorina saxatilis. The gel runs revealed that many of the animals sampled actually were L. saxatilis. Also the number of fast and slow growers from HO that could be run electrophoretically was limited since these were animals labelled for use in other studies.

RESULTS

Of the 30 loci regularly scored for the four pre-oil basins plus HO'83, only 6Pgdh showed a distinct polymorphism (average heterozygosity about 48%). The remaining polymorphic loci show a heterozygous frequency of less than 2.5%. Last fall, when the HO'84 samples were run, a new highly polymorphic Pgm-locus was detected, but these data are yet not included in the present tables.

Mean expected heterozygosity for the pre-oil basins for those 30 loci varied between 1.8 to 2.5% (Table 1), a rather low figure even for marine invertebrates.

The remaining samples were run for only eight loci, all polymorphic to some degree. Mean heterozygosities for these eight loci are given in Table 2 and allele frequencies for each locus in Table 3 which also gives the number of animals scored. In cases where only one or two animals appear to have been scored, the gels were practically unscorable. Either the enzymes were inactive or the bands were too blurred to be scored reliably. In order to run the computer program however, data from at least one homozygous individual was included.

No deviation from Hardy-Weinberg expectation was detected for any sample.

Contingency Chi-square tests revealed a heterogeneity in the 6Pgdh-locus among the pre-oil basins (Table 4). Since all four enclosures were recruited at the same time some physical/chemical factors in the basins may have been different - either at the time of settling, or chronically over time - thus causing selection. Due to this pre-oil interbasin difference, the basin populations of Littorina littorea were not pooled in the further analyses (which could be done for Mytilus edulis). The fjord adults represent a "mean" of the four basin populations implying that the basins had been populated by the same population as the shore outside.

Influence of oil

Adults: One year after oilexposure started animals that were sampled in HO could be split into fast growers and slow growers. Animals that had shown a pronounced growth in oil (>4 mm) were categorized as fast growers, those with a lowered growth (< 2 mm) as slow growers. Comparing allele frequencies for these two groups a pronounced difference was seen at the 6Pgdh-locus (Table 3). Although this difference was not statistically significant ($p = 0.258$, d.f. = 2), the fast growers were significantly different from the background population in the same basin (B1, Tables 4 and 3). On the other hand slow growers in HO'83 could not be statistically distinguished from the B1 pre-oil animals. From these data it would appear that a genetic selection had taken place among those animals that were able to thrive and maintain a healthy growth under oil stress. In order to have this confirmed this same procedure was repeated last fall by examining a number of fast growers (growth >7 mm) and slow growers (growth <4 mm) from HO. Growth was measured from '82 to '84 on animals that by the time of sampling had been exposed to oil for two years. Neither of the two groups were now significantly different from

B1 (when slow and fast growers were pooled, $p = 0.126$, d.f. = 3) although both had higher frequencies of the 96-allele than had the original population in that basin (Table 3). The growth characteristics of the animals sampled in '84 will be further analyzed, particularly to check if any potential selection can be linked to the initial stage (first year) of oil exposure.

Juveniles: No significant heterogeneity was registered between new recruits in the basins (Table 4 and 3). The presence of low concentrations of oil seemed to have no effects on the different genotypes' ability to settle. Nor was there any difference in allele frequencies between basin juveniles and fjord juveniles. The rather low number of new recruits that we were able to sample from the basins, especially the two oil basins, should however, be taken into consideration.

The remaining comparisons in Table show only one significant difference, that is when fjord recruits from '83 are compared to fjord adults from '82. Whether this is caused by a selection of the more rare Pgm-alleles over time, or reflects yearly variations is unknown. The gel-bands of fjord recruits also remain to be proof-read.

The minor heterogeneity reported between samples is too little to give values of genetic identity very different from 1.000, whichever samples are being compared. Only values averaged by biotop is therefore given (Table 5) and shows that the "largest" deviation is found when the pre-oil basins are compared.

CONCLUSION

The presence of a low concentration of oil seemed to have much less effect on the Littorina littorea's ability to survive than it had on Mytilus edulis' ability to survive. This fact made it unfeasible to test whether survival of Littorina was favoured by certain genotypes. One year of oil exposure gave evidence for allelic selection in the 6Pgdh-locus for those animals that grew fast under oil stress. Animals that had been exposed to oil over two years, however, showed no difference in allele frequencies between fast and slow growers. This could indicate a long term adaptation. The latter animals will be checked, however, to see if their growth in the initial year of oil exposure were genotypically linked. The larvae's ability to settle has not been proven to be favoured by the precence of certain gene alleles.

Remaining work

Animals from LO, C2, and C4 sampled after the termination of oil exposure are still to be analyzed. The newly found polymorphic Pgm-locus will tentatively be included in comparing these animals to the HO'84 animals.

Table 1.

LITTORINA LITTOREA

GENETIC VARIABILITY AT 30 LOCI

(STANDARD ERRORS IN PARENTHESES)

POPULATION	MEAN SAMPLE SIZE PER LOCUS	MEAN NO. OF ALLELES PER LOCUS	PERCENTAGE OF LOCI POLYMORPHIC*	MEAN HETEROZYGOSITY	
				DIRECT-COUNT	HDYWBG EXPECTED**
1. BASIN1 NONOIL	56.5 (2.6)	1.2 (.1)	3.3	.021 (.018)	.021 (.017)
2. BASIN2 NONOIL	74.7 (3.4)	1.5 (.1)	3.3	.026 (.018)	.025 (.017)
3. BASIN 3 NONOIL	71.8 (4.3)	1.4 (.1)	3.3	.018 (.014)	.020 (.016)
4. BASIN 4 NONOIL	58.0 (2.9)	1.2 (.1)	3.3	.020 (.017)	.018 (.015)
5. SLOW GROWERS HO	37.6 (1.9)	1.4 (.1)	3.3	.029 (.018)	.028 (.017)
6. FAST GROWERS HO	44.0 (2.8)	1.4 (.1)	6.7	.028 (.018)	.026 (.016)

* A LOCUS IS CONSIDERED POLYMORPHIC IF THE FREQUENCY OF THE MOST COMMON ALLELE DOES NOT EXCEED .95

** UNBIASED ESTIMATE (SEE NEI, 1978)

Table 2. Genetic variability of *Littorina littorea* at 8 loci

(STANDARD ERRORS IN PARENTHESES)

POPULATION	MEAN SAMPLE SIZE PER LOCUS	MEAN NO. OF ALLELES PER LOCUS	PERCENTAGE OF LOCI POLYMORPHIC*	MEAN HETEROZYGOSITY	
				DIRECT-COUNT	HDYNBG EXPECTED**
1. BASIN1 NONOIL	58.3 (5.0)	1.6 (.3)	12.5	.074 (.066)	.072 (.064)
2. BASIN2 NONOIL	78.5 (5.9)	1.9 (.2)	12.5	.083 (.065)	.079 (.062)
3. BASIN3 NONOIL	83.3 (3.9)	1.9 (.4)	12.5	.058 (.051)	.066 (.059)
4. BASIN4 NONOIL	61.4 (3.9)	1.5 (.2)	12.5	.068 (.062)	.062 (.055)
5. ADULT FJORD CTRL	83.0 (11.2)	2.4 (.3)	25.0	.094 (.062)	.095 (.064)
6. HO SLOW GROWERS	43.6 (2.0)	1.8 (.3)	12.5	.090 (.067)	.085 (.062)
7. HO FAST GROWERS	60.3 (1.2)	2.1 (.2)	25.0	.097 (.062)	.091 (.057)
8. NEW RECRUITS HO	13.3 (2.5)	1.9 (.3)	25.0	.096 (.056)	.102 (.062)
9. NEW RECRUITS LO	12.8 (2.5)	1.8 (.3)	50.0	.105 (.048)	.119 (.062)
10. NEW RECRUITS C2	27.9 (5.7)	1.9 (.3)	12.5	.105 (.075)	.092 (.062)
11. NEW RECRUITS C4	19.9 (5.4)	1.5 (.3)	25.0	.085 (.064)	.085 (.065)
12. NEW REC. FJORD	65.6 (11.9)	1.9 (.3)	12.5	.076 (.052)	.083 (.059)
13. HO84 FAST GRWERS	47.0 (6.8)	1.6 (.4)	12.5	.097 (.079)	.081 (.064)
14. HO84 SLOW GRWERS	43.6 (6.6)	1.9 (.3)	12.5	.086 (.066)	.080 (.060)

* A LOCUS IS CONSIDERED POLYMORPHIC IF THE FREQUENCY OF THE MOST COMMON ALLELE DOES NOT EXCEED .95

** UNBIASED ESTIMATE (SEE NEI, 1973)

Table 3. Allele frequencies in 8 polymorphic loci of *Littorina littorea* in 14 samples.

	POPULATION													
	B1	B2	B3	B4	FJRD	HOSG	HOFG	NRHa	NRLO	NRC2	NRC4	NRF1	HOFSSY	HOSGPY
LOCUS	1	2	3	4	5	6	7	8	9	10	11	12	13	14
APH														
(N)	64	91	97	70	100	48	60	17	16	37	33	86	56	55
A	1.000	.989	1.000	.993	.995	1.000	.992	1.000	1.000	.986	1.000	.988	1.000	.991
B	.000	.011	.000	.007	.005	.000	.008	.000	.000	.014	.000	.012	.000	.009
G3PDH														
(N)	59	84	89	67	6	46	63	17	17	34	2	71	56	40
A	1.000	.994	.994	1.000	1.000	1.000	.992	.971	.941	.985	1.000	.965	1.000	1.000
B	.000	.006	.006	.000	.000	.000	.000	.000	.000	.000	.000	.021	.000	.000
C	.000	.000	.000	.000	.000	.000	.008	.029	.059	.015	.000	.014	.000	.000
LAP-2														
(N)	38	63	63	43	90	34	57	2	1	2	2	22	42	50
A	1.000	.976	.992	1.000	.944	.971	.965	1.000	1.000	1.000	1.000	1.000	1.000	.990
B	.000	.024	.008	.000	.056	.029	.035	.000	.000	.000	.000	.000	.000	.000
C	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.010
LAP-3														
(N)	37	53	81	51	92	43	62	17	15	37	23	84	55	38
A	1.000	1.000	1.000	.990	.989	.977	.992	.971	.933	1.000	.935	.988	.964	.974
B	.000	.000	.000	.000	.005	.000	.000	.029	.067	.000	.065	.012	.036	.026
C	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
D	.000	.000	.000	.010	.005	.023	.008	.000	.000	.000	.000	.000	.000	.000
MDH														
(N)	57	61	71	52	92	36	54	2	2	2	2	3	1	1
A	1.000	1.000	1.000	1.000	.995	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	.000	.000	.000	.000	.005	.000	.000	.000	.000	.000	.000	.000	.000	.000
6PGDH														
(N)	71	91	89	72	100	47	63	17	17	37	31	86	54	55
A	.528	.462	.360	.333	.460	.489	.381	.382	.412	.405	.355	.413	.426	.400
B	.451	.527	.629	.667	.510	.500	.611	.588	.559	.568	.597	.587	.546	.591
C	.021	.011	.006	.000	.010	.011	.008	.029	.029	.027	.048	.000	.019	.009
D	.000	.000	.006	.000	.020	.000	.000	.000	.000	.000	.000	.000	.009	.000
PGI														
(N)	71	94	86	71	84	48	60	17	17	37	33	86	56	55
A	.993	.995	1.000	.993	.994	1.000	.950	.941	.971	.959	1.000	.971	.973	.973
B	.007	.005	.000	.000	.000	.000	.017	.029	.029	.014	.000	.017	.000	.009
C	.000	.000	.000	.007	.006	.000	.033	.029	.000	.027	.000	.012	.027	.018
PGM														
(N)	71	91	90	65	100	47	63	17	17	37	33	87	56	55
A	.979	.984	.989	1.000	.965	.968	.984	.971	.941	.959	.985	1.000	1.000	1.000
B	.000	.000	.000	.000	.000	.021	.000	.000	.000	.000	.000	.000	.000	.000
C	.007	.000	.006	.000	.005	.000	.000	.000	.000	.000	.000	.000	.000	.000
D	.014	.016	.006	.000	.030	.011	.016	.029	.059	.041	.015	.000	.000	.000

Table 4. Contingency table analyses of heterogeneity among samples of Littorina littorea using Pearson Chi square statistics.

Samples compared	*Single locus*	*p*	*s.l.*
B1/B2/B3/B4	6pgdh	.007	**
HOSG/HOFG '83	-		
HOFG '83/B1	6Pgdh	.027	*
HOSG '83/B1	-		
HOFG/HOSG '84	-		
HOFG'84/HOSG'84/B1	-		
NRHO/LO/C2/C4	-		
NRFJ/NR-basins	-		
FJRD/NRFJ	Pgm	.045	*

* 1% < p < 5% , ** 0.1% < p < 1%

Table 5.

MATRIX OF SIMILARITY/DISTANCE COEFFICIENTS AVERAGED BY BIOTOP
****

COEFFICIENT: NEI (1978) UNBIASED GENETIC IDENTITY

BIOTOP	NO.OF POPS.	1	2	3	4	5
1 BASINS NONOIL	4	.998 (.995-1.000)				
2 FJORD SAMPLES	2	.999 (.997-1.000)	.999 (.999- .999)			
3 HIGH OIL 83	2	.999 (.997-1.000)	1.000 (.999-1.000)	.999 (.999- .999)		
4 BASIN RECRUITS	4	1.000 (.997-1.000)	1.000 (.999-1.000)	1.000 (.999-1.000)	1.000 (1.000-1.000)	
5 HIGH OIL 84	2	.999 (.998-1.000)	1.000 (.999-1.000)	1.000 (.999-1.000)	1.000 (1.000-1.000)	1.000 (1.000-1.000)

9. POPULATION GENETICS OF MYTILUS EDULIS AT SOLBERGSTRAND

Svein E. Fevolden

INTRODUCTION AND AIM

Genetic differentiation among subpopulations of Mytilus edulis had been widely reported in the literature (list of references in Beaumont et al 1983, Skibinsky et al 1983, and Koehn et al 1984). This species was therefore chosen as one of two initial species to be studied for potential effects of oil pollution on electrophoretic variants at selected gene loci. "Natural" populations of this species were established in the Solbergstrand basins a few years prior to oil exposure. The electrophoretic data was to be used to evaluate the presence of diagnostic differences between oil-polluted and non oil-polluted water.

The survey design was: 1) to compare basin populations of Mytilus before and after a 1-2 year period of oil exposure, and 2) to compare new recruits that successfully settled in the oil-exposed and in the non oil-exposed basins. Controls, both adults and new recruits, were sampled from the fjord.

MATERIAL

The animals sampled in the different years of the experiment were as follows:

1982

Pre-oil basins. Four hundred post juvenile animals were sampled from the basins (100 from each) in the fall of 1982 to provide the background data for the basin populations (although two basins had already been exposed to oil for a month at the time of sampling, the genetic variation was as expected, later shown to be unaffected by this exposure). In the text and tables these pre-oil basin samples are referred to as B1, B2, B3, and B4, while all these basins pooled will be termed B1-4.

Fjord controls. At the same time one hundred post juvenile animals were sampled from the fjord immediately outside the Solbergstrand basins. They are referred to as FJRD.

1983

Survivors HO. About 125 animals that survived the longest in the basin with the high (ca. 200 ppb) oil concentration, HO (=B1 prior to oil dosing), were sampled in June , 1983. The mortality among Mytilus in this basin had been severe. These animals are termed SVHO.

New recruits '83. In late October, 1983, new recruits were sampled from the low (ca. 50 ppb) oil basin, LO (=B3 prior to oil dosing) and from the control basin C4 (=B4 prior to time of oil dosing). Unfortunately, no successful settlement of that year's brood occured in basin HO. Recruits from the fjord outside Solbergstrand were also sampled. The three different groups of new recruits from '83 are termed NRLO, NRC4, and NRFJ.

1984

New recruits '84. New recruits (smallest size group found) were sampled from LO, C2 and C4 in early October 1984. No living Mytilus were found in HO. The number of adult living animals in LO was also too low to be analyzed genetically. It was possible, however to sample some 150 small animals from LO to be compared to C2 and C4. These samples are termed LO84, C284, and C484.

ELECTROPHORETIC PROCEDURES

Immediately after being collected, animals were frozen and kept at -80C until thawed and prepared for electrophoretic examination. For the electrophoretic survey animals were homogenized whole in about an equal volume of chilled distilled water (or in 0.5M Tris-Hcl buffer, pH 7.1) and centrifuged for 20 minutes. The supernatant was reserved and used for starch gel electrophoresis (methods as described by Ayala et al 1972, 1974). The staining procedures were those described in Fevolden and Ayala (1981) and Fevolden (1982). At least 96 animals from each sample were run (except for B1 where only 86 animals were sampled).

Genetic variation was determined by means of measuring frequencies of the different alleles occuring at each locus. The most common allele at each locus was termed 100. The other alleles were termed according to their migration on the gel relatively to the 100 allele. Common criteria for polymorphism were used and normal statistical analyses were employed. Genetic identity, I, was calculated according to Nei's (1972) original formula $I=Iab/\sqrt{IaIb}$ where Iab etc. means arithmetic mean over all loci of $\Sigma a_i b_i$ etc., and a_i represents the frequency of the ith allele in locus k of population A. I varies between 1 (identical allele frequencies) to 0 (no common alleles shared). Genetic distance, $D,= -\ln I$. Nei (1978) later developed formulas for obtaining unbiased estimates of average heterozygosity and genetic distance/identity to take into account the bias caused by e.g. small sample sizes. These later formulas were used in the present text although they "minimize" differences shown by using his original formulas.

RESULTS

Out of 39 loci originally scored, 30 were assayed for animals from the basins prior to oildosing (B1-4, Table 1). Twenty loci were scored for the survivors and for the fjord samples (SVHO and FJRD, Table 1). Because of their small size the new recruits were analyzed only for the more polymorphic loci (Table 2).

Mean heterozygosity in the basin population of Mytilus edulis is about 11.5% (Table 3), while 23% of the loci were polymorphic after the more restrictive criterion (most common allele <95% in frequency). No differences could be seen in overall heterozygosity between the four basins (Table 4). When only the six most polymorphic loci are considered, average expected heterozygosity varied between 35% to 42%.

Very few deviations from Hardy-Weinberg expectations were seen (Table 5) when each sample was treated separately (B1-4 also pooled), meaning that each sample represents, or is recruited from, homogenous populations. When the observed number of heterozygotes are far fewer than the expected, mixed background popula-

tions are often the cause (due to the Wahlund effect). For Pgm-JRP-2 in B2, the deviation among the few (16) animals that could be scored was caused by registration of a rare heterozygote (98/102). The deviation from Hardy-Weinberg in B4 was caused by one registration of a rare homozygote.

Nei's (1978) genetic identity formulas show that no selection was detected between the basins when all 30 loci are considered (Table 6). All values of I are 1.000 meaning that the four basins were inhabited by one and the same population of Mytilus edulis before oil exposure started.

When pre-oil basins are compared to adult fjord controls ('82) some slight differences from total identity values are seen while survivors from HO ('83) are practically identical to the pre-oil basin samples (Table 7). These results are more clearly shown in the cluster analyses (Fig. 1) which use unweighted pair group methods and Nei's (1978) unbiased genetic identity coefficients. All pre-oil basins are clustered together with survivors from the HO basin while the adult fjord controls stand out separately.

Because of the close similarity of the four pre-oil basins, these animals were pooled in the next comparison of six polymorphic loci between non-oil basins, oil basins and fjord controls. Deviations from total identity values are now numerous (Table 8), although the numbers are still close to 1.000. The similarity cluster dendrogram (Fig. 2) shows the same clustering as above of B1-4 and survivors HO (SRHO) with the fjord adults at a little distance indicating a basin effect in genetic structure. Another cluster is formed by all new recruits, where LO and C4 do not seem more similar to each other than to the fjord recruits. A strong basin effect on juveniles is thereby not evident. The '84 recruits from LO and the two control basins are clustered on one line.

Contingency table analyses of heterogeneity among the different subsamples using Pearson's chi-square statistics and a G-test for polymorphic loci (Table 9) show which loci contribute to the differences illustrated in the cluster dendrograms:

The difference between non-oil basins' adults and the adult fjord controls is caused by Lap-3 (not significant on the G-test) and Pgi. For Lap-3 there are more 102 and 104 alleles in the fjord than in the basins while there seems to be a selection for the 96 allele in the basins (table 1). For Pgi there is a surplus of 98 alleles in the basins while there is a 96 surplus in the fjord. The fjord adults have a lower frequency of the 98 Pgi allele than all remaining samples, juveniles as well as adults (Table 1). This could indicate a selection against this allele over time in the natural environment. (The difference in the Pgi locus between oil and non-oil adults that was reported last year was eliminated upon proofreading of the gels.)

The difference between new recruits in LO and C4 in 1983 is caused by one locus, Idh. The 96-allele is significantly more common in the control basin than in the oil basin (Tables 2 and 9). However, one year later ('84) there was no significant difference in the Idh allele frequencies between that year's recruits in oil and non-oil basins (Tables 2 and 9).

Only the 1983 generation of juveniles were compared to juveniles from the fjord. Three loci contributed to differences between basin recruits and fjord recruits (Table 9). For Idh it is the oil basin sample (NRLO) which again has the lowest frequency of the 96 allele. Both the control basin and the fjord

(Table 2)

have a higher frequency of this allele. No selection against this allele was demonstrated among adult individuals during oil-exposure (cf. the lack of significant differences between B1-4 and SVHO). The similar lack of significant differences among the 1984 0-groups from the three basins, indicate that if there is a potential selection against the Idh 96 allele among juveniles under oil-exposure, then other factors are involved in determing whether this selection will actually take place or not.

For Lap-2 98 and 102 alleles are more common among the fjord recruits than in either of the two basins, with and without oil respectively. This could indicate a selection against the rarer Lap alleles in the basins, although the higher frequencies of these alleles observed among the 1984 basin juveniles seem to weaken the evidence. Also the fact that there are more 98 alleles in adult basin animals than in juvenile basin animals (comparison B1-4/NRC4 Table 9, and Table 1 & 2), does not indicate that this allele is selected against in post juvenile stages.

The last locus contributing to differences between 1983 juveniles in basins and fjord, Pgm-JRP3, has fewer 98 alleles in the oil basin than in the fjord. Also in the control basin there are fewer 98-alleles than in the fjord. This latter difference, however, is not significant, nor are NRC4 and NRLO significantly different at this locus. The possibility that this should indicate a basin effect is again weakened by the higher frequencies of this allele observed in the 1984 juveniles from the basins where values are closer to the 1983 fjord juveniles.

CONCLUSION

The few and minor differences detected between oil-exposed and non oil-exposed samples in the present survey do not indicate any strong genetic effect from the supposedly low concentrations of oil used. The oil dosing did indeed have a severe effect on the mortality of Mytilus edulis in the basins. For these gene systems that have been studied, adult animals' ability to survive in oil-polluted water could, however, not be determined by genotypic differences. The strongest evidence for an oil influence was seen among juveniles on the Idh locus. The difference registered between the two years of basin juveniles, do however, indicate that even for this locus, certain other, and unknown, factors must exist for any selection to take place. Also the actual basin effect on Mytilus's genetic structure seems to be marginal for the chosen loci. If there is such an effect it could vary from one year class to the next and also have a different effect upon different life stages.

REFERENCES

Ayala F.J., J.R. Powell, M.L, Tracey, C.A. Mourao & S. Peres-Salas 1972. Enzyme variability in the Drosophila willistoni group. IV. Genic variation in natural populations of Drosophila willistoni. Genetics 70:113-119.

Ayala, F.J., J.W. Valentine, L.G. Barr & G.S. Zumwalt 1974. Genetic variability in a temperate intertidal phoronid, Phoronopsis viridis. Biochem. Genetics 11:413-427.

Beaumont, A.R., C.M. Beveridge & M.D. Budd 1983. Selection and heterozygosity within single families of the mussel Mytilus edulis. Mar. Biol. Letters 4:151-161.

Fevolden, S.E & .F.J. Ayala 1981. Enzyme polymorphism in

Antarctic krill (Euphausiacea); genetic variation between populations and species. Sarsia 66:167-182.

Fevolden, S.E. 1982. Feeding habits and enzyme polymorphism in Thysanoessa raschi andMeganyctiphanes norvegica Crustacea; Euphausiacea). Sarsia 67:1-10.

Koehn, R.K., J.G. Hall, D.J. Innes, and A.J. Zera 1984. Genetic differentiation of Mytilus edulis in eastern North America. Mar. Biol. 79:117-126.

Nei, M. 1972. genetic distance between populations. Am. Nat. 106:283-292.

Nei, M. 1978. Estimation of average heterozygositiy and genetic distance from a small number of individuals. Genetics86:583-590.

Skibinsky, D.O.F., J.A. Beardmore & T.F. Cross 1983. Aspects of the population genetics of Mytilus (Mytilidae; Mollusca) in the British Isles. Biol. J. Limn. Soc. 19:137-183.

Wright, s. 1978. Evolution and the genetics of populations; Vol. 4. Variability within and among natural populations. Univ. Chicago press, Chicago.

Table 1. Allelic frequencies and frequencies of heterozygotes at 30 gene loci assayed for Mytilus edulis.

	B1-4	B1	B2	B3	B4	FJRD	SVHO
Acph-1	72	16	12	23	21		
100	1.000	1.000	1.000	1.000	1.000		
Acph-2	46	12	12	11	11	77	37
98	...	...	...	...	...	.006	...
100	1.000	1.000	1.000	1.000	1.000	.961	1.000
102	...	...	...	...	...	.032	...
H exp	...	...	...	...	...	.076	...
H obs	...	...	...	...	...	.078	...
Acph-3	62	17	19	14	12		
	1.000	1.000	1.000	1.000	1.000		
Adk-4	62	7	17	20	18	78	102
96	.008	...	...	.025	...	.006	.015
98	.323	.286	.353	.300	.333	.199	.240
100	.516	.500	.471	.525	.556	.686	.544
102	.145	.214	.147	.150	.111	.109	.201
104	.008	...	.029	...	...	...	...
H exp	.613	.670	.651	.627	.584	.481	.609
H obs	.645	.857	.647	.650	.556	.449	.578
Ao-1	282	69	61	89	63	57	104
100	.989	.993	1.000	.989	.976	1.000	.976
102	.011	.007	...	.011	.024	...	.024
H exp	.021	.014	...	.022	.047	...	.047
H obs	.021	.014	...	.022	.048	...	.048
Ao-2	179	61	43	42	33		
100	1.000	1.000	1.000	1.000	1.000		
Est-A	333	85	73	86	89	70	122
100	1.000	1.000	1.000	1.000	1.000	1.000	.996
98	...	...	...	...	...	...	.004
H exp	...	...	...	...	...	...	.008
H obs	...	...	...	...	...	...	.008
Est-B	328	86	83	74	85	71	74
98	.002	.006	...	...	...	.007	...
100	.998	.994	1.000	1.000	1.000	.993	1.000
H exp	.003	.012	...	...	...	.014	...
H obs	.003	.012	...	...	...	.014	
Fum-1	221	51	56	70	44	81	113
98	.002	...	.009	...	...	...	.004
100	.998	1.000	.991	1.000	1.000	1.000	.996
H exp	.005	...	.018	...	...	...	.009
H obs	.005	...	.018	...	...	...	.009
Got-1	108	42	13	23	30		
100	1.000	1.000	1.000	1.000	1.000		

Table 1. cont.

	B1-4	B1	B2	B3	B4	FJRD	SVHO
G3pdh	343	85	88	90	80	95	125
98	.001	...	...	...	.006	...	.004
100	.990	.988	.994	.983	.994	.974	.984
102	.009	.012	.006	.017	...	.026	.012
H exp	.020	.023	.011	.033	.013	.052	.032
H obs	.020	.024	.011	.033	.013	.053	.032
Idh-1	325	60	72	96	97	93	125
96	.082	.075	.063	.068	.113	.102	.084
98	.006	.008	.007	...	.010	.016	.008
100	.909	.908	.931	.932	.871	.882	.908
102	.003	.008	...	...	.005	...	...
H exp	.167	.171	.131	.127	.229	.213	.169
H obs	.169	.150	.139	.135	.237	.215	.168
Lap-2	367	85	96	93	93	93	124
96	.004	.006	...	.005	.005	...	.004
98	.080	.071	.094	.070	.086	.097	.077
100	.890	.882	.875	.903	.898	.898	.883
102	.026	.041	.031	.022	.011	.005	.036
H exp	.202	.216	.226	.180	.187	.185	.214
H obs	.193	.188	.208	.172	.204	.183	.210
Lap-3	355	86	86	94	89	84	114
96	.020	.029	.012	.016	.022	...	.031
98	.179	.163	.116	.218	.213	.143	.118
100	.661	.692	.721	.612	.624	.655	.706
102	.137	.105	.151	.149	.140	.179	.145
104	.004	.012	...	.005	...	.024	...
H exp	.513	.486	.446	.559	.548	.522	.468
H obs	.510	.512	.430	.553	.539	.488	.491
Mdh-1	313	75	71	78	89	87	125
96	.005	.007	.014	...	...	...	.008
98	.010	.020	.007	.006	.006	...	...
100	.982	.973	.972	.987	.994	1.000	.992
102	.003	...	.007	.006	...	...	...
H exp	.035	.053	.056	.026	.011	...	.016
H obs	.035	.053	.056	.026	.011	...	.016
Mdh-2	130	17	34	39	40	24	24
100	1.000	1.000	1.000	1.000	1.000	.958	1.000
102	...	...	...	...	...	.042	...
H exp	...	...	...	...	...	.082	...
H obs	...	...	...	...	...	.083	...
Me-1	64	19	17	13	15		
100	1.000	1.000	1.000	1.000	1.000		

Table 1. cont.

	B1-4	B1	B2	B3	B4	FJRD	SVHO
Me-2	269	62	58	76	73	89	125
98	.007	...	...	...	.027	...	...
100	.993	1.000	1.000	1.000	.973	1.000	1.000
H exp	.015	...	...	...	.054	...	...
H obs	.007	...	...	...	.027	...	...
Me-3	51	6	4	25	16		
100	1.000	1.000	1.000	1.000	1.000		
Mpi	217	54	79	43	41	65	123
96	.007	.019	.016	...	...	...	...
98	.009	.019	.006	.012	...	...	...
100	.979	.954	.987	.977	1.000	1.000	1.000
102	.005	.009	...	.012	...	...	...
H exp	.041	.091	.025	.046	...	...	...
H obs	.041	.093	.025	.047	...	...	...
Odh-1	74	17	22	15	20		
100	1.000	1.000	1.000	1.000	1.000		
6Pgdh	337	80	96	92	69	88	113
98	.007	...	.021	.011	.007	.006	.013
100	.984	.994	.979	.989	.971	.983	.978
102	.009	.006	...	...	.022	.011	.004
H exp	.032	.013	.041	.022	.057	.034	.044
H obs	.033	.013	.042	.022	.058	.034	.044
Pgi	348	80	82	88	98	66	102
94	.004	...	.006	.006	.005	.015	.005
96	.109	.094	.122	.125	.097	.167	.123
98	.272	.294	.220	.295	.276	.144	.265
100	.601	.600	.640	.563	.602	.659	.588
102	.013	.013	.012	.011	.015	.015	.020
104	.001	...	...	...	.005	...	...
H exp	.554	.548	.530	.584	.555	.521	.571
H obs	.575	.563	.561	.636	.541	.621	.627
Pgm/LiOH	364	85	91	93	95	94	122
94	.011	.012	.011	.011	.011	.005	.004
96	.070	.065	.044	.086	.084	.053	.029
98	.335	.359	.352	.301	.332	.298	.389
100	.552	.535	.549	.591	.532	.574	.553
102	.032	.029	.044	.011	.042	.064	.025
104	.001	...	...	...	...	.005	...
H exp	.578	.583	.574	.555	.602	.577	.543
H obs	.602	.659	.626	.591	.537	.553	.598

Table 1.cont

	B1-4	B1	B2	B3	B4	FJRD	SVHO
Pgm/JRP-2	35	4	10	9	12	34	48
96	...	...	...	...	...	.044	...
98	.043	...	.050	.056	.042	.132	.042
100	.957	1.000	.950	.944	.958	.809	.917
102	...	...	...	...	...	.015	.042
H exp	.083	...	.100	.111	.083	.331	.158
H obs	.086	...	.100	.111	.083	.265	.167
Pgm/JRP-3	87	12	24	25	26	84	111
96	.006	...	...	...	.019	.018	.014
98	.178	.083	.146	.240	.192	.155	.122
100	.695	.750	.771	.700	.596	.708	.739
102	.092	.083	.083	.060	.135	.089	.108
104	.029	.083	...	...	.060	.030	.018
H exp	.478	.435	.386	.458	.597	.468	.429
H obs	.437	.500	.292	.400	.577	.429	.468
Sdh-1	159	23	34	63	39		
98	.006	...	.044	.008	...		
100	.972	1.000	.941	.960	1.000		
102	.022	...	.015	.032	...		
H exp	.055	...	.114	.077	...		
H obs	.057	...	.118	.079	...		
Sdh-2	62	16	15	26	5		
100	1.000	1.000	1.000	1.000	1.000		
Xdh-1	362	83	95	88	96	96	125
98	.010	.018	.011	.006	.005	...	...
100	.985	.982	.968	.994	.995	.990	1.000
102	.006	...	.021	...	...	.010	...
H exp	.030	.036	.063	.011	.010	.021	...
H obs	.030	.037	.064	.011	.010	.021	...
Prot-1	105	25	32	24	24		
100	1.000	1.000	1.000	1.000	1.000		

B1-4=four basins prior to oil dosing pooled, B1-B4=four pre-oil basins kept separate, FJRD=adult control animals from the fjord, SVHO=survivors in the high oil basin summer 1983.
H exp=frequency of expected heterozygotes, H obs=frequency of observed heterozygotes. Number of scorable animals are on line of locus designation.

Table 2. Allelic frequencies and heterozygous frequencies at six/eight selected loci for new recruits of Mytilus edulis.
NRLO = new recuits LO 1983, NRC4 = new recruits C4 1983, NRFJ = new recruits fjord 1983, LO84 = new recruits LO 1984, C284 = new recruits C2 1984, C484 = new recruits C4 1984.

	NRLO	NRC4	NRFJ	LO84	C284	C484
Adk-4	48	51	49			
98	.156	.147	.286			
100	.698	.706	.592			
102	.135	.147	.122			
104	.010	...	...			
H exp	.475	.463	.559			
H obs	.500	.431	.571			
Idh-1	86	95	92	129	139	119
96	.029	.116	.098	.089	.061	.067
98	.023	.005	.016	.016	.018	.008
100	.948	.874	.880	.895	.921	.924
102	...	.005	...	...	...	...
104	...	...	.005	...	...	...
H exp	.101	.224	.216	.191	.148	.142
H obs	.105	.211	.217	.209	.158	.134
Lap-2	62	92	92	123	141	141
96	...	.005	...	.012	.007	.004
98	.016	.022	.060	.049	.039	.035
100	.984	.973	.918	.935	.943	.947
102	...	...	.022	.004	.011	.011
104	...	...	...	...	...	.004
H exp	.032	.053	.153	.130	.109	.103
H obs	.032	.054	.152	.124	.113	.106
Lap-3	90	92	93	136	133	122
96	.028	.022	...	.004	...	...
98	.139	.152	.124	.118	.128	.127
100	.678	.668	.677	.654	.680	.701
102	.144	.147	.167	.221	.184	.172
104	.011	.011	.032	.004	.008	...
H exp	.502	.511	.500	.511	.448	.465
H obs	.478	.533	.484	.463	.496	.492
Pgi-1	89	94	91	140	132	128
94	.006	.005	.005	.014	.023	...
96	.073	.096	.121	.136	.114	.145
98	.236	.309	.225	.254	.182	.203
100	.663	.559	.615	.582	.670	.641
102	.022	.027	.033	.014	.011	.012
104	...	.005	...	....	....	....
H exp	.502	.586	.558	.580	.506	.529
H obs	.449	.574	.571	.593	.523	.547

Table 2. cont.

	NRLO	NRC4	NRFJ	LO84	C284	C484
Pgm/LiOH	91	86	91	135	122	98
94	.005	...	.005	...	...	.010
96	.038	.035	.038	.007	...	.010
98	.220	.291	.220	.233	.242	.219
100	.665	.640	.676	.715	.717	.714
102	.071	.035	.060	.044	.041	.046
H exp	.506	.507	.493	.434	.427	.442
H obs	.440	.477	.538	.467	.467	.500
Pgm/JRP-2	64	77	62			
96	.008	.000	...			
98	.023	.026	.032			
100	.969	.968	.960			
102	...	.006	.008			
H exp	.061	.064	.079			
H obs	.063	.065	.081			
Pgm/JRP-3	84	90	83	136	133	118
96	.006	.006	.018	.011	.004	.004
98	.077	.100	.193	.169	.158	.127
100	.726	.722	.663	.665	.680	.763
102	.137	.144	.102	.107	.105	.085
104	.048	.028	.024	.040	.038	.021
106	.006	...	...	.007	.015	...
H exp	.448	.449	.515	.517	.501	.396
H obs	.417	.478	.470	.529	.474	.381

Table 3. Summary of genetic variation in the basin population of Mytilus edulis prior to oil-dosing.

Number of loci studied	= 30
Number of individuals	= 378
Mean number of alleles per locus	= 2.53 (s.e. 0.29)
Mean heterozygosity per locus (unbiased estimate)	= 0.115 (s.e. 0.037)
Mean heterozygosity per locus (direct-count estimate)	= 0.116 (s.e. 0.038)
Percentage of loci polymorphic (0.95 criterion)	= 23.33
Percentage of loci polymorphic (0.99 criterion)	= 50.00

Table 4. Mean heterozygosities for the different samples of Mytilus edulis .

		Number of loci included in the mean			
		30	20	8	6
B1-4	exp	.115(.037)	.169(.052)	.398(.075)	.415(.075)
	obs	.116(.038)	.171(.053)	.402(.078)	
B1	exp	.112(.038)	.167(.052)	.387(.083)	
	obs	.122(.043)	.184(.061)	.427(.103)	
B2	exp	.112(.036)	.163(.050)	.381(.073)	
	obs	.111(.036)	.162(.050)	.379(.079)	
B3	exp	.115(.038)	.168(.053)	.400(.078)	
	obs	.116(.039)	.170(.055)	.406(.083)	
B4	exp	.119(.039)	.177(.054)	.423(.077)	
	obs	.115(.038)	.179(.055)	.409(.070)	
FJRD	exp		.179(.048)	.413(.054)	.415(.070)
	obs		.174(.048)	.401(.058)	.415(.074)
SVHO	exp		.166(.050)	.396(.070)	.398(.069)
	obs		.173(.053)	.414(.070)	.426(.079)
NRLO	exp			.328(.078)	.349(.090)
	obs			.310(.072)	.320(.081)
NRC4	exp			.357(.075)	.388(.084)
	obs			.353(.074)	.388(.085)
NRFj	exp			.384(.070)	.406(.071)
	obs			.386(.071)	.406(.971)
L084	exp				.393(.077)
	obs				.399(.076)
C284	obs				.362(.075)
					.372(.075)
C484	obs				.346(.073)
					.360(.079)

Table 5. Chi-square test for deviation from Hardy-Weinberg equilibrium (H-W expected frequency of the different genotypes). -: no significant deviation.

Sample	Number of loci assayed	Significant deviations	probability
B1	30	-	
B2	30	Pgm/Jrp-3	p<5%
B3	30	-	
B4	30	Me-2	p<0.1%
B1-4	30	Me-2	p<0.1%
FJRD	20	-	
SVHO	20	-	
NRLO	8	-	
NRC4	8	-	
LO84	6	-	
C284	6	-	
C484	6	-	

Table 6. Matrix of genetic distance and similarity coefficients between pre-oil basins. Above diagonal: Nei (1978) unbiased genetic distance. Below diagonal: Nei (1978) unbiased genetic identity. 30 loci included.

	B1	B2	B3	B4
B1		.000	.000	.000
B2	1.000		.000	.000
B3	1.000	1.000		.000
B4	1.000	1.000	1.000	

Table 7. Basins compared with fjord (FJRD) and survivors (SVHO) for 20 loci. Above diagonal: Nei (1978) unbiased genetic distance. Below diagonal: Nei (1978) unbiased genetic similarity.

	B1	B2	B3	B4	FJRD	SVHO
B1		.000	.000	.000	.002	.000
B2	1.000		.000	.000	.002	.000
B3	1.000	1.000		.000	.002	.000
B4	1.000	1.000	1.000		.002	.001
FJRD	.998	.998	.998	.998		.002
SVHO	1.000	1.000	1.000	.999	.998	

Table 8. Matrix of Nei's (1978) unbiased genetic distance (above diagonal) and genetic similarity (below diagonal) when pre-oil basins (pooled) are compared to fjord controls, survivors and new recruits. Sample designations as in Table 1.

	B1-4	FJRD	SVHO	NRLO	NRC4	NRFJ	L084	C284	C484
B1-4		.002	.000	.006	.002	.003	.006	.006	.006
FJRD	.998		.003	.004	.005	.001	.004	.002	.002
SVHO	1.000	.997		.007	.002	.006	.008	.008	.006
NRLO	.994	.996	.993		.001	.001	.003	.001	.000
NRC4	.998	.995	.998	.999		.002	.002	.004	.003
NRFJ	.997	.999	.994	.999	.998		.000	.000	.000
L084	.994	.996	.992	.997	.998	1.000		.000	.000
C284	.994	.998	.992	.999	.996	1.000	1.000		.000
C484	.994	.994	1.000	.997	1.000	1.000	1.000	1.000	

Table 9. Contingency table analyses of heterogeneity among samples of Mytilus edulis using Pearson Chi-square statistics, and RxC test of allele frequency independence between samples using G-test.

Pearson Chi-square statistics			G-test		
Samples compared	Single loci	p	G	Df	Sl
B1/B2/B3/B4	-				
B1-4/SVHO	-				
B1-4/FJRD	Lap-3	.013 *	7.24	4	ns
	Pgi	.020 *	12.98	5	*
B1-4/NRC4	Lap-2	.004 **	17.90	3	***
NRC4/NRLO	Idh	.005 *	14.01	3	**
NRC4/NRFJ	Lap-2	.036 *	10.56	3	*
NRLO/NRFJ	Idh-1	.044 *	8.96	3	*
	Lap-2	.041 *	4.28	3	ns
	Pgm-JRP3	.024 *	13.56	5	*
NRFJ/FJRD	-				
L084/C284/C484	-				

Df=degrees of freedom; Sl=significance level; ns=no significance, * 1%<p<5%, ** 0.1%<p<1%, *** p<0.1%

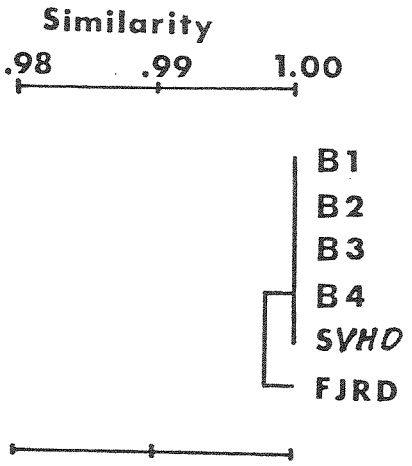


Figure 1. Cluster dendrogram (Nei's unbiased identity coefficients) for comparison of 20 gene loci in Mytilus edulis from pre-oil basins, survivors HO and fjord adults.

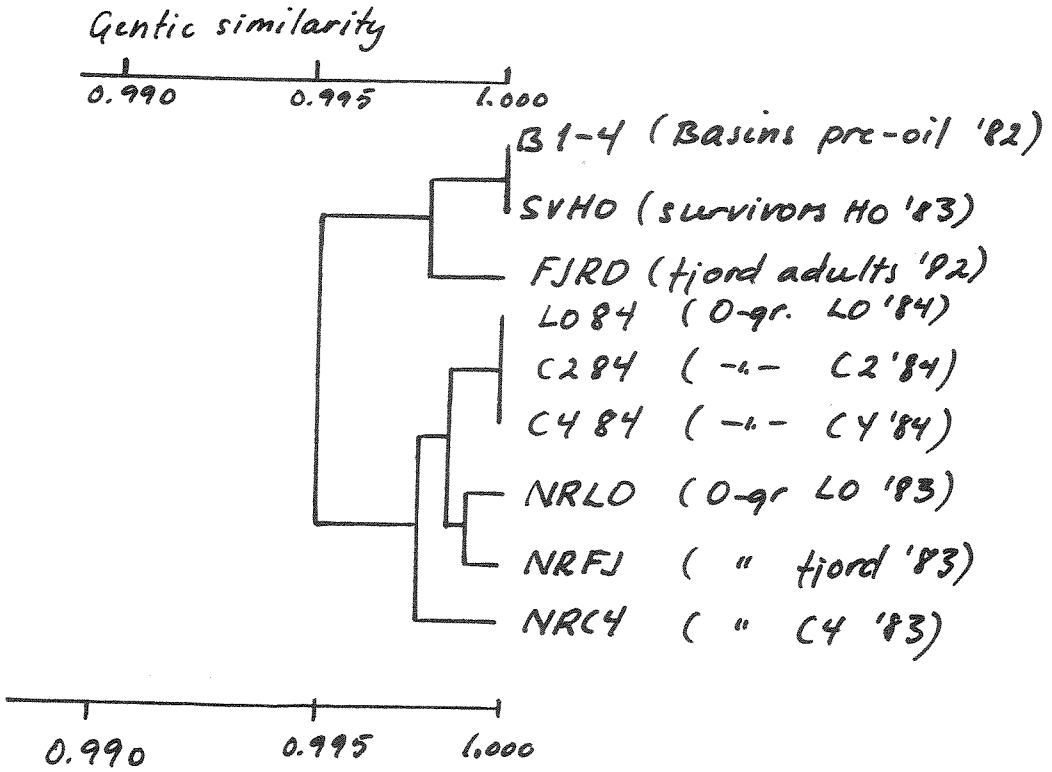


Figure 2. Cluster dendrogram (Nei's unbiased identity coefficients) for comparison of six polymorphic gene loci in Mytilus edulis from basins and fjord.

10. Genetic and demographic variation in an oil polluted population of Balanus balanoides.

Oskar Isfeld Sigurdsson.

Aim and purpose

To investigate the potential effects of low chronic oil pollution on individual growth, survival, mortality and genetic variation in a population of Balanus balanoides.

Work progress

The work has proceeded as scheduled in my Research proposal with exception of the fecundity experiment, which was excluded. In the fall of '83, 200 animals were collected at the Fjord station, just outside the Solbergstrand basins. The animals were homogenized and used to run electrophoretic analyses for 24 enzymes on six different buffer systems. There were 10 multimorphic enzymes found and these were used in the genetic analyses.

At the same time a total of 1250 "newly settled" animals were collected from the Fjord station and from all four basins at Solbergstrand. These were later run in horizontal starch gel electrophoresis.

Also in the fall of '83, 10 photographic quadrats were established on a rock-face in the intertidal zone and 40 on single rocks outside Solbergstrand. The rocks were then transplanted to the four basins. Each quadrat, 15x15cm, was marked with two expansion bolts in holes drilled in the rock. A focal framer (with a fixed ruler for scale definition) on the camera was fitted in the slots of the bolts, providing precise registration of the camera position from survey to survey. Photographs were taken with a flash. Monitoring started in December '83 and ended in September '84 with one survey every month. Individual growth will be measured as change in the opercular area, using enlargements of the negatives and a graphic table. The photographs have not yet been analysed. After the last photographic monitoring a total of 1250 animals from the "surviving" '83 generation on

the quadrats in the basins and the fjord were sampled for electrophoretic analyses.

From the "83 generation", 1200 of the "newly settled" and about 600 of the "surviving" animals from all stations have been analysed electrophoretically. Out of 19 scorable loci, 13 (ca.68%) are polymorpic. Degree of polymorphism appers to be as follows:

most polymorphic loci:	PGI and MPI
▼	GOT-1 and MDH-2
	AO-2, GOT-2, IDH-1, IDH-2 and MDH-2
least " "	AO-1, FUM-1, ME-2 and ODH-2.

These data will be further analysed using a computer program, BIOSYS-1. This program performs most types of electrophoretic data analyses commonly used in biochemical population genetics. The electrophoretic analyses will be finished by the end of this year, or early next year. The data will then be used to detect potential differences in genetic variation between populations from oil polluted and non-oil polluted basins.

11. On *Carcinus maenas*(L.) in the four rock bottom basins at Solbergstrand experimental station.

Moe, K. Follum, O. A. Lystad, E. (UiO)

Introduction

The number of predators inside the basins at S. E. S. are small. They are limited by a planktonic input from the pipelines, or by their own ability to increase the present population. One of the most conspicious predators here is the shore crab, *Carcinus maenas*, which probably influences a lot on the community structure.

Carcinus predates on young *Littorina littorea* (Hylleberg & Christensen 1978) and newly settled *Mytilus edulis* (Elner & Hughes 1978) and is probably eating or at least disturbing newly settled barnacles and other small organisms on the substrate by "scavenging". It has also been observed kicking up the sediment and eating from the "cloud" of detritus it creates by this behaviour (pers. observ.). The long menu for *Carcinus* set up by Crothers (1968) also suggests a long list of possible prey organisms.

One of the most important contributions we expect the crabs to give the basin communities is their ability to disturb or to keep the periwincle population down. Grazing by *Littorina* denudes the substrate in the littoral zone (Lubchenco 1978, Lein 1980) and also disturb newly settled organisms (Hylleberg & Christensen 1978).

We suspect an interaction taking place between *Littorina* grazing and *Carcinus* predation, and the aim of this work was to estimate the crab population in each basin for a comparison with the *Littorina* population and the community structure of benthic organisms.

Material and methods

Crabs from the four rock basins were collected four times in 1984, July, August, September and October. One of the

intentions with this project was to look at the population growth and compare these values of each basin during their active period. Naylor (1962) observed that this periode was at water temperatures abowe 10°C, which in this occasion not was reached until beginning of July after an extremely cold and windy spring.

Two persons with SCUBA equipment sampled the crabs, 30 minutes in each basin, except for October where crabs were sampled in 10 minutes intervals in each basin until there were found less than five crabs in the last interval.

The weight and the greatest carapax width were measured. and the sex was registered for each individuals before they were put back into their respective basins.

In September, it was observed that some of the crabs had burried down into the sediment and were difficult to find, particulary under the wave generator. In the last sample, a rake was used to get as many as possible up from the sediment.

Different growth of macro algae and the varying thickness of the sediment-layer made it difficult to find the crabs easily, but with similar technique at each sample, for the same period of time, we presume that the comparison not will be disturbed.

Results

Population sizes

In October, the sampling was in 10 min. intervals, until only five crabs or less were found in one interval. The results are presented in Table 1.

Table 1. Number of Carcinus sampled in 10 min. intervals in each basin in October.

Basin	10	20	30	40	50	60min.	Tot.
HO	32	12	5				49
C2	61	35	30	34	13	5	178
LO	33	16	8	5			62
C4	19	16	17	13	11	5	81

We expect these data to fit the following equation

$$Y = A - B\ t^{x},$$

where Y is the number of crabs, t is the time and A is the estimated population of crabs when x grows to infinity. The logarithmic form of this equation gives a straight line where A easily can be estimated. This A was used to calculate the Basin Indexes in Tab. 2, and further to estimate the population sizes in each basin for all samples (Fig. 1).

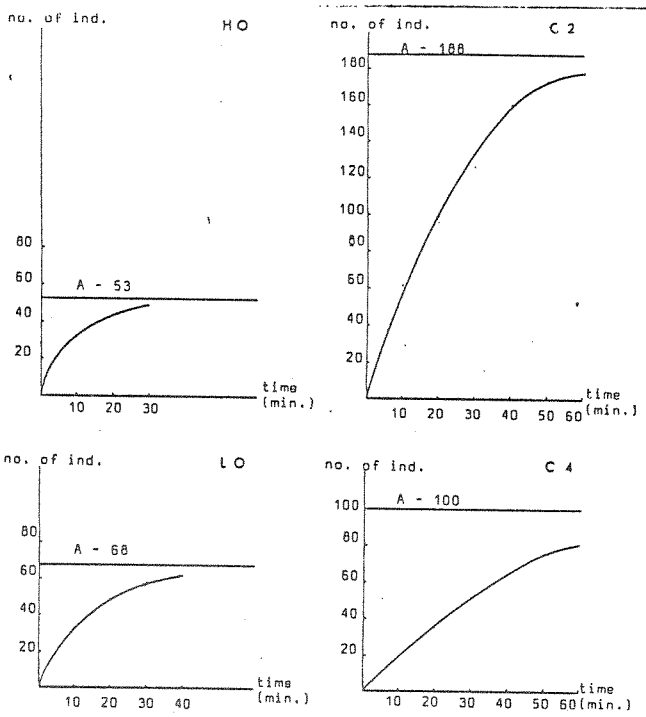


Fig. 1. Accumulated numbers of Carcinus maenas sampled in 10 minutes intervals in October(se Tab. 2.). The graphs are plotted using equation $Y = A - B\ t^{x}$, and A is the estimated total population at this time.

Table 2. The number of sampled and estimated crabs for each basin in July, August, September and October 1984. Sampling time was 30 minutes each time. The basin index was made by dividing number of crabs found after 30 min. in October by the total number of animals in each basin. This index multiplied with the number of sampled crabs gives the estimated values.

Basin		July	Aug.	Sept.	Oct.	Basin Index
HO	Sampled	26	57	59	49	1.08
	Estimate	28	62	64	53	
C2	Sampled	59	88	67	126	1.49
	Estimate	88	131	100	188	
LO	Sampled	56	51	31	57	1.19
	Estimate	67	61	37	68	
C4	Sampled	65	70	63	52	1.92
	Estimate	125	134	121	100	

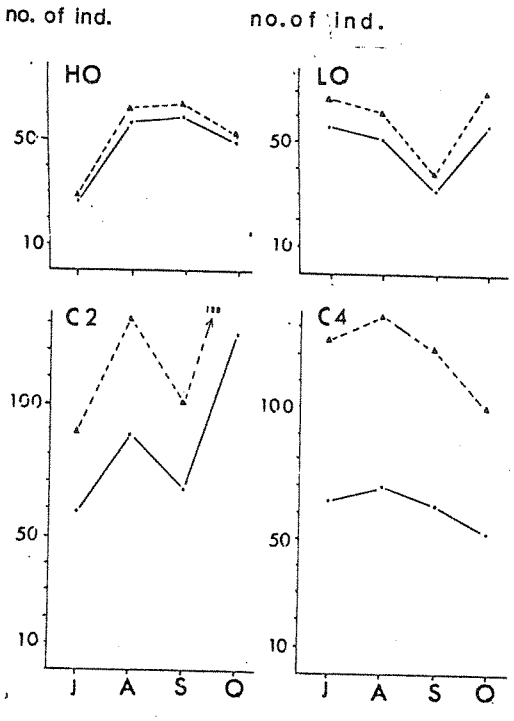


Fig. 2. Number of observed and estimated crabs for July, August, September and October in each basin. •——• : observed crabs. Δ----Δ : estimated crabs from the October data (see also Tab. .).

We expected an increase in number of crabs in each basin during the summer when the newly hatched individuals grew larger and were easier to sample. After the last moulting in autumn, according to previous work (Crothers 1968), some of the oldest generation would die and the population size would decrease. These assumptions seem to fit the data for HO and C4 (Fig. 2). The great variations of numbers found in C2 have two explanations. One is the artificial wall with possibilities for the crabs to migrate below or through to the chamber behind. The other is the very dense cover of algae, mainly Fucus serratus, at the bottom of the basin. The last statement is also a fact for the situation in C4, and explain the distance between observed and estimated values of crabs in C2 and C4. This is not the fact for HO and LO where algal canopy were sparse or absent (and the crabs are easy to sample). The very low number of crabs found in the Sept. sampling in LO is partly due to the tendency of some crabs beeing burried in the sparse sediment, and the accumulation of algae fragments in one end of the basin.

Population growth

The size frequency data for each sampling and all basins are shown in Fig. 3.

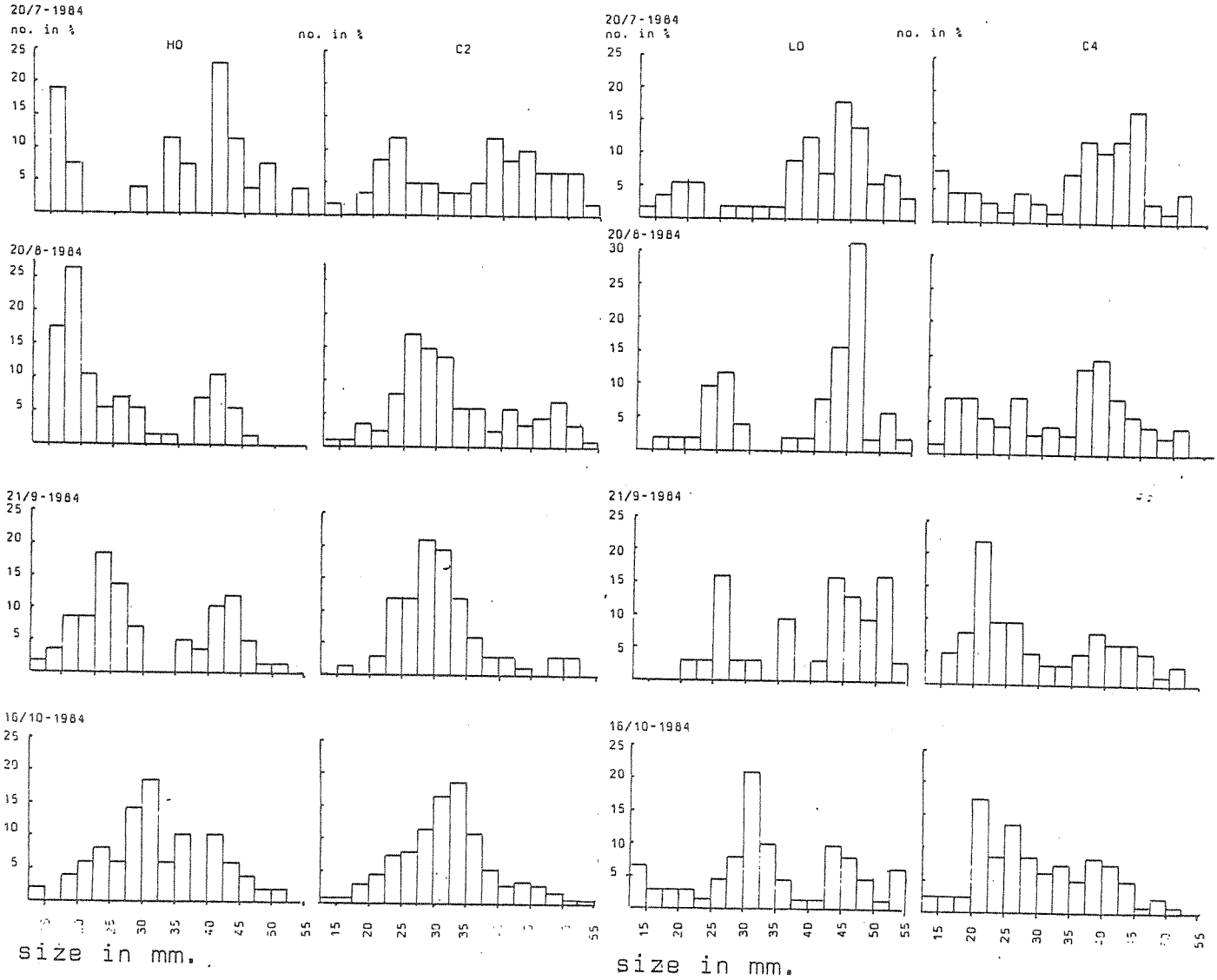


Fig. 3. Histograms of the size frequencies values of Carcinus maenas for each sampling and all basins. Total number represented by the histograms are shown in Table 2.

Completely analysis of the histograms in Fig. 3, are not yet fulfilled. Growth curves and determination of the range of each cohort will be analysed before the Tømte meeting in 1985. Analysis of the histograms based on the method using probability paper gave to many contradictions. (9 generations make a sensational record of longevity for Carcinus maenas.)

Length - Weight relation

In addition to measure the length of each crab, the corresponding weight of the animal was noted. This length-weight relation was thought to give an idea of moulting frequence and resource utilization for the crabs in each basin. The relation between length and weight can be expressed by the equation

$$1) \qquad \text{Length} = a \sqrt[3]{\text{weight}}.$$

The equation gives a curve of parabolic form, which we are allowed to, within the limits of our data, express by the equation

$$2) \qquad \text{Length} = a\ \text{weight}^{b}.$$

The adjustment of our data to this equation (2) gave F values between 500 to 2500 and R^2 values between 0.94 to 1, and gave rise to Fig. 4. (By using equation 2, calculating a and b were simplified utilizing preprogrammed software.)

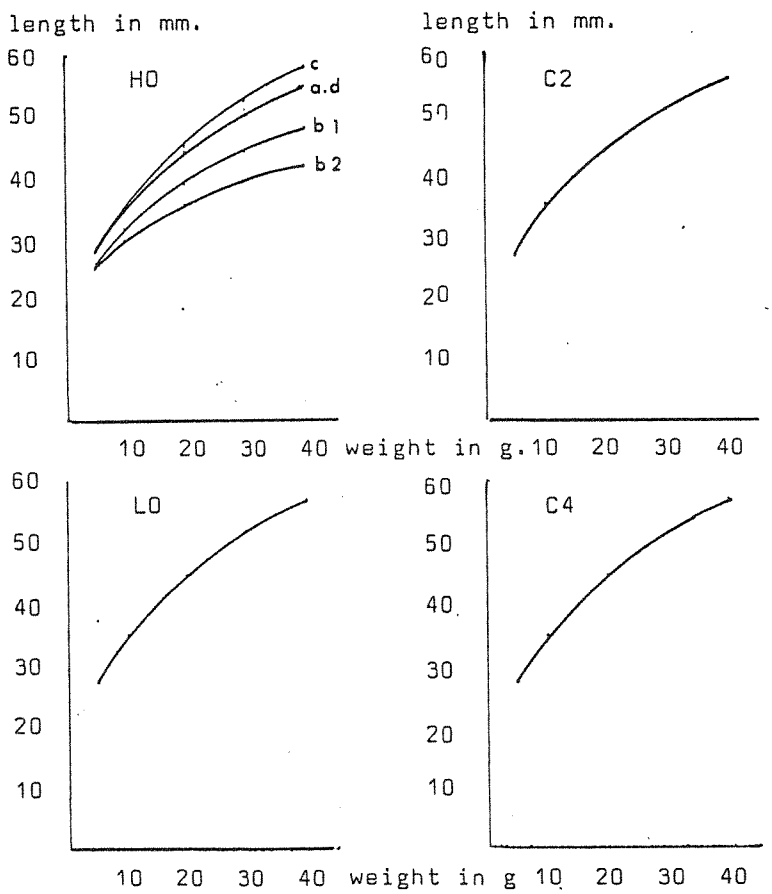


Fig. 4. The length-weight relation expressed by the equation length - a weight^{b}. The differentiation of the curves for each sex and sampling period are not visible on this scale except for HO, where a-20/7 both sexes, b1-females 20/8, b2-males 20/8, c-21/9 both sexes and d-16/10 both sexes.

There are at least three concpicuous features looking at Fig. 4.
1) The similarity of the curves in C2, LO and C4 (a of each sex and sampling period lies between 15.50 and 16.50 and the corresponding b between 0.33 and 0.36). Though there are significances, males in all basins and sampling periods are smaller and lighter than the females, this is not visible on the scale of Fig. 4.
2) The lack of crabs with large carapax width in HO 20/8, indicates a decertion of this size group at a very early stage (Fig.3). This size group is easy to sample so the reason is not lacking sampling technique.
3) The distinction between the length weight relation for the sampling periods in HO. Some crabs sampled 20/8 are heavier than for the rest of the periods (there are somewhat lower F and R^2 values for these curves stressing this deviation), and the males are heavier (and smaller) than the females (b1 and b2 in Fig. 2).

Discussion and conclusion

The sampling of Carcinus maenas in the basins at SES every month from July to Oct. gave us a broad view of the population size in each basin. Though some variation in number catched pr. sampling, we assume that our results are representative for the 1984 season. C2 has a distinct higher population size than the other basins Table 1 and 2, Fig. 1). The C4 value are somewhat higher than LO, and LO's population size is higher than in HO (Table 1 and 2, Fig. 1). The population size of Carcinus maenas seen in association with the population size of Littorina littorea and their predator-prey relations (Lystad and Moe in prep. and Muntz et al. 1965), stress these results. The predation by Carcinus maenas seem to be able to keep the population and recruitment of C2-Littorinas down (C2 has a significant lower population size than the other basins). This situation seems to be more turbid in C4. The condition for Littorina recruits in C4 are very good (the important surface of stair A are very rough and give good shelter), so even a high number of crabs cannot keep the population size down. In HO and LO it is hard to tell either oil contamination or sparse of food keep the population of Carcinus maenas down.
Any conclusion of the population growth in each basin is hard to draw with lacking growth curves. One thing is to be said about the histograms of HO (Fig. 3). The F_0 generation seems to made a "jump" between 21/9 and 16/10. The end of oil dozing

was 27/9 and there may be some connection. The greater part of big crabs in LO may also have an effect on the predator-prey relations. One feature interfere the analysis of growth using probability paper. Crothers (1967) state that Carcinus maenas usually copulate during the last moulting in Aug. and spawn in early spring. Unfortunately, if conditions are good, the shore crab can be spawning also in autumn. This might be the reason for the contradictions in year classes.

The moulting of Carcinus maenas is determined by temperature, PH, Ca^{2+} ions and food resources (Crothers 1967). During this period water is used to increase the pressure on the old integument (taken mainly up by osmoregulation). In addition to increase the pressure, the water are filling up the new space without tissue during the hardening process of the new integument. Tissue will later continously substitute the water.

The volume of water is usually 60% of the volume of the crab. During moulting (the ecdysis part), the water content can rise to 80%. The ecdysis is normally very rapid, with a duration of 4-5 days, but under unnormal conditions, it may last for 16 days (Crothers 1967). Among those factors determining the moulting process of Carcinus maenas at SES, two of them are not common for all basins. The food resources and the oilcontamination.

Our conclusion on the detected distinction in the length-weight relations in HO (Fig. 4) is that the moulting of the shore crab are not synchronized with the populations in the other basins. Substituting the water content during ecdysis, take so long time, in HO, that we are able to detect this weight increase by our monthly sampling. The reason for this delay is assumed to be food shortage (it seems to be hard to survive in HO for young Littorinas) in addition to the lacking ability to utilize the food in oil contaminated environments (as for Littorinas in HO (Bakke 1984)).

LITERATURE

Crothers, J. H. (1967) The biology of the Shore Crab, Carcinus maenas (L.). 1.The background - anatomy, growth and life history. Field studies Vol. 2, no. 4.

Crothers, J. H. (1967) The biology of the Shore Crab, Carcinus maenas (L.). 2. The life of the adult crab. Field studies Vol. 2, no. 5.

Elner, R. W. & Hughes, R. N. (1978) Energy maximixation in the diet of the Shore Crab, Carcinus maenas (L.) J.Anim. Ecol. 47:103-116.

Hylleberg, J. & Christensen, T. (1978) Factors affecting the intraspecific competition and distribution of Littorina littorea. Natura jutlandica 20: 193-203.

Lein, T. E. (1980) The effects of Littorina littorea, grazing on littoral green algae in the inner Oslofjord, Norway. Sarsia 65: 87-92.

Lystad, E. & Moe, K. (1985) Thesis in preparation

Muntz, L., Ebling, F. J. & Kitching, J. A. (1965) The Ecology of Lough Ine. XIV. Predatory activity of large crabs. J.Anim.Ecol. Vol 34: 315-329.

Naylor, E. (1962) Saesonal changes ina a population of Carcinus maenas (L.) in the littoral zone. J.Anim.Ecol 31: 601-609.

12. SUBLETHAL BIOLOGICAL EFFECTS AND RECOVERY OF MUSSELS (MYTILUS EDULIS) FOLLOWING CHRONIC EXPOSURE TO PETROLEUM HYDROCARBONS: PHYSIOLOGICAL RESPONSES.

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INTRODUCTION.

Previous laboratory studies at IMER have shown that physiological and cellular processes of Mytilus edulis are affected by chronic exposure to low and environmentally realistic concentrations of the water-accommodated fraction of North Sea crude oil (Widdows et al. 1982; Bayne et al. 1982). The main objectives of this project at the Solbergstrand experimental station were:
a) to investigate the sublethal physiological responses of Mytilus edulis following chronic exposure to two concentrations of petroleum hydrocarbons, and
b) to study the recovery of Mytilus edulis both in terms of hydrocarbon contamination of the body tissues and the toxic effects of petroleum hydrocarbons.

In May 1983 the effects of oil exposure and the short-term (10 days) recovery of Mytilus edulis were studied and the results presented in Progress Report No 4 of the Littoral Rock Community Project.

In May 1984 the chronic effects of oil exposure and the long-term (53 days) recovery of physiological responses and tissue hydrocarbon concentrations were determined at intervals in order to establish whether there was a simple relationship between the rate of recovery of physiological responses and the rate of depuration of hydrocarbons from the various body tissues of mussels.

MATERIAL AND METHODS

During the period of tissue sampling and physiological measurement in May 1984 the seawater temperature in the Solbergstrand experimental basins was 10°C, salinities ranged from 22.5 to 27‰ and suspended particulate load was 1.12mg l^{-1} (± 0.15 S.E.) of which 38% was particulate organic matter (POM).

Physiological responses, such as rates of feeding, food absorption efficiency, respiration and excretion, were determined using procedures previously described (Widdows et al, 1982). Feeding rate was measured in terms of clearance rate, or the volume of water cleared of particles per hour, in a flow through system. Food absorption efficiency was calculated by the method of Conover, which compares the proportion of organic matter in the food and faeces. Respiration rates were measured in closed respirometers by recording the decline in oxygen concentration with a Radiometer oxygen probe and amplifier linked to a chart recorder. Excretion rates were determined by placing individuals in 200ml of 0.4µm membrane filtered seawater for 3 hours and analysing the ammonia concentration in the water by the phenol-hypochlorite method.

The energy budget of an animal represents an integration of the basic physiological responses such as feeding, food absorption, respiration, excretion and production. Each component can be

converted into energy equivalents (Joules h^{-1}) and the energy available for growth and production gametes, termed the 'scope for growth', is estimated from the difference between the energy absorbed and the energy expenditure via respiration and excretion.

All physiological responses were corrected to a 'standard size' of animal (0.5g), which approximates to the mean body weight, using appropriate weight exponents.

Mussels were collected from Solbergstrand, caged and exposed to high (basin 1) and low (basin 3) oil concentrations in August 1983. After ~7 months exposure, 15 individuals from each basin (high oil and low oil) were transferred to basin 4 (control) on 22nd March and 24th April 1984. In May 1984 physiological measurements were performed on hydrocarbon exposed mussels from basin 1 and 3, mussels previously exposed to high and low oil conditions and maintained for 22 days and 55 days in basins 4 (control), and control mussels from basin 4.

Following measurement of physiological responses, the mussels were dissected into three tissues (digestive gland, gill, remaining tissue), weighed and stored at -30°C prior to the extraction of hydrocarbons by steam distillation and analysis by HPLC. In addition, the shell length and weight of the two main storage tissues (digestive gland and mantle) were determined.

RESULTS AND DISCUSSION

A. Effects of Chronic Oil exposure.

The effects of 9 months chronic exposure to low (~30µg l^{-1}) and high (~130µg l^{-1}) oil concentrations on the physiological responses of *Mytilus edulis* are illustrated in Figs. 1 - 4 (day 0).

There was a marked reduction ($P<0.001$) in the suspension-feeding (=clearance rate) of *Mytilus edulis* in response to hydrocarbon exposure (Fig. 1). Mussels exposed to low oil had a clearance rate 67% of the control and those exposed to high oil had a clearance rate 34% of the control group. This reduction was similar to that recorded in May 1983, when clearance rate was 51% and 42% of the control, for low and high oil conditions respectively. The food absorption efficiency of mussels exposed to high oil (33% $\pm$ 6) was lower than the absorption efficiencies recorded for mussels from the control, low oil and both the high and low oil recovery groups, which had a mean of 45% $\pm$ 1.5.

There were no significant differences between the mean rate of oxygen consumption by mussels from the oil exposed and control conditions, but there was an increase in the variance in the 'high oil' group.

The rate of ammonia excretion by mussels exposed to 'low oil' was low (4.0µg NH_4-N h^{-1})and similar to the control group. In contrast, the 'high oil' mussels had a very high rate of ammonia excretion (19µg NH_4-N h^{-1}) and this was reflected in a low ratio of oxygen consumed to nitrogen excreted (O:N = 18) which is indicative of a severe stress condition involving a high protein catabolism relative to lipid and carbohydrate.

The conversion of feeding, digestion, respiration and excretion to energy equivalants allows the construction of an energy budget and the calculation of scope for growth from the difference between the energy gains from feeding and digestive processes and the energy expenditure and loss through respiration and excretion. The scope for growth of high oil exposed mussels was negative, indicating utilization of body reserves for maintenance and survival. This is confirmed by the lower tissue weight, the relatively smaller size of storage tissues such as digestive gland and mantle (Table 1) and the lower O:N ratio indicative of a relatively high protein catabolism. The mussels exposed to low oil had a significantly reduced scope for growth but this was positive and therefore did not reflect a severe stress condition. Consequently the effect of oil exposure on tissue weight and relative size of digestive gland and mantle (Table 1) was less in this low oil group.

B. Recovery from Oil Exposure.

Recovery experiments carried out in the previous year (May 1983) had shown that a) recovery of scope for growth was only partially completed over a period of 10 days in 'clean' water (control-basin 4), and b) mussels recovered from 'high oil' exposure faster than those exposed to low oil.

The longer-term recovery of physiological responses such as feeding rate, respiration, ammonia excretion and scope for growth are presented in Figs. 1 - 4.

There is evidence of a slight overshoot in the clearance rate of mussels from the 'high oil' group after 22 days of recovery. (Fig. 1). This confirms the previous findings which showed a rapid recovery by the 'high oil' group and a slight overshoot in clearance rate after 5 and 10 days.

However, the 'low oil' group of mussels recovered more slowly and their clearance rates were significantly lower than the controls after 5 and 10 days but were similar after 22 days recovery. After 55 days there was no significant difference between the clearance rates of the controls and both 'oil recovery' groups.

Absorption efficiencies recovered and were similar to the control group after 22 days. In contrast to the clearance rate, there was an overshoot in the rate of oxygen consumption by mussels after 22 and 55 days of recovery from exposure to low oil, but there was no apparent overshoot in the respiration rate of 'high oil'

recovered mussels (Fig. 2). This overshoot in the metabolic rate appears to be related to an enhanced reproductive activity during recovery from stress induced by low oil exposure (see report by D. Lowe). After 53 days recovery there was a higher proportion of developing and ripe gametes in the mantles of the 'low oil' group and this would increase their weight-specific metabolic rate.

The rate of ammonia excretion by mussels during recovery from low oil exposure was significantly higher than the controls and the low oil exposed mussels. After 22 days the increase was 5-fold and after 55 days declined to a rate 2-fold higher than controls. This increase in ammonia excretion reflected the overall increase in metabolic activity in this group and the O:N ratio of 19 after 22 days was indicative of the utilization (catabolism) of protein for maintenance and the production of gametes. After 55 days the O:N ratio increased to 58 and this suggests a more 'balanced catabolism' of substrates. The high rate of ammonia excretion and the low O:N ratio ($\sim$20) recorded for the 'high oil' exposed mussels was maintained during 22 days of recovery, but after 55 days the rate of ammonia excretion was reduced and the O:N increased to 50.

The overall performance and recovery of oil exposed mussels is integrated by means of the energy budget and expressed in terms of scope for growth. Fig. 4 illustrates the difference between the rate of recovery of low and high oil exposed mussels. Recovery from low oil exposure was gradual and took approximately one to two months, whereas recovery from high oil exposure was more rapid and showed a slight 'overshoot' phenomenon. This 'overshoot' has been termed 'catch-up' growth in previous studies of mammalian recovery from stress and disease. The observed 'catch-up' growth in mussels may be explained in terms of weight-specific processes and morphometrics, the relative sizes of organs and tissues, after a period of stress. During exposure to high oil concentrations, mussels were severely stressed and body reserves were utilized resulting in de-growth. However, the gill area and therefore the size of the ciliary pump would be maintained and consequently the weight specific the weight-specific pumping (= clearance) rate would increase. For example, during early recovery ($\leq$22 days) from high oil exposure, the weight-specific clearance rate was higher ((4.35 l h^{-1} for a standard body size of 0.5g) than the rate for control mussels (3.75 l h^{-1}), whereas when expressed as litres per animal per hour the clearance rate was low (4.48 l h^{-1}) compared to the control group (4.80 l h^{-1}). However, the metabolic rate, which is more directly proportional to body mass, showed little change in weight-specific rate during the recovery period. In the later phase of recovery (22 to 55 days) the weight-specific clearance rate declined (Fig. 1) as body tissues were built-up (Table 1) and the size of the gills relative to other body tissues returned to the 'normal' morphometric condition.

C) Analysis of body tissues for hydrocarbons.

The tissue hydrocarbon concentrations (Table 2) show that the control mussels had a measurable 'background' level of hydrocarbon contamination. Mussels exposed to the low oil concentration accumulated 17µg of 2-ringed aromatic hydrocarbons per g wet tissue mass and those exposed to the high oil concentration accumulated 28µg g^{-1} wet mass. There was a negative correlation between tissue hydrocarbon concentration (the dose) and scope for growth (the response).

After 5 days in clean water (basin 4) there was little depuration of hydrocarbons by the mussels from both oil exposed conditions, but after 22 days recovery the high oil exposed group had a lower tissue concentration (1.7µg g^{-1} wet mass) than the low oil recovery group (3.43µg g^{-1} wet mass). The rapid decline in tissue hydrocarbon concentration in the 'high oil' recovery group was concomitant with the rapid recovery in terms of the physiological responses such as clearance rate and scope for growth. The marked decline in tissue concentration between 5d and 22 days was probably a combination of a high rate of hydrocarbon depuration and a 'dilution effect' caused by tissue growth and 'catch-up' growth associated with the recovery of this 'high oil' group. After 55 days the tissue hydrocarbon concentrations were relatively low in both 'recovery' groups and the scope for growth values were high and not significantly different from the controls.

CONCLUSIONS

1) There was a significant dose-dependent reduction in the feeding rate and scope for growth of Mytilus edulis exposed to the two oil concentrations (30 and 130µg l^{-1}). The decline in these physiological responses was inversely related to the concentration of hydrocarbons in the tissues.

2) During a period of 10 days in clean water the physiological responses of Mytilus edulis only partially recovered. The lack of an immediate recovery suggests that the stress responses were not directly related to the hydrocarbon concentrations in the water but appear to be a result of tissue hydrocarbon concentrations.

3) After 22 days recovery there was a marked reduction in the tissue hydrocarbon concentration of the high oil group and this was concomitant with a rapid increase in scope for growth. There was evidence of a slight overshoot in scope for growth which may give rise to 'catch-up' growth. The rapid decline in tissue hydrocarbon concentration within 22 days was probably the combination of a high depuration rate and a 'dilution effect' due to tissue growth.

4) Recovery of low oil exposed mussels was slower both in terms of tissue dupuration and physiological performance. Recovery of both high and low oil groups was complete after ~55 days.

Table 1. Effect of oil exposure on body mass and the relative size of storage tissues. (Mean ± S.E. ; n=15)

Condition	Shell length (cm)	Dry mass (g)	% Digestive Gland	% Mantle
Control	4.84 ±.11	0.811 ±.050	12.11 ±.53	16.09 ±1.38
Low Oil Exposed	4.68 ±.113	0.767 ±.056	10.77 ±.71	14.82 ±.91
High Oil Exposed	4.49 ±.29	0.499 ±.128	9.67 ±.45	14.5 ±1.24
Low Oil Recovery (22d)	4.56 ±.19	0.714 ±.122	-	-
High Oil Recovery (22d)	4.28 ±.19	0.565 ±.055	-	-
Low Oil Recovery (55d)	5.28 ±.134	1.019 ±.081	-	-
High Oil Recovery (55d)	5.14 ±.28	0.899 ±.090	-	-

Table 2. Concentration of hydrocarbons in the Body Tissues of Mytilus edulis following exposure to 30 µg l^{-1} (low oil) and 130 µg l^{-1} (high oil) and 22 days and 55 days recovery (May 1984).

Sampling time and Condition		Concentration of 2-1 ringed aromatic hydrocarbons ug g^{-1} wet weight of tissue with gills and digestive gland removed. (2,3-dimethylnaphthalene equivalents).
Time '0'	Control	0.34 ± 0.01
	Low Oil	16.94 ± 1.48
	High Oil	27.70 ± 2.78
5 days Recovery	Low Oil	10.33 ± 0.03
	High Oil	33.13 ± 1.06
22 days Recovery	Low Oil	3.43 ± 0.77
	High Oil	1.71 ± 0.03
55 days Recovery	Low Oil	1.04 ± 0.10
	High Oil	2.27 ± 0.38

Mean ± range (n=2 pooled samples of 10 to 14 mussels). 1 µg 2,3dimethylnaphthalene ≡ 7.66 µg aromatic hydrocarbons.

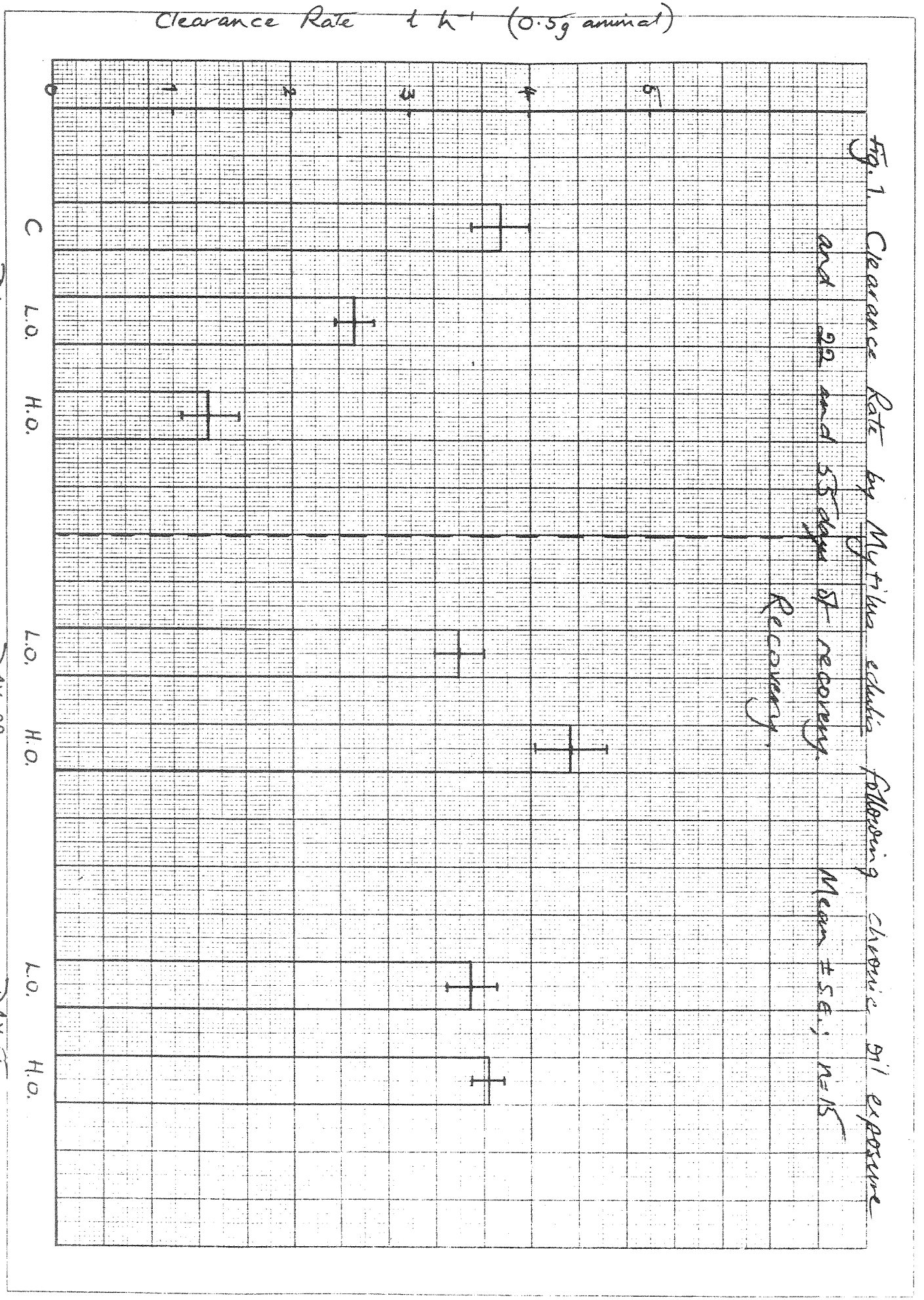

Fig. 1. Clearance Rate by Mytilus edulis following chronic 91 exposure and 22 and 55 days of recovery. Mean ± S.E.; N=15

$\dot{V}O_2$ $ml\,O_2\,h^{-1}$ (0.5g animal).

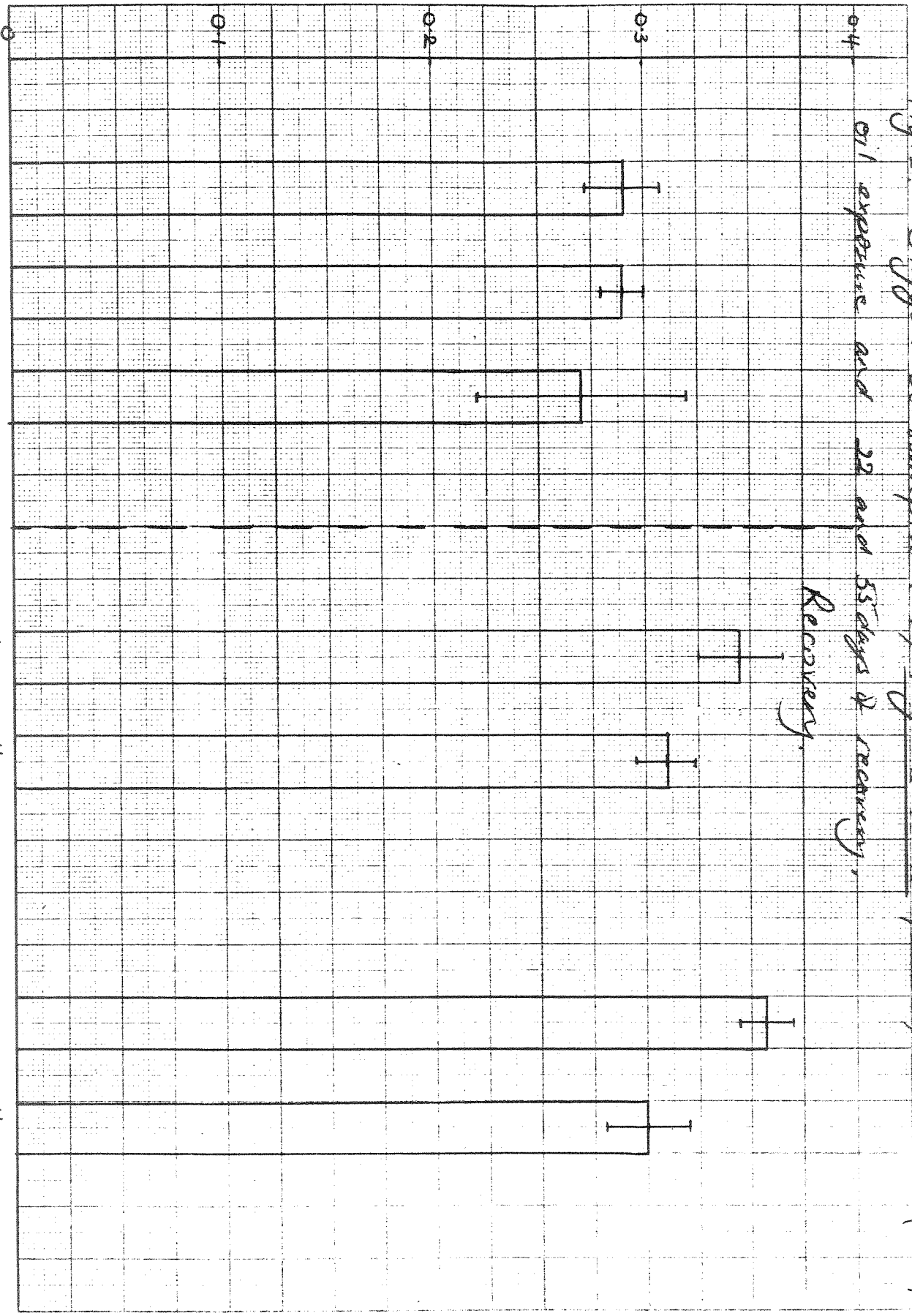

Fig. 2. Oxygen consumption by *Mytilus edulis* following chronic exposure to acid (pH 5.2) and 55 days of recovery.

MAY 1984

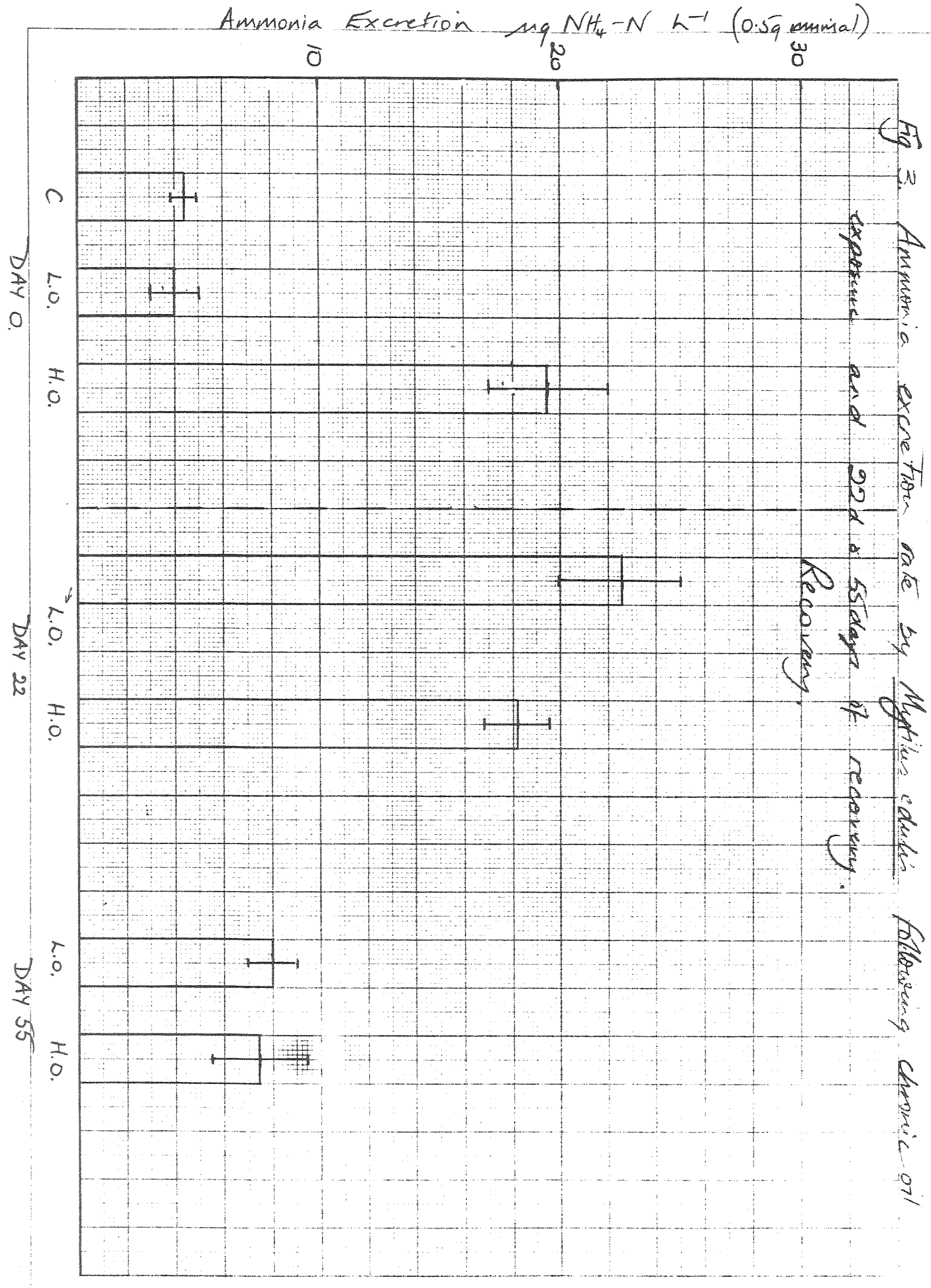

Fig 3. Ammonia excretion rate by Mytilus edulis following chronic oil exposure and 22 days 55 days of recovery. Recovery

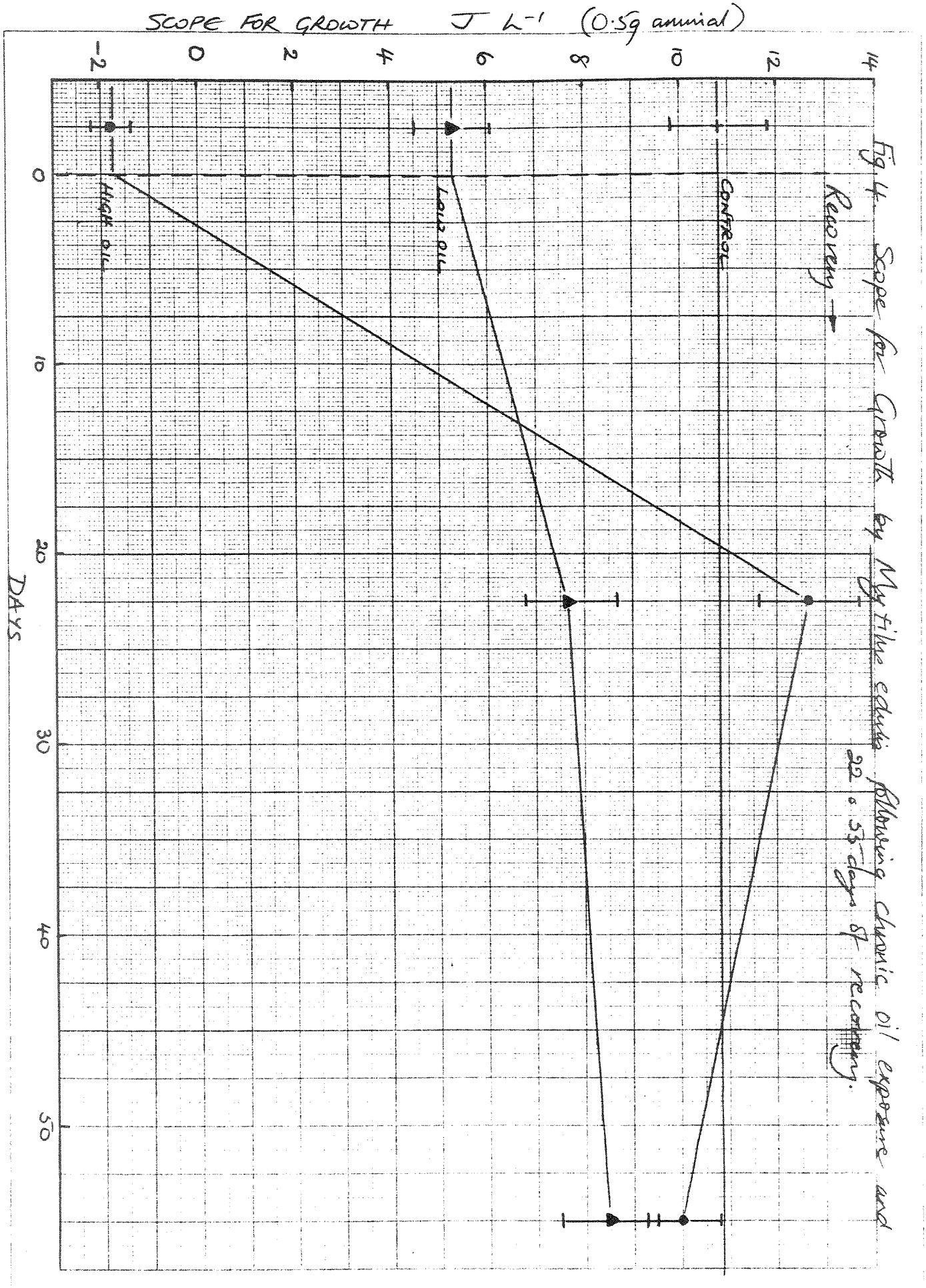

Fig. 4. Scope for Growth by Mytilus edulis following chronic oil exposure and 22 & 53 days of recovery.

13. *ENERGY BALANCE IN LITTORINA LITTOREA*

Torgeir Bakke
NIVA

Purpose

To investigate the energy uptake and loss of the four basin populations of Littorina littorea and one shore population near MRS at two months intervals during the dosing period and, after that, until complete redcovery.

To investigate if oil has a long term effect on any of the main pathways of energy conversion in the individual such as food uptake, assimilation, respiration or excretion, and whether there is a seasonal change in the sensitivity of any of these processes towards the oil.

To link the individual energy budget to the measurements of individual growth and mortality (Lystad & Moe), to the effects of grazing on the substrate microlayer (Bokn, Pedersen) and to biochemical or cytochemical stress indices in Littorina (Moore, Lowe, Livingstone).

To link effects on energy utilization in Littorina to tissue levels of oil hydrocarbons.

Methods

The investigation is in principle based on separate measurements on 10 marked individuals of average size from each population on each occasion. The same individuals are used for all the following measurements in order to link the elements of energy conversion in each individual.

Food uptake

Estimate of food uptake has been based on the green alga Ulva lactuca as food source. This species is ranked high as preferred food for L. littorea (Lubchenco 1978), and is common in all the basins. Prior to the feeding experiment the animals were kept in cages with Ulva as the only food for at least 3 days. Individual feeding rate was estimated

by incubating the snails singly with a prewighed piece of Ulva (about 70 mg ww) in 70ml, gauze-covered vessels which were placed for free water exchange in the appropriate basin. Feeding time was 1-3 days dependent on feeding rate (i.e., until a high percentage of the food was consumed) after which the remaining Ulva was reweighed. For each set of 10 individuals tested, 5 vessels were prepared with only Ulva to adjust for growth of food. The control pieces of Ulva were afterwards used to determine wet weight dry weight ratio and organic content of the food (from loss on ignition). Food consumption was calculated on basis of loss of Ulva wet weight, adjusted for mean growth of Ulva during feeding and converted to energy uptake (Joules/day.ind).

Repeated weighing of Ulva pieces after quick blotting of excess water gave a coefficient of variation of 1.81% of the wet weight mean. This level of precision was considered adequate.

Faecal production and assimilation efficiency

One group of 50 individuals from each poulation was kept caged with Ulva as food for at least 3 days prior to the measurements. The faecal material produced from each group was collected quantitatively after the group had been incubated in GF/C filtered sea water of appropriate temperature for 2-4 hours. The faecal material was filtered onto GF/C filters for drying and analysis of total dry weight and organic content (loss by ignition). Assimilation efficiency was calculated by the short-cut expression of Conover (1966) based on the organic fraction of food and faeces. Faeces recovery was also done occasionally from the feeding and ammonia excretion experiments, but the assimilation efficiency estimates are based on the main faeces sampling.

Respiration

Individual aquatic oxygen consumption was estimated in closed chambers (60ml) supplied with a magnetic stirrer and an YSI mod. 5331 oxygen probe, connected to a pen recorder. The chamber was inmmersed in a water bath of the appropriate temperature. Each animal was kept in the chamber for a time long enough to give a reliable linear recorder track, usually 20-40 minutes. Oxygen consumption was estimated from the slope of the reading and converted to Joules/day of a standard size individual.

Excretion

Individual ammonia excretion rates were estimated by incubation for 2 hours in beakers with 70ml GF/C filtered sea water immersed in a water bath. Two control beakers with no animals were always included. The water samples were afterwards preserved with 8N sulfuric acid prior to analysis at NIVA. Analysis of the NH_4^+ concentration was performed ater the Solorzano method (Koroleff 1970). Excretion rate calculations were based on differences between test and control concentrations of ammonia and converted to Joules/day of a standard size individual.

Individual size

Shell length was estimated to the nearest 0.1mm. All the wild population animals were at the end of the measurements sacrificed for dry weight (48 hours at 70 C) and ash content (2 hours at 450 C). Occasionally the basin animals were sacrificed for the same purpose, but usually length/weight relationship was obtained from equations developed in the Littorina population study (Lystad & Moe). Tissue weights were obtained with operculum included.

All the energy conversion elements were calculated on the individual basis (except assimilation efficiency) and converted to a standard individual of 21.9mm length (mean of all basins) and 180.35mg dry tissue weight.

The relation between shell length and individual volume was developed once for use in obtaining net chamber water volume when calculating respiration rate.

Scope for growth (SFG)

The estimated individual Joules/day values of feeding rate, respiration, and excretion, and the population value of assimilation efficiency were used to calculate the potential energy available for somatic and reproductive growth at one point in time (SFG) by the expression:

((food consumed)x(assimilation efficiency))-(respiration+excretion)

The individual SFG values were calculated as Joules/hour adjusted to a standard size individual.

Statistical tests

Differences between mean population values at corresponding dates have been tested for significance by use of one way analysis of variance and by the non-parametric Mann-Whitney U test.

Results

The individuals used in the measurements were picked at random within a median size interval from the following populations:

HO: The winkles in the high oil basin
LO: The winkles in the low oil basin
C2: The winkles in the control basin between HO and LO
C4: The winkles in the other control basin
W: The winkles on the shore outside of the stone pier north of the pump house.

Energy measurements have been performed during the following periods:

10-30 April	7°C
7-15 June	14-16°C
9-16 August	19-20°C
9-16 October	11-12°C

Feeding rates

The seasonal variation of feeding in the wild population indicated an increase from April to maximum in June, followed by a gradual decrease till October (Figure 1). The basin populations more or less followed the same trend, but C4 and in particular HO showed an increase from August to October. In HO this increase was significant (1%) and could represent short term recovery from a very bad energy condition in August.

In April the lowest feeding was found in the two oiled populations. The C2 animals consumed slightly more Ulva (not significantly higher than HO). The C4 and W populations consumed significantly higher amounts of food than C2 and the two oiled populations.

In June feeding rates were generally higher than in April. The oiled populations showed equal feeding intensity, the C2 populations somewhat higher (not significant), and the C4 and W populations significantly higher than both the oiled (1% level) and the C2 (5% level) populations.

In August feeding was slower, but the trend was the same as in June. The C2 population had increased its consumption relative to the other populations (significantly higher than HO and LO and not significantly different from C4 and W).

In October, two weeks after the end of dosing, the HO consumption had increased to the same level as the wild population and higher than the C2. LO had the lowest feeding intensity and C4 had the highest (significantly higher than LO and C2).

Although the feeding rate estimates seem adequate to show relative differences in feeding intensity, it is difficult to judge how well they reflect natural feeding rate. In the experiments the food surface constituted about 10% of the total crawling surface, whereas in the basins one must expect that the snails crawl over potential food most of the time. If the snails have no preference for the Ulva surface in crawling, the feeding estimates should be adjusted one order of magnitude up. If they stayed on the Ulva pieces most of the time, the estimates can be considered reasonably accurate. These matters should be looked into more closely in follow-up experiments, but for the consideration of differences between populations and with time such information is not imperative.

The results could indicate a negative relation between feeding intensity, population size,and possibly individual size, but a discussion on this should be done in view of the population size estimates (Bokn & Moy) and population structure characteristics (Lystad & Moe).

Assimilation

Assimilation efficiency was generally high when the snails fed on Ulva (Figure 2), especially in April (above 80% in all populations). The general trend was a somewhat lower efficiency during summer followed by an increase between August and October, i.e., an inverse relation to the feeding rates. The lowest efficiencies were found in the oiled populations during dosing, but complete recovery seemed to have occurred at least in HO by October.

The high efficiencies found with pure Ulva could be considered overestimates of the efficiencies of snails grazing naturally on a mixed surface cover film. Yet, it has been reported from choice experiments that L. littorea is very food selective (Lubchenco 1978,

K. Jansson pers.inf.) The real food consumed is therefore assumed to consist of pure algal material even when grazing on a surface, and should therefore be not too different from Ulva in organic content.

The assimilation efficiencies strengthened the difference between oiled and controlled populations when used to calculate the amounts of energy really assimilated by the animals during dosing. Also the impression of HO recovery in October was strengthened by looking at energy assimilated.

Production of faeces

Generally faecal production ranged between 5 and 50 mg dw/day.ind. Although there seemed to be a slight positive correlation between amounts of faeces produced and assimilation efficiency, the regression between these was not significant.

Respiration

The general seasonal trend in oxygen consumption (Figure 3) showed values around 100-150 $\mu l O_2$/h per individual equivalent to a loss in energy of 50-70 Joules/day during April, an increase to a maximum of about 290 $\mu l O_2$/h (135 Joules/day) in June in the oiled populations and a subsequent decline to the April levels in October. The wild population had the lowest respiratory loss throughout the summer (significantly below the other populations). Only in June did the oiled populations show higher respiratory loss than the controls, and then only significant with respect to the W population.

Ammonia excretion

The loss of ammonia was largest in April (Figure 4) and to some extent most conspicuous in the LO population (significantly higher than all the other populations) with a mean excretion of 13.2 µg N/h (7.9 Joules/day). The excretion decreased from April to June and kept steady after that. Genereally the excretion of HO and LO was within the control range in June - October, the one exception being HO having significantly higher excretion than the others in August. Thus, one can state that ammonia excretion, indicative of protein catabolism, was highest at the onset of the feeding season in April, and that only during August was there any indication of elevated excretion (less feeding) at oil exposure.

Scope for growth (SFG)

A preliminar regression analysis of the SFG as function of the scope elements: assimilated energy, respiration, and excretion showed that the equation

SFG=0.0362(feed.rate)-0.0455(respiration)+0.0631(excretion)-0.12

explained 99.7% of the scope variation. Feeding rate accounted for 98.7% of the total SS (sum of squares) of the regression. Therefore the SFG levels and seasonal variation primarily reflected the variation in feeding rates (Figure 4 as compared to Figure 1). In addition, assimilation efficiency, respiration and excretion of the oiled populations generally shifted the SFG towards more adverse conditions than in the control populations up to and including August.

Thus the mean SFG for the HO and LO populations was significantly lower than that of C4 and W till the end of dosing, and also generally lower than C2 (significant in April and August, not in June). In October the HO SFG was level with the controls, the LO still significantly below.

The difference in SFG among the control populations was also clear with C2 always significantly below one or both of C4 and W.

Conclusions

The physiology measurements reported here have shown that during the last 6 months of oil dosage the energy conditions in the two oiled populations were clearly less favourable than in all control populations. Although not tested statistically the HO population showed a gradual decrease in scope for growth with time, the LO population showed a slight increase. There was also a considerable variation among the control populations with C2 being most similar to the oiled and W being least similar.

The dominating element in the scope for growth estimates was feeding rate. Since the estimated feeding rates also are considered to be on the lower side of the real feeding rates, it seems clear that all factors influencing feeding will have serious implications for the amount of energy available for growth. The main factors which seemed to affect feeding were oil dosing, population size and individual size but consideration of the relative importance of the latter two has not yet been made.

Respiration was less affected by oil than feeding, and seemed only to respond to the dosing in June. Here the main difference was found between the wild population and all basin populations indicating a basin effect of respiration enhancement.

Ammonia excretion seemed not to be affected by oil with the possible exception of the spring situation when elevated excretion was found in the HO, LO and C4 populations indicating high protein catabolism just at the start of the main feeding season. Although not conclusive the oxygen to nitrogen mole ratio also showed the smallest values in April in all populations indicating the most intensive utilization of protein reserves.

The last period of measurements was made 14 days after oil dosing had terminated and should therefore cover a possible initial recovery. The measurements showed that HO feeding rates went up significantly from August to October, with a corresponding increase in the scope for growth, whereas feeding in the other populations either decreased (C2, W) or stayed the same (LO, C4). Respiration and excretion did not change to any degree that could be linked to oil recovery.

The reason why the HO recovery in feeding was not reflected in the LO population too, is at the moment uncertain. The fast HO recovery may have been a compensation for more adverse energy conditions than in LO before dosing stopped, as found in August, or an overshoot (hormesis) after the high exposure stopped. It may also be that the adverse energy conditions in LO during summer was caused by the combined effects of oil and low population size (possibly also large individual size) and that the low energy level in October reflected the latter. Evidence for this could be the fact that also the C2 population showed feeding rates in October equivalent to those of the LO. The structure of these two populations seems very similar with few, large individuals compared to the other populations.

References

Conover, R.J., 1966. Assimilation of organic matter by zooplankton. Limnol. Oceanogr. 11, 338-345.

Lubchenco, J., 1978. Plant species diversity in a nmarine intertidal community: importance of herbivore food preference and algal competitive abilities. Amer. Natur. 112, 23-39.

Koroleff, F., 1970. In: Information on techniques and methods for sea water analysis. Charlottenlund Interlaboratory Report No 3, 1970, 19-22.

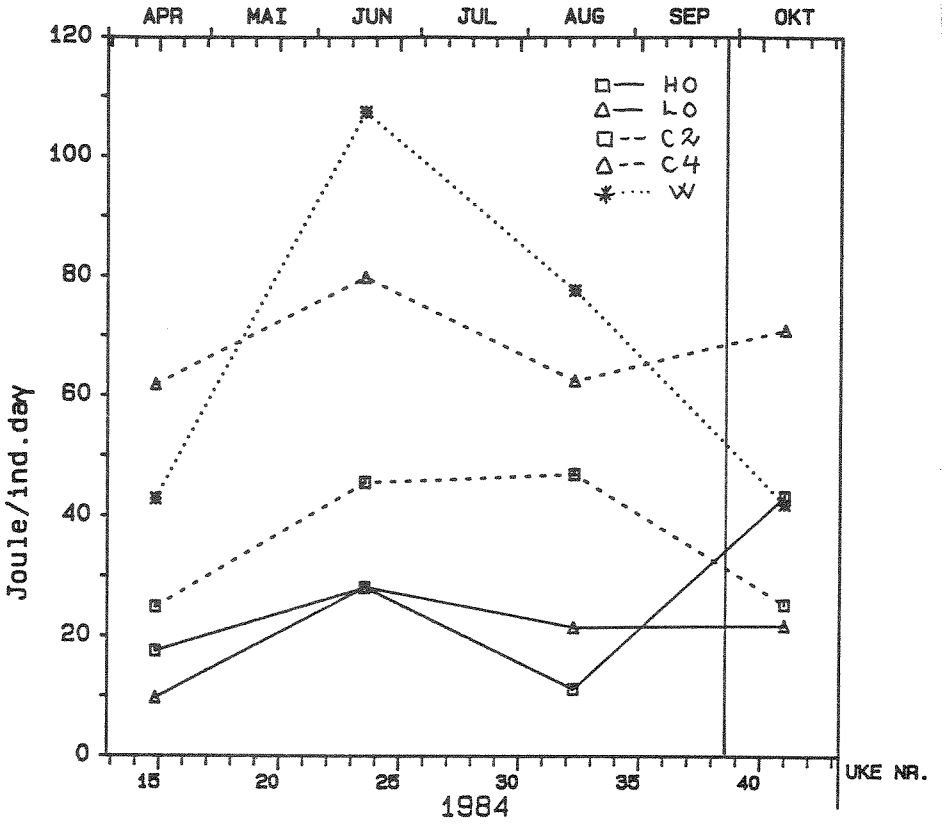

Figure 1. Mean individual feeding rate in Littorina littorea, with Ulva lactuca as food. Each value is the mean of 10 individuals adjusted to standard size. The lines between the points are drawn for clarity only and do not indicate how the means fluctuate.

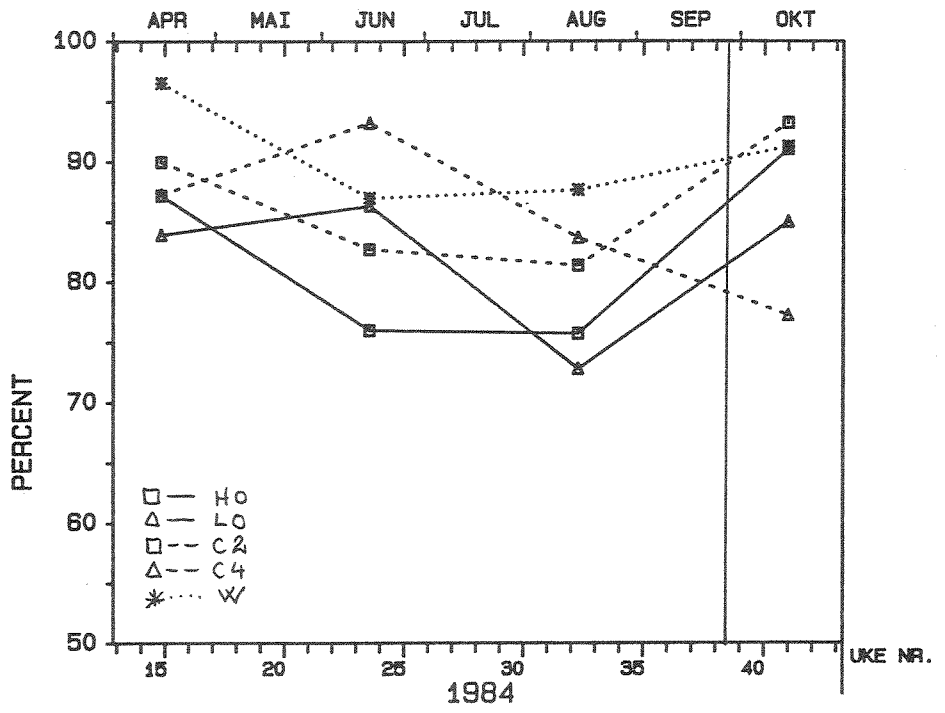

Figure 2. Assimilation efficiency in Littorina littorea when fed on Ulva lactuca. Each point is a single value based on the organic content of Ulva and of the faeces produced by groups of 50 individuals.

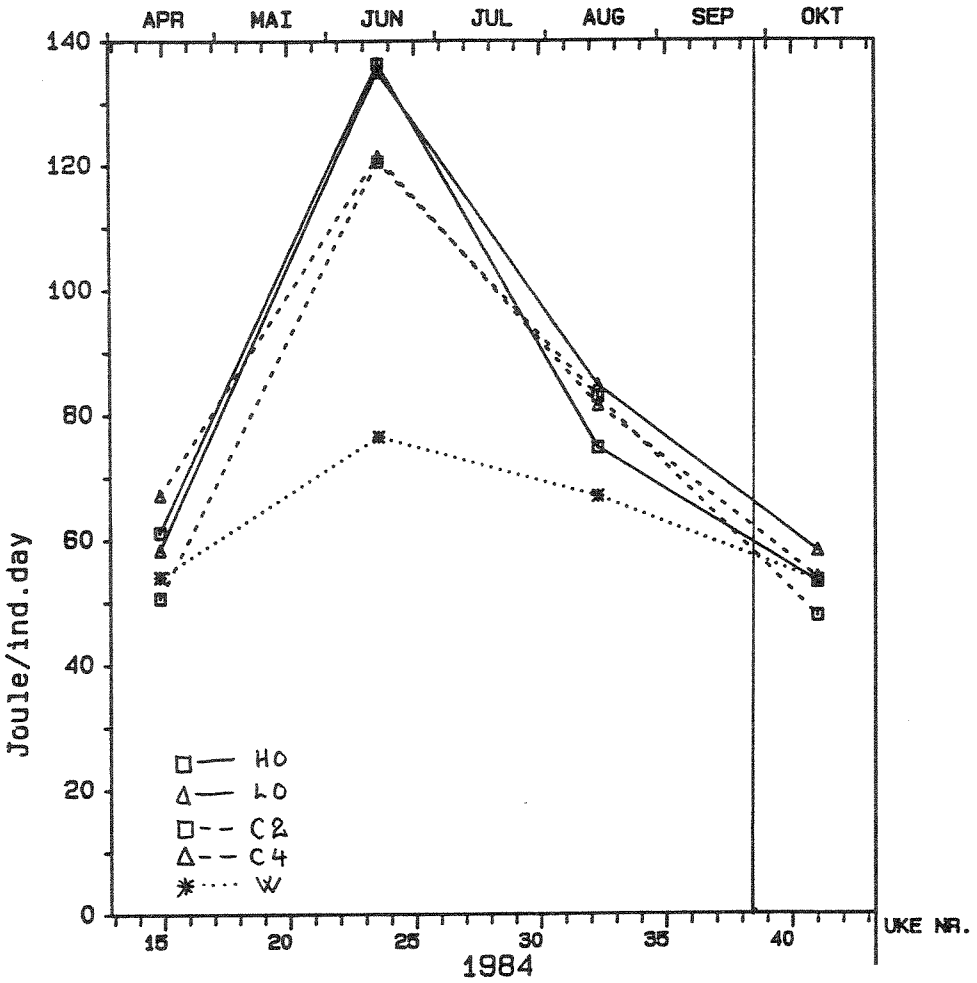

Figure 3. Mean individual aquatic respiration rate in Littorina littorea, Each value is the mean of 10 individuals adjusted to standard size. The lines between the points are drawn for clarity only and do not indicate how the means fluctuate.

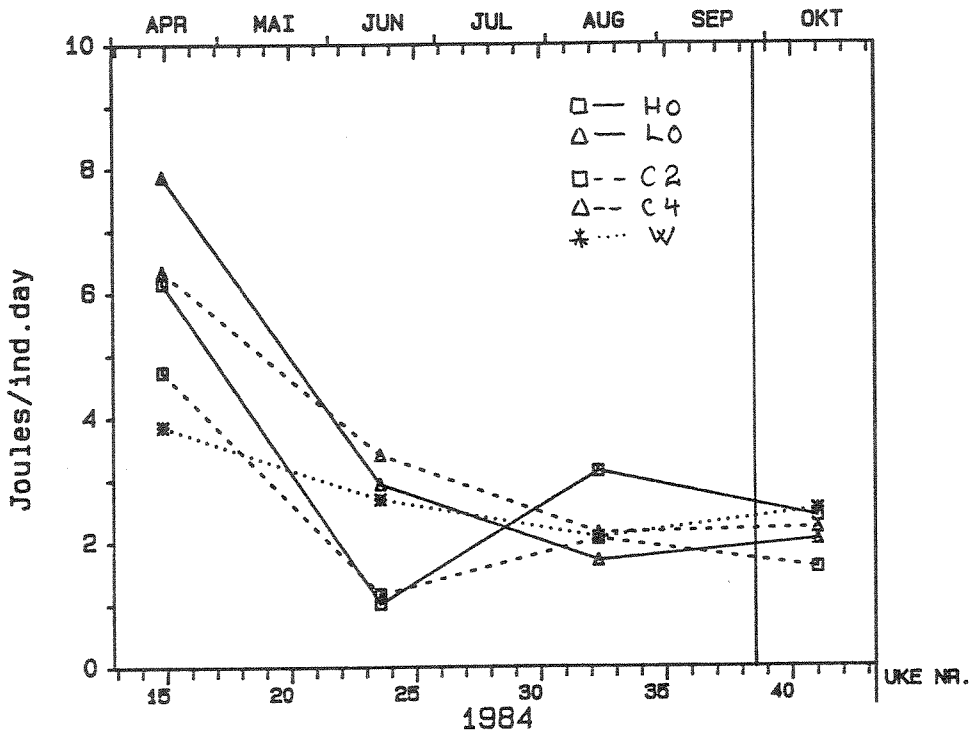

Figure 4. Mean individual ammonia excretion rate in Littorina littorea, Each value is the mean of 10 individuals adjusted to standard size. The lines between the points are drawn for clarity only and do not indicate how the means fluctuate.

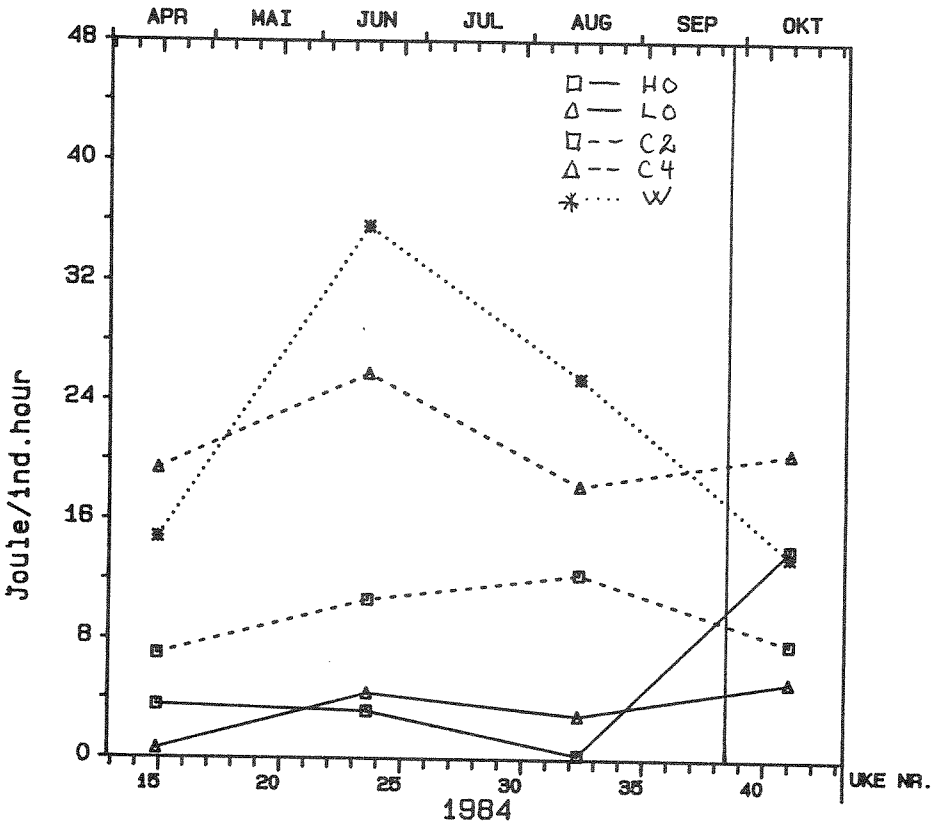

Figure 5. Mean individual scope for growth in Littorina littorea. Each value is the mean of 10 individuals adjusted to standard size. The lines between the points are drawn for clarity only and do not indicate how the means fluctuate.

14. Sublethal cellular and molecular effects and short-term recovery of mussels (*Mytilus edulis*) and periwinkles (*Littorina littorea*) following chronic exposure to petroleum hydrocarbons.

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General Introduction

Previous studies at IMER have shown that cellular processes of mussels and periwinkles are affected by chronic exposure to low concentrations of oil-derived hydrocarbons, both in the laboratory and in the field (Lowe et al, 1981; Moore and Clarke, 1982: Bayne et al, 1982, Moore et al, 1982, 1984a,b).

The last report (1983) showed that comparable cellular and subcellular effects had occurred in the experimentally exposed mussels and periwinkles at Solbergstrand. This report reflects the continuing investigations and also introduces a new component, namely the investigation of the cytochrome P-450 detoxication/toxication system.

The main objectives of this continued investigation were:

1. to investigate the sublethal cellular and molecular responses at two concentrations of hydrocarbons on a seasonal basis,

2. to study short-term recovery from and exposure to hydrocarbons in both species.

Part 1: Subcellular cytochemical reactions to hydrocarbon induced cell injury - M. Moore.

Introduction

Oil-derived hydrocarbons have been demonstrated to have deleterious effects on lysosomal structure and function and NADPH -neotetrazolium (cytochrome P-450) reductase (a component of the detoxication/toxication system in the endoplasmic reticulum of both mussels and periwinkles (Moore, 1980; Moore et al, 1982, 1984a).

The present report continues the investigation of these species at the Solbergstrand Experimental Station, and concentrates on the reproductibility of measurements of effects on lysosomal stability and NADPH-neotetrazolium reductase (NTR). Effort is also focussed on the detailed response patterns to short-term exposure to and recovery from hydrocarbons.

Materials and Methods

Mussels and littorinids were sampled in January, June and September 1984 for examination of the effects of hydrocarbons. Short-term exposure experiments were carried out in January on mussels (∿25ppb) and littorinids (∿25ppb and ∿100ppb) for 8 and 7 days respectively. Mussels were transferred in cages and littorinids in polythene bottles with numerous holes to allow free passage of water. Littorinids were provided with excess Ulva lactuca from the appropriate treatment basin. Short-term recovery

in the littorinids was also studied in January over a 7 day period as described in the last report. Long-term recovery in mussels (∿25ppb) was examined in June after a period of 53 days as previously described.

Results and Discussion

Effects of hydrocarbon exposure

Lysosomal membrane stability was significantly reduced in the digestive cells of both mussels and periwinkles at both hydrocarbon concentrations (Tables 1 and 4). There was no evidence of any seasonal differences in the periwinkles, however, the mussels did show a dose-related difference in the January sample with lower lysosomal stability at the higher oil concentration (Table 1). There was no evidence of a lysosomal dose-related response in the periwinkles (Table 4).

NADPH-neotetrazolium reductase (NTR) is a cytochemical marker for NADPH-cytochrome P-450 reductase, a component of the microsomal detoxication/toxication system. Activity for this enzyme was significantly elevated in both mussels and littorinids in January (Table 7 and 9). With the periwinkles there was also evidence of a dose-related effect (Table 9). These data are in good agreement with the biochemical determinations for NADPH-cytochrome c (P-450) reductase described in the report by Livingstone (see Part 2),

Short-term exposure to hydrocarbons

Exposure of mussels to ∿30ppb hydrocarbons resulted in a large decrease in lysosomal stability after 1 day (Table 2). There was no further change up to the end of the experiment at 8 days. With the periwinkles at both ∿25ppb and ∿100ppb the effects on the lysosomal stability were similar (Table 5). At ∿25ppb, however, there was a significant increase in lysosomal stability between 2 and 7 days of exposure, possibly representing recovery from an overshoot and the establishment of a new steady-state (Table 5). Evidence for this effect derives further support from data on quantative structural aspects of the digestive cells (Lowe, Part 3).

NADPH-NTR data is as yet only available for the short-term exposure of mussels in January. These results do not show any significant differences in NTR levels (Table 8). This data is in agreement with the biochemical determinations (see Livingstone - Part 2).

Short-term recovery from hydrocarbons.

Transfer of periwinkles from the low oil (∿25ppb) and high oil (∿100ppb) conditions to the control basin (B2) resulted in a dramatic recovery of lysosomal stability between 3 and 4 days for both treatments (Table 6). This abrupt restabilisation of the membranes is supported by experimental results on recovery from phenanthrene exposure (Moore and Mayernik, in preparation).

Long-term recovery from hydrocarbons.

Transfer of mussels from the low oil condition (~25ppb, exposed 86 days) to the control system (B4) for 53 days resulted in complete recovery of lysosomal stability in the digestive cells (Table 3).

Conclusions

1. Mussels and periwinkles continue to show evidence of perturbation of lysosomal function in the digestive cells.

2. NADPH-NTR results are in agreement with the biochemical data for both species.

3. Short-term exposure to hydrocarbons results in rapid (1 day) destabilization of lysosomes.

4. Short-term recovery of lysosomal stability in periwinkles occurs dramatically between 3 and 4 days at both oil concentrations.

5. Lysosomal stability in mussels was fully restored after 53 days in the control system. Mussels do not show any evidence of recovery in the short term (see previous report).

6. Measurable effects have been demonstrated in both mussels and periwinkles which are consistent with previous data. Details of the response times for recovery and exposure have been elucidated.

Other Work

There is considerable material still to be analysed from the June and September visits. This will be reported later.

References

Bayne B L, Widdows J, Moore M N, Salkeld P, Worrall C M, Donkin P (1982) Some ecological consequences of the physiological and biochemical effects of petroleum compounds on marine molluscs. Phil Trans R Soc 297B: 219 - 239.

Lowe D M, Moore M N, Clarke K R, (1981) Effects of oil on digestive cells in mussels: quantitative alterations in cellular and lysosomal structure. Aquat Toxical 1: 213 - 226.

Moore M N, (1980) Cytochemical determination of cellular responses to environmental stressors in marine organisms. Rapp P-v Reun Cons int Explor Mer 179: 7 - 15.

Moore M N, Clarke K R, (1982) Use of microstereology and quantitative cytochemistry to determine the effects of crude oil derived aromatic hydrocarbons on lysosomal structure and function in a marine bivalve mollusc, Mytilus edulis. Histochem J 14: 713 - 718.

Moore M N, Pipe R K, Farrar S V, (1982) Lysosomal and microsomal responses to environmental factors in Littorina littorea from Sullom Voe. Mar Pollut Bull 13: 340 - 345.

Moore M N, Pipe R K, Farrar S V, Thomson S, Donkin P, (1984a) Lysosomal and microsomal responses in Littorina littorea: further investigations of environmental effects in the vicinity of the Sullom Voe Oil Terminal and the effects of experimental exposure to phenanthrene. In: Wastes in the ocean, Vol. 4. John Wiles, New York, In press.

Moore M N, Widdows J, Cleary J J, Pipe R K, Salkeld P N, Donkin P, Farrar S V, Evans S V, Thomson P E, (1984b) Responses of the mussel Mytilus edulis to copper and phenanthrene: interactive effects. Mar Environ Res 14: 167 - 183.

Table 1. Lysosomal stability in the digestive cells of the mussel : effects of hydrocarbons.

Treatment		Lysosomal Stability (Labilization period of latent β-hexosaminidase in minutes, =5) [A]	
Control	Jan. 1984	25 (25, 25)	
	Jun. 1984	20.4(2, 25)	
25ppb	Jan. 1984	5 (5, 5)**	
	Jun. 1984	2.6(2, 5)*	PSO.01
100ppb	Jan. 1984	2 (2, 2)**	
	Jun. 1984	2 (2, 2)*	

A Numbers in parenthesis are lowest and highest values

* P $\leq$0.05, Mann-Whitney U-test, comparing with control value

** P $\leq$0.01

Table 2 Lysosomal stability in the digestive cells of the mussel : effects of short-term exposure to 30ppb hydrocarbons (Jan. 1984).

Treatment		Lysosomal Stability (Labilization period of latent β-hexosaminidase in minutes, (n=5))
		A
Control	0d	25 (25, 25)
	7d I	22.5(15, 25)
	7d II	25 (25, 25)
25ppd	1d	2 (2, 2)**
	2d	3.6(2, 5)**
	4d	2 (2, 2)**
	8d	2 (2, 2)**

A Numbers in parenthesis are lowest and highest values.

** $P < 0.01$, Mann-Whitney U-test, comparing with control 0d values for 1, 2 and 4d exposure and control 7d II for 8d exposure.

Table 3 Lysosomal stability in the digestive cells of the mussel: effects of a long-term recovery period in clean seawater.

Treatment	Lysosomal Stability (Labilization period of latent β-hexosaminidase in minutes, (n=5)
Control	20.4(2, 25)[A]
∿25ppb: 53d Recovery	25 (25, 25)

A Numbers in parenthesis are lowest and highest values.

Table 4 Lysosomal stability in the digestive cells of L.littorea: effects of hydrocarbons.

Treatment		Lysosomal Stability (Labilization period of latent β-glucuronidase in minutes, n=5)
Control	Jan. 1984	24 (20, 25)[A]
	Jun. 1984	25 (25, 25)
	Sep. 1984	25 (25, 25) n=7
25ppb	Jan. 1984	2.6 (2, $\overline{5}$)**
	Jun. 1984	2 (2, 2)**
	Sep. 1984	2 (2, 2)** n=7
100ppb	Jan. 1984	2 (2, 2)**
	Jun. 1984	2.6(2, 5)**
	Sep. 1984	2.9(2, 5)** n=7

A Numbers in parenthesis are lowest and highest values

** P $\leq$0.01, Mann-Whitney U-test, comparing with control value.

Table 5 Lysosomal stability in the digestive cells of L.littorea: effects of short-term exposure to ∿25ppb and ∿100ppb hydrocarbons (Jan. 1984).

Treatment		Lysosomal Stability (Labilization period of latent -glucuronidase in minutes, n=5) A	
Control	1d	25 (25, 25)	
	7d	23 (20, 25)	
∿25ppd	1d	2 (2, 2)**	
	2d	2 (2, 2)**	
	4d	2.6(2, 5)**	P ≤0.05
	7d	4.4(2, 5)**	
∿100ppb	1d	2.6(2, 5)**	
	2d	2 (2, 2)**	
	4d	2 (2, 2)**	
	7d	2.6(2, 5)**	

A Numbers in parenthesis are lowest and highest values.

** P ≤0.01, Mann-Whitney U-test, comparing with control 1d values for 1d, and 2d and control 7d values for 4d and 7d.

Table 6 Lysosomal stability in the digestive cells of L. littorea: effects of a recovery period in clean seawater (Jan. 1984).

Treatment		Lysosomal Stability (Labilization period of latent β-glucuronidase in minutes, n=5) [A]	
Control	1d	25 (25, 25)	
	7d	23 (20, 25)	
∿25ppd	1d	4.4(2, 5)**	
	2d	5 (5, 5)**	
	3d	6 (5,10)**	
			P ≤0.01
	4d	22 (15, 25)	
	7d	25 (25, 25)	
∿100ppb	1d	2 (2, 2)**	
	2d	2.6(2, 5)**	
	3d	2 (2, 2)**	
			P ≤0.01
	4d	23 (20,2 5)	
	7d	24 (20, 25)	

A Numbers in parenthesis are lowest and highest values.

** P ≤0.01, Mann-Whitney U-test, comparing with control 1d values for 1,2 and 3d and control 7d values for 4 and 7d.

Table 7 NADPH-neotetrazolium reductase (NTR) cytochrome P-450 reductase) activity in mussel digestive cells: effects of hydrocarbons (Jan. 1984).

Treatment	NTR activity.
Control	11.1 ± 2.3 [A]
∿25ppb	15.3 ± 1.4*
∿100ppb	20.4 ± 9.2*

A Activity expressed in relative units of absorbance (Mean ± SD, n=5)

* P ≤0.05, Mann-Whitney U-test, comparing with control value.

Table 8 NADPH-neotetrazolium reductase (NTR; cytochrome P-450 reductase) activity in mussel digestive cells: effects of short-term exposure to ~25ppb hydrocarbons (Jan. 1984)

Treatment		NTR activity A
Control	0d	6.0 ± 2.3
	7d	7.5 ± 4.3
~25ppd	1d	7.6 ± 4.4
	2d	12.2 ± 7.6
	4d	11.7 ± 5.7
	8d	10.1 ± 2.9

A Activity expressed in relative units of absorbance (Mean ± SD, n=5).

Table 9 NADPH-neotetrazolium reductase (NTR; cytochrome P-450 reductase) activity in the digestive cells of L.littorea: effects of hydrocarbons (Jan. 1984)

Treatment	NTR activity [A]	
Control	7.0 ± 2.3	
∿25ppd	20.4 ± 3.0**	P ≤0.05
∿100ppb	27.6 ± 2.2**	

A Activity expressed in relative units of absorbance (Mean ± SD, n=5).

** P ≤0.01, Mann-Whitney U-test, comparing with control value.

Part 2. Responses of the biochemical detoxication system - David Livingstone

Introduction

Organic pollutants such as polynuclear aromatic hydrocarbons (PNAH) are taken up and accumulated in the tissues of marine organisms. These compounds are often lipophilic and toxic and there is a requirement for their detoxication and/or elimination from the animal. This may be achieved by a number of apparently universally distributed enzyme systems that generally function to convert apolar chemicals to more water-soluble and excretable metabolites. The first phase of metabolism involves oxidation by various monooxygenase reactions, (epoxidation, hydroxylation, dealkylation) and is catalyzed by the cytochrome P-450 monooxygenase or mixed function oxidase (MFO) enzyme system (Sato and Omura, 1978). The MFO system requires molecular oxygen and the coenzyme NADPH (reduced nicotinamide dinucleotide phosphate), in addition to the organic substrate, and an example of a reaction is:

$$R + O_2 + NADPH \longrightarrow NADP^+ + H_2O + R\text{-}OH$$

where R is a PNAH and R-OH is the hydroxlated product.

The MFO system is a multi-component enzyme system that is membrane bound in the endoplasmic reticulum of the cell. Its functions include both the metabolism of exogenous foreign organic compounds (so-called xenobiotics) and endogenous compounds (fatty acid desaturation, steroid synthesis). It can be measured by the MFO activity towards a particular substrate (the assay commonly used in xenobiotic studies is benzo(a)pyrene hydroxylase) and by the activities and concentrations of its protein components i.e. cytochrome P-450 reductase and cytochrome P-450 (catalyze the xenobiotic reaction) and cytochrome b_5 reductase and cytochrome b_5 (catalyze the endogenous reactions but are also involved in the xenobiotic metabolism.) Paradoxically during the course of these and other subsequent metabolic transformations of the xenobiotics, reactive electrophilic intermediates may be formed which are more toxic, mutagenic or carcinogenic than the parent compound (Sims and Grover, 1974), and the MFO system and other detoxication enzymes must therefore be viewed as part of a detoxication/toxication system, the usefulness of which depends on the metabolites produced. An important feature of the MFO system is that its activity and the activities and concentrations of its components may be increased by exposure of the animal to the xenobiotic or pollutant, and the nature and consequences of this induction phenomenon have been investigated in a variety of non-marine (Snyder and Remmer, 1982) and marine organisms (Lee, 1981; Stegeman, 1981).

Bivalve and gastropod molluscs possess MFO systems (Livingstone 1984a) and, in the case of the common mussel Mytilus edulis,

it is primarily localized in the endoplasmic reticulum of the digestive gland or hepatopancreas and varies with sex and season (Livingstone and Farrar, 1984). The existence, functioning and inducibility of molluscan MFO systems have been studied in recent years for three main reasons, viz, the possibility of using changes in enzyme activities or amounts as specific indicators of stress or biological effect in enviromental impact assessment studies (Bayne et al, 1979; Lee et al., 1980; Livingstone et al 1984), the investigation of the relationship between pollution and the occurence of neoplastic diseases (Mix et al., 1981; Anderson and Döös, 1983) and the use of bivalves as bioconcentrators and indicators of marine pollutant levels (Burns and Smith, 1981; Knutzen and Sortland, 1982). Indications of inducibility of molluscan MFO systems have been obtained (Anderson, 1978; Moore, 1980; Gilewicz et al., 1984) but the information is very limited and the Solbergstrand experimental station facility provided an oppurtunity to address a number of important questions (see objectives below). Of particular importance was that because of the design of the facility, viz. natural food levels, wave and tidal simulation, outdoor tanks, exposure experiments could be carried out with little or no likelihood of general stress effects, often associated with long-term laboratory experiments, interfering with the biochemical responses.

Objectives

1. To investigate the existence and responses of the MFO system of the digestive gland of M. edulis and Littorina littorea to short-term and long-term exposure to diesel oil.

2. To investigate if the MFO responses are dependant on season (both species), sex (M. edulis only) and oil dosage (water levels, tissue levels, exposure time) (both species).

3. To examine short-term and long-term recovery of the MFO system in both species.

4. To assess the data in relation to the possible use of the various measurements of the MFO system as specific indicators of biological effect in enviromental monitoring programmes.

Materials and Methods

A. Experiments

Three trips were made to Solbergstrand in January, June and September 1984 to encompass seasonal canges in reproductve state, temperature and salinity. On each occasion mussels and winkles were sampled from the control, low-oil and high-oil basins; on most occasions samples were also taken from the fjord. The mussels had been placed in baskets prior to sampling for the following periods of time: JAN sample (approx. 4 months), JUN

sample (approx. 4½ months), SEPT sample (approx. 7 and 2½ months). In addition the following experiments were carried out (N.B. fjord animals were used for short-term exposure expts.)

JAN 84

1. 8 day exposure of mussels to low oil.
2. 8 day recovery of long-term low oil mussels.

JUN 84

Mussels were sexed and male and females sampled separately.

1. 6 day exposure of mussels and winkles to low and high oil.
2. 6 day recovery of low oil mussels.
3. 53 day recovery of low oil mussels (these animals were transfered prior to JUN visit.)

SEPT 84

1. 5 day exposure of mussels and winkles to low and high oil.

B. Sampling procedure

Material was taken for enzyme analysis and tissue PAH determination. For the former, digestive glands were dissected out of mussels and winkles, damp-dried on filter paper, the crystalline style removed if obvious and the tissues immediately frozen in liquid-nitrogen. The samples were transported back to IMER, Plymouth on dry ice and stored at - 70°C prior to biochemical analysis. Samples for PNAH analysis were obtained by first washing off the winkles and mussels in acetone and then storing the whole winkles or in the case of the mussels dissecting out the tissues and storing these. Tissues and animals were transported back to IMER on dry ice.

C. Biochemical and chemical analysis

On homogenization of the tissue the endoplasmic reticulum breaks up and is obtained as the microsomal fraction by high-speed ultracentrifugation. Microsomes were prepared and assayed essentially as described in Livingstone and Farrar (1984) with the exception of benzo(a)pyrene hydroxylase (BPH) which was measured fluormetrically by the method of Dehnen et al (1973). The following measurements were made: total microsomal protein, cytochrome P-450 and b_5 content, BPH activity and NADPH-cytochrome c (NADPH-CYTCRED), NADH-cytochrome c (NADH-CYTCRED) and NADH-ferricyanide (NADH-FERRIRED) reductase activities; N.B. the reductive assays employ artificial electron-acceptors (cytochrome c or potassium ferricyanide) and generally speaking NADPH-CYTCRED is a measure of P-450 reductase activity, NADH-CYTCRED reflects aspects of both P-450 and b_5 reductase activity and NADH-FERRIRED least reflects aspects of the xenobiotic MFO system.

PNAH were extracted by steam distillation and measured by HPLC as described in Donkin and Evans (1984).

For biochemical analysis, depending on availability, pools of tissues of 3-8 animals were used for each sample and generally 4 or 5 samples were taken per sampling time or condition. Two pools of 5 animals each were used for PNAH analysis.

D. Statistics

Groups of values were compared by one-way analysis of variance.

Results

Material is still being analysed from the JUN and SEPT trips and data from the JAN trip only are presented. Insufficient material was available for mussels in the high oil condition and a comparison between control and low oil only was possible.

1. Tissue PNAH's (Table 1)

PNAH's were accumulated in the tissues of winkles and mussels with the accumulation greater in the high oil condition. PNAH's were rapidly taken up by the mussels in the 8 day exposure experiment despite the low temperatures at that time of the year.

The tissue concentrations of mussels in the control basin seemed high (e.g. compared with 0.7 to 0.04 $\mu g\ g^{-1}$ wet wt. 2,3 - dimethylnaphthalene equivalents for field mussels from the Shetlands - see Livingstone, 1984b). No data are available for fjord mussels or winkles (samples have been subsequently taken) or for the 8 day recovery experiment.

2. Mussel responses to long-term exposure and short-term recovery (Table 2).

Marked changes occured following exposure of mussels to 25ppb diesel oil for 4 months. Elevations were seen in cytochromes P-450 and b_5 and in NADH-CYTCRED and NADPH-CYTCRED activities: in contrast, BPH activity was unchanged. The cytochrome b_5 and NADH-CYTCRED activity remained high following 8 days recovery of the low oil mussels but the cytochrome P-450 returned to control levels and the NADPH-CYTCRED difference was no longer significant. Although no data are available on tissue PNAH following recovery, in comparable experiments carried out in JUN trip the concentrations were little affected by 6 days depuration.

3. Mussel responses to short-term exposure (Table 3).

Fewer changes were observed for the short-term exposure experiment. Surprisingly, declines occurred in cytochrome b_5 and BPH activity with exposure but the same changes also occurred in fjord animals transferred to the control basin. No change occurred in cytochrome P-450 with transfer of fjord mussels to the control basin and considering the pooled data for these two conditions versus the pooled data for day 1 and day 8 exposed mussels, the cytochrome P-450 concentrations were higher in the exposed mussels ($P\ 0.1 > 0.05$; $n=10$).

4. Winkle responses to long-term exposure (Table 4).

A marked increase occurred in NADPH-CYTCRED activity with exposure and the increases were greatest in the high oil condition. Increases also occurred in cytochrome b_5 at 100ppb and were indicated for cytochrome P-450 although it was not possible to quantify them due to the unusual nature of the spectra obtained. BPH activity was unchanged.

Discussion and Conclusions

The experiments clearly show that the MFO systems of M. edulis and L. littorea are inducable and respond to environmental PNAH's. The increases in cytochrome P-450 and b_5 represent the first statistical demonstration of these changes and the increases in NADPH-CYTCRED activity support the data on NADPH-neotetrazolium reductase activity of other studies (e.g. Moore, 1980; this report) (N.B. neotetrazolium reductase activity measures P-450 reductase activity). The lack of change in BPH is perhaps surprising but is consistent with our other studies on this enzyme activity (Livingstone, 1984a); the decline in BPH with transfer of mussels from the fjord to the control basin (Table 3) is interesting and may indicate that the activity is very sensitive to membrane structural changes, possibly brought about by the action of hydrolytic enzymes released from temporarily destabilized lysosomes.

With respect to the use of the MFO system of molluscs as a specific index of biological effect, the changes in cytochrome P-450 and NADPH-CYTCRED activity offer potential. Cytochrome P-450 was most responsive showing elevations after 1 day of exposure to diesel oil (Table 3) and returning to control levels after 8 days of recovery (Table 2). The changes in NADPH-CYTCRED activity were very marked in winkles and dose-related. The general trend of a difficulty in demonstrating changes in BPH activity but a ready response of the cytochromes and of NADPH-CYTCRED activity has now been observed in four species of bivalve and gastropod molluscs viz., M. edulis, L. littorea, Cardium edule and Thais haemostoma (Livingstone, 1984a).

Publication of Material

The following paper is in preparation.

"Responses of the cytochrome P-450 monooxygenase system to diesel oil in the common mussel Mytilus edulis L., and the periwinkle Littorina littorea L." by D.R. Livingstone, M.N. Moore, D.M. Lowe, S.V. Farrar & C. Nasci.

In addition, data from Table 2 has been used in : "Responses of the Detoxication/Toxication Enzyme Systems of Molluscs to Organic Pollutants and Xenobiotics" by D.R. Livingstone. Mar. Pollut. Bull., in press.,
(and some of the data has also been presented in lectures or posters at the following meetings).

1. "Benzo(a)pyrene Hydroxylase and the Monooxygenase System in Marine Molluscs by D.R. Livingstone & S.V. Farrar. In 6th. Int. Symp. on Microsomes and Drug Oxidations, Brighton, England (5-10th August, 1984. Published as Supplement No 1. to Xenobiotica, Vol. 14 (1984).

2. "Responses of Molluscan Mixed Function Oxidase Systems to Organic Xenobiotics" by D.R. Livingstone. In NATO Advanced Study Institute on "Strategies and Advanced Techniques for Marine Pollution Studies", Beaulieu Sur Mer, France (4-14th October, 1984).

Future Trips

A trip is planned for 24th - 29th November 1984 to sample mussels and winkles for recovery.

References

1. Anderson, R.S. (1978). EPA Ecol. Res. Ser. Monogr. (EPA-600/3-78-009).

2. Anderson, R.S. & Doos, J.E. (1983). Mutat. Res., 116, 247 - 256.

3. Bayne, B.L., Moore, M.N. Widdows, J., Livingstone, D.R. & Salkeld, P. (1979). Phil. Trans. R. Soc. Lond. B. Biol. Sci., 286, 562 - 581.

4. Burns, K.A., & Smith, J.L. (1981). Estuar. Cstl. Shelf. Sci, 13, 433 - 443.

5. Dehnen, W., Tomingas, R. & Roos, J. (1973). Analyt Biochem., 53, 373 - 383.

6. Donkin, P. & Evans, S.W. (1984). Anal. Chim. Acta, 156, 207 - 219.

7. Gilewicz, M., Guillaume, J.R., Carles, D., Leveau, M. & Bertrand, J.C. (1984). Mar. Biol., 80, 155 - 159.

8. Knutzen, J. & Sortland, B. (1982). Water Res., 16, 421 - 428.

9. Lee, R.F. (1981). Mar. Biol. Lett., 2, 87 - 105.

10. Lee, R.F., Davies, J.M., Freeman, H.C., Ivanovici, A., Moore, M.N., Stegeman, J. & Uthe. J.F. (1980). Rapp. P.V. Réun. Cons. Int. Explor. Mer., 179, 48 - 55.

11. Livingstone, D.R. (1984a). Responses of the Detoxication/Toxication Enzyme Systems of Molluscs to Organic Pollutants and Xenobiotics. Mar. Pollut. Bull, in press.

12. Livingstone, D.R. (1984b). Biochemical Differences in Field Populations of the Common Mussel Mytilus edulis L. Exposed to Hydrocarbons: Some Considerations of Biochemical Monitoring. In Toxins, Drugs and Pollutants in Marine Animals (Bolis et al., eds), Springer - Verlag, Berlin, ~~in press.~~ pp 161 - 175.

13. Livingstone, D.R., Brown, D.A., Burns, K.A. & Ivanovici, A.M. (1984). Biochemical procedures. In effects of Stress and Pollution on Marine Animals: a manual of methods. Praeger Press, U.K., in press.

14. Livingstone,D.R. & Farrar, S.V. (1984). Tissue and subcellular distribution of enzyme activities of mixed-function oxygenase and benzo(a)pyrene metabolism in the common mussel Mytilus edulis L., Sci. Total Environ., 39, 209-235.

15. Mix, M.C., Schaffer, R.L. & Hemingway, S.J. (1981). In Phyletic Approaches to cancer (Dawe et al., eds), pp167 - 177. Japan Scientific Societies Press, Tokyo.

16. Moore, M.N. (1980). Rapp P.V. Reun. Cons. Int. Explor. Mer, 179, 7-15.

17. Sato, R & Omura, T. (1978). Cytochrome P-450 Academic Press, New York. 233pp.

18. Sims, P. & Grover, P.L. (1974). Adv. Cancer Res., 20, 165-274.

19. Stegeman, J.J. (1981). In Polycyclic Hydrocarbons and Cancer (Gelbion & T'So, eds), pp1 - 60, Vol. 3 Academic Press, New York.

20. Synder, R. & Remmer, H (1982). In Hepatic Cytochrome P-450 Monooxygenase System (Schenkman & Kupfer, eds.), pp 227-268. Pergamon Press, Oxford.

Table 1. Polyaromatic hydrocarbon concentrations of whole tissues of M. edulis and L. littorea exposed to diesel oil (JAN. 84).

Species	Condition	Exposure time	2-aromatic rings* (ug g^{-1} wet wt.)	3-aromatic rings* (ug g^{-1} wet wt.)
M. edulis	Control	4 months	2.1 ± 0.3	0.05 ± 0.01
M. edulis	25ppb	4 months	14.7 ± 2.4	3.4 ± 0.4
M. edulis	100ppb	4 months	25.4 ± 0	7.4 ± 0.1
M. edulis	25ppb	8 days	13.8 ± 1.6	2.4 ± 0.3
L. Littorea	Control	†	0.25 ± 0.1	0.025 ± 0
L. Littorea	25ppb	16 months	6.0 ± 0.8	0.56 ± 0.5
L. Littorea	100ppb	16 months	12.5 ± 0.6	1.5 ± 0.2

* Concentrations in, respectively, 2,3-dimethylnaphthalene and 1-methylphenanthrene equivalents. Values are means ± range (n = 2) and each sample is the pooled material of 5 animals.
† Animals placed in control basin at the setting-up of the Littoral Rock Community Project.

Table 2. Responses of digestive gland microsomal MFO system of mussels (M. edulis) exposed to 25ppb diesel oil for four months and subsequently recovered for eight days in control basin (JAN. 84).

Microsomal parameter	Control	25ppb exposed	Recovered 8days
total protein[1]	5.21 ± 0.60 (6)	5.21 ± 0.29 (7)	5.29 ± 0.23 (5)
NADH-FERRIRED[2]	741 ± 33 (6)	772 ± 18 (7)	838 ± 69 (5)
NADH-CYTCRED[2]	95 ± 8 (4)	116 ± 4** (7)	124 ± 10* (5)
NADPH-CYTCRED[2]	10.3 ± 1.2 (4)	13.5 ± 0.9** (5)	12.2 ± 1.1 (5)
P-450[3]	46.6 ± 14.3 (5)	92.0 ± 10.9** (7)	34.2 ± 5.9 (5)
b_5[3]	25.8 ± 2.3 (5)	40.2 ± 5.8* (5)	43.2 ± 2.8** (5)
BPH[4]	54 ± 22 (6)	56 ± 4 (5)	58 ± 4 (5)

Values are means ± SEM, number of samples in brackets. One-way analysis of variance of 25ppb exposed or recovered condition versus control condition; * P 0.1<>0.05, ** P<0.05. [1]mg g^{-1} wet wt., [2]nmoles min^{-1} mg^{-1} protein, [3]pmoles mg^{-1} protein, [4]arbitary fluorescence per unit time.

Table 3. Responses of digestive gland microsomal MFO system of mussels (M. edulis) exposed to 25ppb diesel oil for one to eight days (JAN. 84).

Microsomal parameter	Fjord sample	Fjord transferred to control basin for 8 days	Day 1 exposed	Day 8 exposed
Total protein[1]	4.80 ± 0.27	4.95 ± 0.27	4.37 ± 0.25	4.46 ± 0.19
NADH-FERRIRED[2]	973 ± 56	990 ± 23	939 ± 32	1012 ± 71
NADH-CYTCRED[2]	120 ± 7	123 ± 7	101 ± 2**	129 ± 5
NADPH-CYTCRED[2]	8.5 ± 0.8	7.5 ± 0.4	7.6 ± 0.8	7.9 ± 0.3
P-450[3]	35.3 ± 10.6	36.9 ± 10.3	59.8 ± 9.6	49.6 ± 8.5
b_5[3]	36.5 ± 1.4	25.1 ± 3.5**	27.2 ± 1.5**	27.7 ± 2.9**
BPH[4]	102 ± 14	56 ± 15*	27 ± 5**	49 ± 11**

Values are means ± SEM, number of samples =5 except where indicated otherwise in brackets. One-way analysis of variance of exposed or transferred day 8 control versus Fjord sample; * P 0.1<> 0.05, ** P <0.05. [1]mg g^{-1} wet wt., [2]nmoles min^{-1} mg^{-1} protein, [3]pmoles mg^{-1} protein, [4]arbitrary fluorescence per unit time.

Table 4. Responses of digestive gland microsomal MFO system of winkles (*L. littorea*) exposed to 25ppb and 100ppb diesel oil for 4 months (JAN. 84).

Microsamal parameter	Control	25ppb	100ppb
total protein[1]	6.56 ± 0.31	7.56 ± 0.23	7.09 ± 0.84
NADH-FERRIRED[2]	1272 ± 99	1222 ± 88	1247 ± 296
NADH-CYTCRED[2]	117 ± 15	129 ± 14	128 ± 27
NADPH-CYTCRED[2]	7.5 ± 0.4	11.3 ± 0.7*	21.8 ± 1.9**
b_5[3]	26.4 ± 2.2	28.1 ± 2.7	48.0 ± 7.8**
BPH[4]	46 ± 9	71 ± 16	50 ± 6

Values are means ± SEM, number of samples =5 for Control and 25ppb, and =4 for 100ppb. One-way analysis of variance of Control condition versus 25ppb or 100ppb; * P 0.1<>0.05, ** P<0.05. [1]mg g^{-1} wet wt., [2]nmoles min^{-1} mg^{-1} protein, [3]pmoles mg^{-1} protein, [4]arbitrary fluorescence per unit time.

Part 3: Cellular Reactions - D. Lowe

Objectives

To investigate and quantify cellular reactions in the reproductive system, nutrient storage matrix and digestive tissues of Mytilus edulis and Littorina littorea following exposure to petroleum hydrocarbons.

Materials and Methods

To date during 1984 three collection trips have been made to Solbergstrand Experimental Station and a series of short and long term exposure/recovery studies carried out on both Littorina and Mytilus. As on previous occasions mussels were collected at the end of one visit and maintained in large cages in the HO, LO, and C4 basins for use during the next visit. However, sufficient mussels were collected in August 1983 for sampling on both January and June 1984 visits. Similarly sufficient numbers were collected in June 1984 for the September and forthcoming November visits. Littorina was plentiful in the various basins and no special collections were necessary.

Sampling Programme

January 84

Mytilus (50-60mm size class)

Exposure Studies
Control Basin 148 days, Low Oil Basin 1,2,4 and 148 days, High Oil 148 days.

Recovery Studies (50-60mm size class)

Low Oil Basin 4 and 8 days.

Fjord Reference Age Related Studies
Size Classes 30-40, 40-50, 50-60, 60-70, and 70-80.

Littorina

Recovery/ Exposure Studies

Control Basin, 1,2,4,7, days plus resident animals, Low Oil 1,2,4,7, days plus resident animals. High Oil 1,2,4,7, days plus resident animals.

June 84

Mytilus

Exposure Studies (50-60mm size class)

Control Basin 4,143 & 296 days, Low Oil 1,3,5,143 & 296 days, High Oil 1,3,5, & 143 days.

Recovery Studies (50-60mm size class)
Low Oil→Control 5 & 53 days.

Size/Age Related Studies
Fjord Reference, Control Basin, Low Oil Basin & High Oil Basin

Size Classes 30-40, 40-50, 50-60,60-70.

Littorina

Recovery/Exposure Studies
Control Basin 1,3,5 days plus residents, Low Oil 1,3,5 days plus residents & High Oil 1,3,5 days plus residents.

Results

This report will confine itself primarily with the cellular reactions exhibited in the reproductive and storage cell matrix of Mytilus and digestive epithelial cell responses in Littornia.

The stereological analysis of Mytilus mantle tissue sections measured differences in the relative proportions of the following cell types.

1. vesicular connective tissue cells
2. adipogranular cells
3. developing gamete
4. ripe gamete
5. evacuated follicles
6. atretic follicles

Where applicable the volume fraction data for the various cell types was volume transformed to take into account the natural variability in the size of the mantle and digestive gland tissue within a single size class. Alternatively when comparisons were made across size classes the stereology data was not volume transformed as this would give spurious results. All results were tested for significance using a one way analysis of variance with a Scheffe s-test or a Tukey t-test as appropriate.

JAN 1984

Samples were collected from the HO,LO & C4 basins and from the Fjord reference site. Stereological analysis of mantle sections demonstrated that there was no significant difference between the reproductive or storage cell status of the Fjord mussels or those from C4 basin. However, the C4 mussels had significantly more ($P<0.5$) ripe gamete than either the LO or HO exposed mussels and significantly more ($P<0.5$) developing gamete than the HO exposed mussels. There was no significant difference in levels of V.C.T. cells between treatment groups although A.D.G. cells were significantly higher in the C4 basin mussels than in the HO basin.

Material collected from the recovery/exposure studies was restricted to the digestive gland and these samples have yet to be completed.

JUNE 1984

During the course of the June visit a size range of mussels was sacrificed from both the Fjord reference site and the C4,LO & HO basins to asses the effects of Oil exposure on various age classes. In addition, mussels were examined following 53 days recovery from LO exposure and 296 days exposure to LO.

Short term exposure/recovery studies, i.e. up to 5 days, were also undertaken on both Mytilus and Littorina. Once again the results reported here will concentrate on the effects of exposure on reproductive potential in mussels from the various studies.

All mussels sampled were treated as individuals within a treatment and not, as previously, pooled per treatment. This course was undertaken as females exhibit atretic gametes, when under stress, and this could lead to an under or over estimate of fecundity within a sample group depending on the balance of the sexes. The weight and displaced volumes of all mantles and digestive glands was obtained from each animal and the corresponding stereology data volume transformed. Results presented here refer only to the reproductive analysis.

The results of the displaced mantle volumes indicated that there was generally no significant difference between the sexes within treatments with the exception of the animals from C4 and LO basins where the males had significantly ($P<0.5$) larger mantles than the females.

Vesicular Connective Tissue (V.C.T.)

Whilst there was no significant difference between the sexes within treatment groups there was considerable variability between treatments. There was no significant difference between the Fjord reference site, C4 (143 days), C4 (296 days) and the 53

day recovery group of mussels. However, HO & 296 day LO treatments had significantly less ($P<0.5$) V.C.T., than any of the unexposed groups.

Adipogranular Cells (ADG)

Once again there was no significant difference between the sexes. There were, however, marked treatment effects. 143 day C4 mussels were not significantly different from either the 296 day C4 or recovery group, however all of these had significantly more ($P< 0.5$) A.D.G., tissue than both of the LO conditions and the HO animals.

In summary then mussels from the C4 basin were not significantly different in terms of stored nutrient potential from equivalent sized Fjord animals. Secondly, mussels maintained in the system for 296 days did not appear to suffer from the containment indicating than the basins represent a good operating experimental system. Thirdly, the observed effects on the storage matrix following 143 days exposure to LO did not worsen following 296 days exposure in that system. Finally the recovery studies indicate that following 53 days depuration from LO exposure there was a complete recovery in the stored nutrient potential of the mussels.

Developing Gametes

There was a significant difference in the volume of developing gamete, in both sexes, between the treatments. There was however no signifiant difference between the Fjord reference, 143 day C4 and 296 day C4 and also between 143 day LO and 296 day LO. The 53 day recovery mussels had significantly more developing gamete than any other treatment group.

Ripe Gametes

The volume of ripe gamete was significantly greater ($P<0.5$) in males than females in the C4, HO, LO and 53 day recovery mussels. Females did not differ between treatment groups males, however, did exhibit treatment effects, C4 and 53 day recovery males, although not different from one another, had significantly more ($P<0.5$) ripe gametes. than 296 day C4 or Fjord reference males. L.O. (143 day) animals had significantly more ripe gamete the 296 day LO but were not any different to HO treated animals.

Evacuated Follicles

There was no significant difference between treatments or between the sexes indicating that the differences measured between treatments does not reflect prior spawning activity.

Atretic Follicles

This category of cells is only found in females. There was no difference between C4 (143 days), C4 (296 days), Fjord reference or 53 day recovery mussels. Animals from the 143 day LO group exhibited significantly ($P<0.5$) more gametogenic atresion than the 296 days LO animals but were not any different from the HO group. Both LO and HO mussels had significantly more gametogenic atresion than either of the C4 basin groups.

In summary then there was no appreciable difference in the levels of gamete material between the 143 day C4, 296 day C4 and the Fjord reference mussels indicating once again that the facility does not impose a stress on the mussels. Exposure to L.O. for 296 days appears to be no more detremental than L.O. exposure for 143 days, however, gametogenic atresion was higher in those mussels exposed for the shorter period of time.

Pathology

During the course of these investigations all the sacrificed mussels were screened for indicators of histopathology. There were no signs of any neoplastic or non-neoplastic growth disorders.

Mortalities in caged mussels

During the September 1984 visit mortality estimates were made in the three cages in C4, LO & HO basins of animals transplanted to those conditions in January and June 1984.

Condition	Month Caged	% Mortality	Exposure Period (in days)
C4	JAN	0	227
	JUN	0	80
LO	JAN	12.6	227
	JUN	14.5	80
HO	JAN	27.3	227
	JUN	71.1	80

These observations are significant in that they implicate oil as a major contributor to mortality in the mussels. Crab and starfish predation, both significant in mussel mortality, were largely excluded from the cages and environmental factors, e.g. temp, salinity and seston, were no different between the three conditions.

Summary Table of Volume Transformed Stereology Data.

Sample mean ± standard error.

Condition	Devel	Ripe	ADG	VCT	Evac.Foll	Atretic*
C4 143d	0.10 ± 0.03	0.77 ± 0.27	0.11 ± 0.03	0.53 ± 0.07	0.01 ± 0.01	0.05 ± 0.01
C4 296d	0.09 ± 0.03	0.24 ± 0.05	0.08 ± 0.04	0.37 ± 0.04	0.01 ± 0.01	0.09 ± 0.03
LO 143d	0.13 ± 0.04	0.44 ± 0.16	0.05 ± 0.04	0.38 ± 0.07	0.02 ± 0.01	0.35 ± 0.13
LO 296d	0.22 ± 0.08	0.42 ± 0.07	0.01 ± 0.01	0.28 ± 0.05	0.01 ± 0.01	0.03 ± 0.01
HO 143d	0.13 ± 0.03	0.30 ± 0.11	0.01 ± 0.01	0.14 ± 0.02	0.03 ± 0.01	0.31 ± 0.09
Recovery	0.28 ± 0.08	0.66 ± 0.18	0.11 ± 0.05	0.54 ± 0.04	0.01 ± 0.01	0.01 ± 0.01
Fjord Ref.	0.16 ± 0.32	0.20 ± 0.04	0.05 ± 0.02	0.49 ± 0.05	0.02 ± 0.01	0.19 ± 0.12

* Females only

Littorina Investigations

Materials and Methods

In both the exposure and recovery studies carried out on Littorina littorea the same method of containment of the samples was employed i.e. a one litre plastic bottle was drilled with holes sufficient in size and number to allow a good flow of water but not large enough to permit escape. The bottles were suspended in the basins at a height such that the snails could migrate to a non submerged area of the container regardless of the state of the tide. All bottles contained Ulva lactuca as a potential food source.

Snails for the exposure study were collected from Oslo Fjord and placed in the C4, LO and HO basins for periods of 1, 2, 4 and 7 days. Recovery snails were taken from the C4, LO and HO basins and maintained in basin C4 for periods of 1, 2, 3, 4 and 7 days. The animals were sacrificed, fixed in Bakers formol calcium (+ 2.5% NaCl) and sections (0.5µm) cut following embedding in L.R. White Resin. Sections were stained in Lees methylene blue/basic fuchsin and examined on a Leitz Ortholux microscope using x 50 and x 100 oil objectives.

For the purpose of this investigation it was necessary to re-examine the three phases of the digestive process (i.e. absorption, digestion and fragmentation) originally described in L. littorea by Merdsoy and Farley (1973). This course of action was necessitated as their original description was based on snails collected during a period of feeding and migration whereas the Solbergstrand snails were collected during a non-feeding period. Secondly Merdsoy and Farley concentrated their efforts primarily on the digestive cells and gave only scant attention to the secretory cells of the digestive epithelium.

The appearance of the digestive epithelium in Littorina littorea differs considerably between the feeding and non-feeding periods. During the spring and summer months when the snails are feeding the general mass of the digestive epithelium is very large with only a small tubule lumen. In contrast during the winter non-feeding period the epithelial cells are much reduced in size and the lumen therefore occupies considerably more space. Large chromophilic granules which tend to occur in the apical region of the digestive cells migrate down the full length of the cell during a feeding period whereas they descend no more than half way during the non-feeding period.

Waste vacuoles are in abundance and other, much larger vacuoles are also to be found giving rise to the descriptive term of fragmentation to describe this final phase of the digestive cycle. This condition was not observed in any of the animals studies during a non-feeding period. Secretory cells also exhibited seasonal differences in that during feeding they were large and packed with granules whereas during non-feeding periods

they were comparitively small and somewhat deplete of the secretory granules. Relatively few vacuoles are to be found in the feeding snail secretory cells regardless of the digestive phase, however, they were numerous and frequently quite large in a non-feeding snail.

These observations indicate that digestive epithelial cell activity is greatly enhanced during periods of feeding, as compared to non-feeding periods, and that during non-feeding periods the digestive epithelial cells still maintain some phasic activity although at a much reduced rate.

Results

Control animals

The apical region of the digestive cells had a well defined apical microvillous region beneath which was a band of cytoplasm, with enhanced staining characteristics, which contained large chromophilic granules. Vacuoles in the mid and lower regions of the cytoplasm were generally small although a few larger vacuoles were present. The secretory cells were of a similar size to the digestive cells and contained many chromophilic granules and a few small vacuoles. In digestive cells with small apical vacuoles the apical granules tended to be numerous. In addition this digestive phase was associated with the secretory cells having few cytoplasmic granules but many vacuoles.

Recovery Studies Low Oil

Day 1

The digestive cells exhibited extensive apical vacuolation interspersed with a large irregular granules. The microvillous was irregular.
Secretory cells appeared atrophic and had numerous vacuoles although, in some animals, granules were also apparent.

Day 2

Digestive cell vacuolation was extensive, however, the apical granules were more regular in shape. Secretory cells exhibited considerable vacuolation and atrophy was apparent.

Day 3

The microvillous appeared to be recovering although there was still considerable vacuolation in the apical cytoplasm of the digestive cells. Secretory cell atrophy was greatly reduced and the cells were gorged with granules and a few vacuoles.

Day 4

Other than a general improvement there was little change from Day 3.

Day 7

An area of enhanced staining in the apical cytoplasm and a well defined microvillous was observed in the digestive cells. Some large vacuoles were still present in the digestive cell cytoplasm and there were many large apical granules. Compared to Day 4 there was an increase in secretory cell vacolation and a decrease in granulation.

Conclusions

Day 3 showed indications of a return to structural normality which was confirmed by Day 4.

Recovery Studies: High Oil

Day 1

The microvillous border appeared to be disrupted due, possibly to the large numbers of apical vacuoles in the digestive cells. Vacuoles in the mid region of the cytoplasm were large and interspersed with waste vacuoles. The large apical granules were regular in shape. Secretory cells exhibited few granules but numerous small vacuoles.

Day 2

There was a general increase in the size of the apical and mid region vacuoles of the digestive cell cytoplasm. Similarly secretory cell vacuolation had increased.

Day 3

A further increase in vacuolation of both digestive and secretory cells was aparent with numerous waste vacuoles in the digestive cells.

Day 4

Apical staining in the digestive tubules was slight with a few small vacuoles. The mid region contained waste vacuoles. There was a reduction in secretory cell vacuolation and an increase in granulation.

Day 7

There was a slight increase in the general apical staining properties of the digestive cells and apical vacuolation was extensive. There was a general reduction in the size of the mid region vacuoles in digestive cells. Secretory cell vacuolation was extensive with little evidence of the secretory granules.

Conclusions

By Day 4 there were indications of a return to structural normality evidenced by a general reduction in apical vacuolation in the digestive cell cytoplasm and an increase in secretory granules.

Exposure Studies: High Oil

Day 1

Although exposure initiated a variable response between animals there was evidence to indicate general disruption of the microvillous border. The large cytoplasmic granules in the digestive cells became irregular in shape and were surronded by a halo of unstained material, very large vacuoles were also apparent in the mid region of the cytoplasm. Large vacuoles were also observed in the secretory cells which contained few granules. There were also indications of general epithelial atrophy.

Day 2

The major difference from Day 1 was an increase in the size and numbers of mid and lower region vacuoles in the digestive cells. Also secretory cells exhibited a slight increase in granulation and a decrease in vacuolation.

Day 4

There was a sharp increase in the numbers of apical vacuoles in the digestive cells and apical granules a exhibiting halo effect. Additionally the apical granules were more irregular in shape and mid region vacuoles tended to be very large. The secretory cells were devoid of granules in some animals and for both cells atrophy was extensive.

Day 7

Neither the digestive or secretory cells were greatly changed from Day 4.

Conclusions

Whilst the trend towards increased vacuolation appears to be indicative of snails voiding unwanted material animals in this study appear to be achieving it with only limited success.

Exposure Studies: Low Oil

Day 1

The digestive cells exhibited an increase in apical vacuolation and disruption of the microvillous border. Many of the large apical granules were irregular in shape and were surrounded by a halo of unstained cytoplasm. The secretory cells did not appear significantly different from the controls.

Day 2

There was an increase in apical vacuolation and irregularity of the large granules. The microvillous border appeared further disrupted. Secretory cell vacuolation was unchanged but granulation had increased somewhat.

Day 4

The numbers of large apical granules was much reduced with an associated increase in apical vacuolation. Some very large vacuoles were observed in the distal portion of the digestive cell cytoplasm and the secretory cell vacuoles showed evidence of coalescing to form larger vacuoles. There was indications of secretory cell atrophy.

Day 7

There was a reduction in apical granulation and an increase in vaculation resulting in a pale apical cytoplasm in the digestive cells. The secretory cells were unchanged from Day 4.

Conclusions

The indications are that by Day 4 the epithelium had become somewhat more stable, although a return to structural normality was not apparent.

SECTION II

SUBLITTORAL SOFT BOTTOM PROJECT

15. General introduction

The first attempt to establish a soft bottom community in the mesocosm at Solbergstrand was performed in April 1983. The sediment brought into the mesocosm was sampled with a Day-grab (a modified Smith.McIntyre type grab without a spring release). The sediment was placed in experimental boxes on board our research vessel, transported to Solbergstrand and placed in the mesocosm. This method for collecting sediment and bringing it into the mesocosm disturbed the sediment considerably but was the only available method at the time.In July and August 1983 some of these boxes turned anoxic due to inadequate water circulation inside the mesocosm (cf.Berge 1983).

A new water supply system was constructed in the basins (a brief description is found in Berge 1983) and new sediment was brought into the basins in December 1983. In order to improve the sediment collecting procedure and thereby reduce the disturbance to the sediment, a USNEL box-corer was used. The box corer was placed at our disposal by Kristineberg Biological station in Sweden. The box-corer takes samples of 0.25 m^2 sections of sediment and brings these sections relativly undisturbed to the surface. Because the box-corer was available to us for only a short time period we were not able to modify the experimental boxes to match the surface area of the box-corer, thus some disturbance of the sediment was unavoidable.

The main experiment in the mesocosm in 1984 has been an organic enrichment experiment performed on the sediment boxes obtained in Desember 1983. This experiment is still not terminated and all samples are not yet analyzed. A preliminary report is however presented in section 2 of this status report.

It seems that the new water circulation system built in the mesocosm has improved the oxygen conditions in the mesocosm sufficiently. There have been no indication of boxes turning anoxic.

Despite the problems of anoxia encountered in some of the first boxes brought into the soft bottom mesocosm at Solbergstrand, several investigations have been performed in the these boxes. These investigations have all been initiated by the observation of an interesting biological phenomena which are not likely to be observed in the field because of lack of accessability and the difficulty of subtidal visual observation. It is believed that these investigations show the unique possibilities for performing detailed ecological investigations in the mesocosm. In the following paragraphs I will give a background of these investigations.

In the experimental boxes which were filled with sediment in April 1983 the terebellid polychaete Streblpsoma bairdi established prominent faecal mounds. This polychaete has tentacles which can extend at least 30 cm from the center of its mound and thus may potentially disturb a surface area of 0.3 m^2. This is aproximatelly the same surface area as sampled by the USNEL box-corer which in the future will be used for community

establishment in the mesocosm. This means that one individual of S.bairdi may potentialy effect a surface area equivalent to an experimental box and thus may bias our results. In order to gain some general information on meifauna abundance in the mesocosm , R.W.Warwich and J.M.Gee from IMER in Plymouth was invited to Solbergstrand. We found it apropriate to perform an investigation on the small-scale effect of S.bairdi on macrofauna and meiofauna abundance, and sediment characteristics inside the mesocosm. A manuscript based on this investigation is almost completed and an abstract of the main results from this investigation is presented in section 3 of this status report.

The feeding behaviour of S.bairdi has also been studied using timelaps photography. This investigation has been performed by H.Rumohr from Kiel, W.G,Ambrose, Jr.(UiO) and J.A.Berge (UiO). H.Rumohr is processing the data. However no report on this investigation is yet available.

An extremly rare interstitial bryozoan (Monobryzoon limicola) was found in the first boxes brought into the mesocosm. This gave us a unique opportunity to sample this species quantitatively and discuss some hypotheses regarding why this species is so seldom found in the field. This work has resulted in a manuscript which will be submitted to Sarsia for publication. The manuscript is found in section 4 of this report.

During the summer 1984 a small branched tubelike structure was observed on the sediment surface in several of the experimental boxes. This structure has later been identified as the test of a foraminifera of the genus Pelosina. We have not yet been able to identify the animal to the species level but it may possibly be a new species (B.Christiansen pers.com.). The spatial distribution of this species in the mesocosm has been investigated by Sofia Gamito from Portugal who has been visiting the University of Oslo for two months. Her visit has been financed by a grant from the Norwegian Ministry of foreign affairs. A manuscript based on this investigation is prepared by S.Gamito,John S.Gray and John A.Berge.

Reference

J.A.Berge (1984) Soft bottom experiments. Status report pr Desember 1.,1983. In:Long term effects of oil on marine benthic communities in enclosures. NIVA report nr. 0-82007.

16. Organic enrichment of subtidal sediments with powdered Aschophyllum nodosum. An experimental study in the soft bottom mesocosm at Solbergstrand.

J.A.Berge, M.Schaanning, and K.Sandøy

Introduction

Subtidal areas of the continental shelf are supplied with organic matter in the form of sedimenting particles. These particles may originate from primary or secondary production in the water column, or from benthic and terrestrial primary production transported to the subtidal benthos. The amount of organic material available to subtidal areas are a fundamental factor which sets the ultimate limits for secondary production in the subtidal. Other environmental factors (polution,anoxia ,currents, biological interactions) may prevent secondary production to reach the limit set by the food supply. Transport of contaminants from the surface water masses to the subtidal benthos may be linked to sedimenting particles (Gearing et al.1980), thus showing the importance of this process. In future experiments at Solbergstrand it is intended to study the faunal and porewater responses to natural and antropogenic substances arriving at the sediment surface.

In the spring of 1984 we had 20 experimental boxes with sediment collected by the USNEL box-corer available for experimentation. Since future experiments to be carried out in the mesocosm will be related to natural and antrophogenic substances incorporated with subtidal sediments, we decided to start a pilot experiment by dosing boxes with organic matter. Our intention with this experiment was (1) to see how the fauna and porewater in the sediment in the mesocosm behave in response to organic enrichment and (2) gain some quantitative information on how much the sediment brought into the mesocosm can receive before the porewater chemistry is changed sufficiently to effect macrofauna densities. Experience from these experiments will be used in designing future experiments in the mesocosm.

Material and Methods

Sediments for the experiments were collected with a USNEL-box-corer at Bjørnehodebukta in the Oslofjord 12 km north of Solbergstrand (Figure 1) on the 13 December 1983. The box-corer samples 0.25 m^2 of the sediment to a depth adjusted to aproximatly 35 cm. The sediment were placed in plastic boxes (x) with

a surface area of 0.54 m^2 and a depth of 29 cm. The box-corer penetrates the sediment 10 cm more than the inner hight of the boxes and because the surface area of the experimental boxes is larger than the surface area of the box-corer, the sediment slides out laterally when introduced into the boxes. The sediment depth in the boxes is thus reduced to aproximatly 10 cm below the rim of the experimental boxes.One box-corer sample was placed in each box. In addition aproximatly 20 l of sediment from another box-corer sample was introduced into the box in order to fill a depression at one end, thus creating a sediment depth of 20 cm throughout the entire box. A total of 20 boxes of sediment were brought into the soft bottom mesocosm at Solbergstrand on the day of sampling.

The powdered Aschophyllum nodosum used was purchased from Alge producter A/S, Kristiansund, Norway. The product used (A120) contains 31-32 % carbon , 0.9 % nitrogen and 8.3% water. The mean chemical composition of the powder as claimed by the manufacturer and analyzed at Institutt for marin biokjemi,NTH,Trondheim is presented in table 1. The particle size of the powdered algea is less than 120 µm. Two levels of dosing were used, a high level equivalent to 200 gC/m^2 yr and a low level of 50 gC/m^2 yr. Eight boxes were used for each dosing level. Four boxes were not dosed and used as controls. The low level dosage is approximatly the amount of carbon the benthic system receives in a year in the Kiler Bight (Smetacek,1980).

The water level in the mesocosm was drained down on the day of dosing so that the sediment in the boxes were only covered with water up to the rim of the box (10 cm). A rectangular frame of the same dimensions as the surface area of the experimental boxes was placed on top of each box in order to extend the sides up above the water surface in the box. The calculated amount of powdered Aschophyllum was sprinkled in each box as evenly as possible using a box with a perforated lid . The powdered Aschophyllum accumulated on the water surface and was allowed to settle down to the sediment surface for 6 hours before the water level in the basins was raised again. The boxes receiving different treatments were assigned out randomly.

On each sampling date four boxes (2 high dosage and 2 low dosage) were picked randomly for sampling. Core samples were taken in these boxes for chemical and faunal analysis (samlping scheme can be seen in table 2). After taking the cores ,the remaining sediment in each box was sieved through a 1 mm sieve in order to quantify the macrofuna in the boxes.Core samples were sectioned at 3 cm intervals , preserved in 10 % neutralized formalin and stored seperatly. All core samples were stained with Rose Bengal and washed through a double set of 500 µm and 250 µm sieves. The fauna was sorted and identified under a binocular dissecting micrscope.

Cores for chemical analyses were collected in 25 cm long plexiglass tubes with an inner diameter of 6 cm. In general three cores were taken from each box. One was immediately processed for electrode measurements (pH, Eh, $Es^{=}$) within the overlying water and down the core at 2 cm intervals. The other two were stored at -20 °C and transported to the laboratory in Oslo for subsampling 2-20 weeks later.

The two cores were sectioned in 1, 2 and 4cm thick slices. Corresponding samples were put together and thawed in a nitrogen atmosphere before squeezing through PVC reinforced filters with a pore size of .45µm, using nitrogen gas pressure.

The water content was determined gravimetrically by drying to constant weight of a homogenized sample. Alkalinity was determined by acid addition to aliquots of the pore water and titration of the excess acid. Dissolved inorganic nutrient species (NH4, NO2, NO3 and PO4) were determined on appropriately diluted pore water using common methods for seawater analyses, modified for the autoanalyser used at our department.

The squeezed sediment was freze-dried, homogenized and stored in sealed glass containers. CHN analyses will be carried out using a Carlo-Erba elemental analyser. Inorganic carbon will be determined by acidification and analyses of the liberated CO2 on an IR gas analyser. The difference between these two carbon determinations will be taken as the organic carbon content of the sediments. Organic nitrogen will be assumed to correspond to the nitrogen determined on the elemental analyser.

Dissolved sulphide was determined using a Radiometer sulphide ion selective electrode. Several calibrations of the electrode in strongly alkaline sulphide standard solutions gave curve equations corresponding closely to:

$$Es^{=} = .66 + .0295\ pS^{=}$$

Total dissolved sulphide could thus be calculated from the equation:

$$pS_t = 13.9 - pH - (Es^{=} + .66)/.0295 + \log(10^{(7-pH)} + 2.22)$$

in which $pS_t = -\log([H_2S] + [HS^-] + [S^{=}])$.

The great advantage of the method is the simplicity of the measurements, but the accuracy is probably not better than $\pm .5\ pS_t$ units.

PRELIMINARY RESULTS AND DISCUSSION.

The results of the chemical analyses available by the time of writing this report are cited in tables 3 and 4. Selected profiles of pore water composition are shown in fig.2 (a, b and c). In fig.3 (a and b) the variations with time of the pore water composition is shown for the top 4cm, the 4-8cm layer and below 8cm.

The addition of the low dose (50 gC/m^2) of powdered algae produced no clear change of the chemical properties of the pore water. The redox boundary remained at 2-4 cm depth. Weak sulphide activities and a slight increase in alkalinity and the concentrations of PO4 and NH4 were generally observed within the deeper strata. High concentrations of nitrite were found at depths corresponding to the upper part of the redox gradient.

In the high dose boxes (200 gC/m^2), however, changes relative to the low dose boxes were observed from the first sampling, which was done only two days after the addition of the algae. Concentrations of ammonia, phosphate and alkalinity were considerably higher within the 0-4 cm layer, and the pH was slightly lower than in the low dose boxes.

Three weeks later ammona, phosphate and alkalinity had decreased in the 0-1 cm section, but the subsurface levels of PO4 and At had increased. Simultaneously the redox cline had become displaced towards the sediment-water interface, at which mats of bacteria, probably sulphide oxidizing Beggiatoa, were visible. High sulphide ion activities were observed throughout the cores, but not in the overlying water.

Maxima of pS_t and At as well as remarkable pH-minima had evolved at 2-4 cm depth. In box nr. EA5 pH values as low as 2.0 and 3.4 were recorded at 2-4 and 4-6 cm depths, respectively. The rest of the pH values in the two high dose boxes ranged between 5.91 and 7.15. Under normal circumstances, bicarbonate ions is the major contributor to the alkalinity of marine samples, but at a pH of 2-3 the equilibrium would be displaced towards carbonic acid which does not contribute to alkalinity. Thus it might seem odd to observe high alkalinities in samples having a very low pH value. It should be noted, however that after squeezing the pH of the pore water ranged between 7 and 8 throughout these cores. (Apparently some reactions (e.g. dissolution of carbonates or silica minerals) which could increase the pH of the pore water had proceeded during storage and subsampling). Thus the pH of the pore water, from which the subsample for alkalinity determination was taken, were normal, and the high alkalinities observed in the acidic sediment could still result from high concentrations of $HCO3^-$ ions.

Consistant with the high rates of decomposition of organic matter indicated by some parameters, we would expect the presence of high concentrations of ammonium ions in the high dose boxes sampled in May. However, a general decrease in the abundancy of

this specie was observed during the first three weeks of the experiment. Within the most acidic pore water environment concentrations of NH_4 were well below initial values. To our knowledge, such observations have not been reported in the litterature, and so far we have no explanation to the phenomenon.

Another 2.5 months later, the increase relative to initial concentrations of alkalinity, sulphide and phosphate was observed at even greater depths in the high dose boxes. Broad peaks of At and PO_4 were observed at 6-10cm and 2-6cm depth, respectively. High concentrations of PO_4 were again present at the surface and subsurface minima of NH_4 and At as well as PO_4 were observed at 1-2cm depth.

The results show that the addition of 200 gC/m^2 to the sediment surface strongly alters the pore water environment. Mineralisation products penetrated during the experiment to succesively greater depths. The altered pH of the pore water down to depths of at least 14 cm as early as three weeks after the manipulation, is most likely to be a result of a rapid (downwards) transport of protons due to the high self-diffusion coefficient (5-28 times the coefficients of most other ions, Li and Gregory, 1974) and the steep concentration gradients provided by the acidification of the surface layer. In comparison, three months after the manipulation, increased levels of At and PO_4 could not be detected at depths greater than 12 and 7 cm, respectively. Further discussion of the details of the pore water profiles, is postponed untill all results are available.

The low pH observed in box nr.EA5, is abnormal compared to pH values found in natural marine sediments. Extreme pH values may alter the chemical equilibria controlling pore water composition and may also have direct and unwanted effects on the organisms living there.

The alginic acid content of 20-26% (table 1), is according to G.Skjaak-Bræk, Institute of Marine Biochemistry, NTH (pers. comm.), present as calcium salt and should therefore, not contribute to the pH-lowering. Furthermore, these acids are weak acids and could thus hardly account for a pH as low as those recorded in EA5.

Assuming the transformation of 50% of the sulphur content of the seaweed meal (table 1) to sulphuric acid, the load of 200 gC/m^2 would imply an input of ca .2 mol H_2SO_4/m^2. Evenly distributed within the top 5 cm of the sediments the resulting pH would be ca 2. Thus, a heavy production of sulphuric acid might break down the carbonate buffer system and produce low pH values.

A simple experiment was performed to investigate the impact of the seaweed meal on the pH of aqueous solutions. Aliquots of the seaweed meal was suspended in destilled water and in filtered seawater with an initial pH of 8.2. The suspensions were left at room temperature on a mechanical shaker, and the pH was occasionally measured over a two weeks time interval. During the incubation period the pH in the destilled water suspension ranged between 6.5 and 6.8, whereas in the seawater suspensions pH values of 5.8 to 6.1 were recorded. The facts that the lowering of the pH occured immediately and that the variations during the

incubation period were small, indicates that the acidification was not produced by biological activity. At one occasssion a temporary lowering to a pH of 3.5 was observed in the seawater suspensions, but we did not succeed in reproducing this result.

However, replacing the commercial Ascophyllum nodosum meal, with freeze-dried, powdered Enteromorpha intestinalis, the seawater suspensions produced a range of pH of 7.7-8.6 during a 10 days incubation period. If cations of the seawater exchange with hydrogen ions of the Ascophyllum meal, the lower pH of the seawater suspensions as compared to the destilled water would be expected. Whatever the cause of the acidification is, it seems that the problem in a future experiment could be overcome by the use of Enteromorpha intestinalis, which is abundant in littoral communities of the Oslofjord.

The macrofaunal abundances found in the boxes are given in table 5 and 6. Community parameters are presented graphically in Figure 4 and 5. A total of 91 species were found in the 10 boxes sieved. This is approximatly the number of species found in 5 replicate grab samples (Day grab, 0.1 m^2) at Bjørnehodebukta (Valderhaug and Gray 1984). The total abundance of individuals in boxes dosed with 50 gC/m^2 did not change discernibly from May to September, however the number of species seems to increase linearly and may indicate recruitment. In the boxes dosed with 200gC/m^2 both total abundance and number of species were reduced in May (1 month after dosing). In September both density and number of species had increased compared to the results from May. In May the number of individuals and the number of different species in boxes dosed with 200 gC/m^2 were lower than in boxes dosed with 50 gC/m^2. This is due to a higher mortality in the high dose boxes than in the lowe dose boxes (cf. Table 8). In september the total density is higher in the high dose boxes as compared to the low dose boxes, whereas the number of species is still lower in the high dose boxes. The increased density in the high dose boxes is mainly caused by a dramatic increase in density of Capitella capitata which is virtually non excisting in the low dose boxes.

Mean macrofauna density in Bjørnehodebukta are relative constant throughout the year with a mean annual abundance of 1953 individuals pr 0.5 m^2 (Valderhaug and Gray 1984). Because of to few boxes only core samples have been taken in control boxes. No whole control boxes have been sacrified for sieving yet (Control boxes will be sieved on the last sampling day). Comparison of field and mesocosm samples are therefore performed between mesocosm samples from the lowe dose boxes in September and the field results of Valderhaug and Gray 1984.

The sediment in a box comes originally from one box-corer sample with a sufrace area of 0.25 m^2, in addition to this approximatly 20 l of sediment (surface are=0.1 m^2) was put in each box. Thus the sediment in one experimental box is comparable to a surface area of 0.35 m^2 in the field. The surface area of a box is 0.54 m^2. If the surface area of 0.35 m^2 is used as a basis for our comparison the mean density in the low dose boxes

in September (264 individuals pr. box) is approximatly 20 % of the mean annual field density of 1953 individuals pr 0.5 m^2 . If we use the surface area of the box as the basis for our comparison the density is approximatly 12.5 % of the field density. It is thus clear that the total density in the low dose boxes in September is lower than in the field's annual mean. No single species is significantly more abundant in the low dose boxes as compared to the field, however there are species which are abundant in the field which are not represented in the boxes.

By comparing the density found in the core samples (See table 7)(sieved with a 500 µm sieve) with the density found in in the whole boxes (sieved with 1 mm sieve) it is clear that only 10 - 29 % of the individuals are retained by using a 1mm sieve. Only negligible amounts of the permanent meiofauna are retained on a 500 µm sieve.

The addition of powdered algae to the sediment surface has caused changes in the pore water chemistry of the sediment particulary pH, Eh and the concentration of H_2S (Table 3). The increased mortality in the boxes dosed with 200gC/m^2 is caused by one or a combination of these changes. It seems that in the high dose boxes both macrofauna density and number of species increased after the initially increased mortality. We conclude that a dose of 200gC/m^2 of powdered A.nodosum to the sediment changes the porewater chemistry so that the macrofaunal community is stressed and community parameters are changed. This causes a reduction in number of species and total density. However, while pore water chemistry is normalizing the community shows signs of restoration. The lowe dosage did neither result in dramatic changes in porewater chemistry nor in major macrofaunal responses.

To avoid major changes in porewater chemistry and increased mortality, future experiments should not receive doses of more than 50 gC/m^2 of A.nodosum.

The method used for establishing the community in the mesocosm was not ideal because of the incompatibility between the size of the box-corer and the experimental boxes. The sediment was disturbed in the process of transporting it from the corer to the experimental boxes. This could explain some of the differences between mesocosm and field densities. For future experimentation an improved system for bringing undisturbed sections of the bottom into the mesocosm have been designed and tested with good results. In this system liners are placed inside the box-core prior to sampling. When the box-corer returns to the surface after sampling the liner with the sediment is removed and a bottom plate fastened to the bottom of the liner so that it functions as a box which can be placed inside the mesocosm. Using this procedure the disturbance caused by moving the sediment from the box-corer into another box is avoided.

References

Gearing,P.J, J.N.Gearing, R.J.Pruell, T.L.Wade and J.G.Quinn (1980) Partitioning of No.2 Fuel oil in Controlled Estuarine Ecosystems. Sediment and Suspended Particulate Matter.

Environmental Science & Technology, 14(9), 1129-1136

Li,Y.H. and S.Gregory (1974) Diffusion of ions in sea water and deep-sea sediments.
Geochim.Cosmochim.Acta,38(1),703-714.

Smetacek,V., (1980) Annual cycle of sedimentation in relation to plankton ecology in Western Kiel Bight.

Ophelia,Suppl.1, 65-76.

Valderhaug,V.A. and J.S.Gray. (1984) Stable macrofauna community structure despite fluctuating food supply in subtidal soft sediments of Oslofjord,Norway.

Mar.Biol.,84,307-322.

Table 1. Average composition of norwegian seaweed meal, produced from Ascophyllum nodosum .

We herewith verify that Norwegian seaweed meal (Ascophyllum nodosum) according to numerous analysis is expected to show the average composition given below.

Component	Content	Component	Content
Moisture	12-15 %	Crude fibre	< 8 %
Ash	17-20 %	Crude protein	5-10 %
Alginic acid	20-26 %	Ether extract	2- 4 %
Mannitol	5- 8 %	Fucoidin	~ 10 %
Laminaran	2- 5 %	N-free extractives	45-60 %
S	2.5-3.5 %	Caloric value	0.56 SFU/kg*
K	2- 3 %	Ascorbic acid	500-2000 mg/kg
Cl	3.1-4.4 %	Carotene	30- 60 mg/kg
Na	3- 4 %	Biotin	0.1- 0.4 mg/kg
Mg	0.5-0.9 %	Folic acid	0.1- 0.5 mg/kg
Ca	1- 3 %	Folinic acid	0.1- 0.5 mg/kg
P	0.1-0.15 %	Niacin	10- 30 mg/kg
B	40-100 mg/kg	Riboflavin	5- 10 mg/kg
Co	1- 10 mg/kg	Thiamin	1- 5 mg/kg
Cu	1- 10 mg/kg	Tocopherols	150- 300 mg/kg
Fe	150-1000 mg/kg	Vit. B12	0.004 mg/kg
Mn	10- 50 mg/kg	Vit. K	~ 10 mg/kg
I	700-1200 mg/kg	V	1.5- 3 mg/kg
Zn	50- 200 mg/kg	Ni	2- 5 mg/kg
Mo	0.3- 1 mg/kg	Ba	15- 50 mg/kg

*SFU = Scandinavian feed units.

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Analysis performed at the University of Oslo gives a total carbon content of 31-32 % and a total nitrogen content of 0.9 %.

Table 2. Sampling scheme for the organic enrichment experiments in the soft bottom mesocosm at Solbergstrand in 1984. Number of boxes cored = (n). The dosing level in the boxes sampled are denoted with 0 , 50 and 200 indicating the grams of carbon /m² added to the boxes.

	Date	No of boxes sieved			No of cores samles in each box		
		50	200	0	50	200	0
Community establishment	13.12.83	-	-	-	-	-	-
1. sampling							
Fauna		-	-	-	-	-	3(4)
Dosing	24.04.84	-	-	-	-	-	-
2. sampling							
Chemistry	26.04.84				3(1)	3(1)	-
Fauna	30.04.84	1	1	-	8(2)	8(2)	-
3. sampling							
Chemistry	24.05.84	-	-	-	3(1)	3(1)	-
Fauna	24.04.84	2	2	-	8(2)	8(2)	-
4. sampling							
Chemistry	15.08.84				3(1)	3(1)	-
Fauna	04.09.84	2	2	-	8(2)	8(2)	4(2)
5. sampling							
Chemistry	21.11.84	-	-	-	3(1)	3(1)	3(1)
Fauna	22.11.84	-	-	-	8(2)	8(2)	8(2)

Table 3. Electrode measurements. $pS_t = -\log([H_2S] + [HS^-] + [S^=])$. pS_t was calculated from pH and Es= (see text). Potentials are referred to the standard hydrogen electrode.

Core nr.: EA8 Date: 26.04.84 Dose: 50gC/m

Depth (cm)	pH	Eh	Es=	pS_t
		---- mV ----		
OW	8.00	313	nd	-
0-2	7.48	256	nd	-
2-4	7.42	-74	-301	5.27
4-6	7.40	-73	-303	5.18
6-8	7.49	-101	-285	5.90
8-10	7.57	-111	-261	6.80
10-14	7.65	-166	-351	3.84

Corenr.: WA3 Date: 26.04.84 Dose: 50 gC/m

Depth (cm)	pH	Eh	Es	pS
		---- mV ----		
OW	7.93	344	-	-
0-2	7.54	59	-191	-
2-4	7.40	-96	-249	7.01
4-6	7.40	-111	-304	5.15
6-8	7.52	-146	-311	5.05
8-10	7.57	-111	-289	5.85
10-12	7.68	-171	-337	4.34
12-14	7.54	-166	-368	3.12
14-18	7.60	-154	-372	3.07

Core nr.: EA4 Date: 26.04.84 Dose: 200 gC/m

Depth (cm)	pH	Eh	Es	pS
		---- mV ----		
OW	7.98	367	nd	-
0-2	7.39	109	-141	-
2-4	7.27	-106	-296	5.27
4-6	7.32	-146	-259	6.58
6-8	7.48	-125	-251	7.04
8-10	7.58	-66	-226	-
10-14	7.61	129	-176	-

Core nr.: WA4 Date: 26.04.84 Dose: 200 gC/m

Depth (cm)	pH	Eh	Es	pS
		---- mV ----		
OW	8.08	278	-	-
0-2	7.30	141	-161	-
2-4	7.35	-65	-323	4.45
4-6	7.38	-89	-322	4.52
6-8	7.48	-21	-290	5.72
8-10	7.55	-86	-249	7.18
10-14	7.73	89	-276	6.47

Table 3 . Continued...

Core nr.: EA3 Date: 24.05.84 Dose: 50 gC/m

Depth (cm)	pH	Eh ---- mV ----	Es	pS
OW	7.94	400	-	-
0-2	7.47	263	-65	-
2-4	7.49	138	-52	-
4-6	7.59	23	-222	-
6-8	7.66	108	-206	-
8-10	7.78	-52	-202	-
10-14	7.70	183	-227	-

Core nr.: WA1 Date: 24.05.84 Dose: 50 gC/m

Depth (cm)	pH	Eh ---- mV ----	Es	pS
OW	7.71	249	-129	-
0-2	7.05	43	-235	7.08
2-4	7.04	-37	-260	6.22
4-6	7.14	-52	-277	5.77
6-8	7.10	-142	-322	4.19
8-10	7.26	-133	-351	3.41
10-12	7.17	-112	-366	2.79
12-14	7.21	-97	-367	2.80

Core nr.: EA5 Date: 24.05.84 Dose: 200 gC/m

Depth (cm)	pH	Eh ---- mV ----	Es	pS
OW	7.44	113	-326	4.46*
0-2	6.16	-42	-307	3.28
2-4	2.0	137	-102	2.03**
4-6	3.4	69	-153	3.10**
6-8	6.29	-107	-315	3.23
8-10	5.91	-63	-289	3.44
10-12	7.02	-127	-317	4.26
12-14	7.00	-14	-293	5.05

Core nr.: WA7 Date: 24.05.84 Dose: 200 gC/m

Depth (cm)	pH	Eh ---- mV ----	Es	pS
0-2	6.57	-72	-331	3.14
2-4	6.64	-128	-350	2.61
4-6	6.83	-147	-348	2.95
6-8	7.09	-131	-351	3.20
8-10	7.15	-144	-352	3.24

* Probably polluted with pore water during sampling.

** Values are uncertain because of low sulphide ion activity in acid environment.

Table 3. Continued...

Core nr.: C5 Date: 24.05.84 Dose: 0

Depth (cm)	pH	Eh (mV)	Es (mV)	pS
OW	7.26	293	-107	-
0-2	7.20	93	-121	-
2-4	7.03	-17	-248	6.61
4-6	7.28	-25	-226	7.73
6-8	7.14	-22	-260	6.34
10-14	7.25	-82	-327	4.21

Core nr.: EA5 Date: 15.08.84 Dose: 50 gC/m

Depth (cm)	pH	Eh (mV)	Es (mV)	pS
OW	7.69	314	-115	-
0-2	7.15	208	-119	-
2-4	6.96	80	-229	7.15
4-6	7.15	-35	-270	6.00
6-8	7.19	-86	-330	4.02
8-10	7.24	-67	-319	4.45
10-14	7.30	-36	-262	6.45

Core nr.: WA8 Date: 15.08.84 Dose: 50 gC/m

Depth (cm)	pH	Eh (mV)	Es (mV)	pS
OW	7.69	380	-	-
0-2	7.41	283	-24	-
2-4	7.36	106	-19	-
4-6	7.40	46	-284	5.82
6-8	7.34	-4	-244	7.11
8-10	7.30	-15	-302	5.10

Core nr.: EA7 Date: 15.08.84 Dose: 200 gC/m

Depth (cm)	pH	Eh (mV)	Es (mV)	pS
OW	7.70	234	-123	-
0-2	7.00	-69	-363	2.66
2-4	6.85	-136	-361	2.52
4-6	7.11	-159	-336	3.71
6-8	7.17	-150	-373	2.55
8-10	7.41	-159	-367	3.02
10-13	7.34	-136	-378	2.57
13-15	7.39	-139	-369	2.93

Table 3. Continued...

Core nr.: WA6 Date: 15.08.84 Dose: 200 gC/m

Depth (cm)	pH	Eh ---- mV	Es ----	pS
OW	7.79	321	-	-
0-2	6.88	-80	-376	2.05
2-4	6.97	-124	-371	2.35
4-6	7.07	-129	-382	2.11
6-8	7.24	-138	-380	2.38
8-10	7.20	-134	-358	3.10
10-12	7.41	-113	-370	2.92
12-14	7.47	-106	-361	3.29

Core nr.: C8 Date: 15.08.84 Dose: 0

Depth (cm)	pH	Eh ---- mV	Es ----	pS
OW	7.77	328	-	-
0-2	7.14	174	-94	-
2-4	7.26	34	-134	-
4-6	7.41	-110	-325	4.46
6-8	7.38	-163	-374	2.76
8-10	7.48	-169	-385	2.49

Table 4. Pore water analyses. "nd" means not detectable; "-" means not analysed.

Core nr.: EA8 Date: 26.04.84 Dose: 50 gC/m

Depth (cm)	Water content (Wt %)	Alkal-inity (meq)	PO4	NH4	NO2	NO3
			ugat/l			
0-1	69.4	-	4.5	42	.2	
1-2	58.7	2.64	6.9	63	1.1	
2-4	51.4	2.42	10.4	103	.1	
4-6	48.3	2.14	13.6	79	.1	
6-8	50.2	2.32	12.4	82	-	
8-10	48.9	2.28	21.7	103	-	
10-14	45.0	2.89	16.9	194	-	

Core nr.: WA3 Date: 26.04.84 Dose: 50 gC/m

Depth (cm)	Water content (Wt %)	Alkal-inity (meq)	PO4	NH4	NO2	NO3
			ugat/l			
OW	-	-	1.9	6.3	.2	
0-1	70.1	3.50	3.6	57	3.0	
1-2	59.5	3.71	3.3	83	3.4	
2-4	53.2	3.09	4.5	133	.4	
4-6	49.3	2.64	7.8	90	.3	
6-8	47.9	2.72	17.5	73	.5	
8-10	47.9	2.84	26.2	87	.4	
10-14	45.7	2.96	19.6	113	.6	

Core nr.: EA4 Date: 26.04.84 Dose: 200 gC/m

Depth (cm)	Water content (Wt %)	Alkal-inity (meq)	PO4	NH4	NO2	NO3
			ugat/l			
OW	-	-	2.9	15.6	.2	
0-1	82.2	3.65	41.2	214	1.9	
1-2	65.0	4.22	21.7	364	1.6	
2-4	56.4	3.16	14.5	219	.2	
4-6	49.3	2.63	9.1	122	nd	
6-8	47.3	2.88	7.8	144	.1	
8-10	45.3	2.98	12.3	151	.1	
10-14	42.1	2.91	10.0	134	.4	

Table 4. Continued...

Core nr.: WA4	Date: 26.04.84		Dose: 200 gC/m			
Depth (cm)	Water content (Wt %)	Alkal-inity (meq)	PO4	NH4	NO2	NO3
			ugat/l			
OW	-	-	3.1	8.2	.3	
0-1	78.9	3.69	18.1	104	1.5	
1-2	66.9	3.69	11.6	333	3.6	
2-4	53.3	3.39	9.4	254	.8	
4-6	49.1	2.63	6.3	152	.1	
6-8	49.0	2.71	9.1	118	-	
8-10	50.1	2.74	13.3	99	-	
10-14	46.8	2.73	15.4	87	-	

Core nr.: EA3	Date: 24.05.84		Dose: 50 gC/m			
Depth (cm)	Water content (Wt %)	Alkal-inity (meq)	PO4	NH4	NO2	NO3
			ugat/l			
OW	-	-	1.5	7.4	1.1	
0-1	74.9	3.51	5.0	124	5.4	
1-2	66.5	3.05	9.7	122	.6	
2-4	59.2	3.13	9.7	108	.1	
4-6	52.5	3.00	8.6	141	.1	
6-8	48.1	2.82	9.2	110	-	
8-10	48.9	2.71	20.4	93	-	
10-14	44.4	2.81	12.7	71	-	
14-18	42.6	2.87	16.1	87	-	

Core nr.: WA1	Date: 24.05.84		Dose: 50 gC/m			
Depth (cm)	Water content (Wt %)	Alkal-inity (meq)	PO4	NH4	NO2	NO3
			ugat/l			
OW	-	-	1.2	9.4	.6	
0-1	76.7	3.25	13.7	123	2.5	
1-2	65.6	2.90	15.7	170	.4	
2-4	59.3	3.01	20.7	136	.1	
4-6	49.9	3.23	16.9	132	-	
6-8	47.1	3.54	18.4	142	.2	
8-10	43.7	3.53	23.5	135	.1	
10-14	45.0	3.60	20.2	-	.2	

Table 4. Continued...

Core nr.: EA5 Date: 24.05.84 Dose: 200 gC/m

Depth (cm)	Water content (Wt %)	Alkal-inity (meq)	PO4	NH4	NO2	NO3
			ugat/l			
OW	-	-	3.3	4.8	.2	
0-1	75.6	3.03	24.6	31	.7	
1-2	62.5	5.44	26.8	13	.5	
2-4	54.0	7.88	20.5	40	.1	
4-6	50.1	6.72	15.5	142	.2	
6-8	47.7	5.50	14.6	189	.1	
8-10	47.7	4.32	13.4	166	.1	
10-14	46.5	3.94	22.8	181	.1	

Core nr.: WA7 Date: 24.05.84 Dose: 200 gC/m

Depth (cm)	Water content (Wt %)	Alkal-inity (meq)	PO4	NH4	NO2	NO3
			ugat/l			
OW	-	-	2.9	-	.4	
0-1	77.0	3.42	28.5	56	1.7	
1-2	62.2	4.12	23.1	123	1.1	
2-4	54.7	5.78	19.6	94	.3	
4-6	51.4	5.52	15.1	94	.1	
6-8	48.2	5.07	13.7	107	-	
8-10	44.2	4.22	13.0	93	-	
10-14	42.8	4.05	9.7	109	-	

Core nr.: EA6 Date: 15.08.84 Dose: 50 gC/m

Depth (cm)	Water content (Wt %)	Alkal-inity (meq)	PO4	NH4	NO2	NO3
			ugat/l			
0-1	71.9	3.07	15.1	168	2.8	
1-2	62.6	2.43	12.7	125	1.2	
2-4	56.6	2.57	9.4	90	.2	
4-6	53.7	2.72	20.2	134	.2	
6-8	50.3	2.77	12.7	121	.2	
8-10	47.3	2.80	14.5	118	.1	
10-14	46.9	2.95	10.9	118	nd	

Table 4. Continued...

Core nr.: WA8 Date: 15.08.84 Dose: 50 gC/m

Depth (cm)	Water content (Wt %)	Alkal-inity (meq)	PO4	NH4	NO2	NO3
			ugat/l			
OW	-	-	.9	3.4	.2	
0-1	73.2	4.20	9.7	114	6.2	
1-2	62.8	2.78	9.4	113	1.1	
2-4	57.2	2.92	10.9	87	.3	
4-6	51.0	2.85	12.5	101	.1	
6-8	46.5	2.66	9.5	82	-	
8-10	45.2	2.97	13.4	110	-	
10-14	46.0	3.32	13.9	128	-	

Core nr.: EA7 Date: 15.08.84 Dose: 200 gC/m

Depth (cm)	Water content (Wt %)	Alkal-inity (meq)	PO4	NH4	NO2	NO3
			ugat/l			
OW	-	-	1.9	5.8	.3	
0-1	84.7	3.16	45.5	135	1.6	
1-2	67.7	3.15	14.6	98	.6	
2-4	56.3	3.56	16.3	75	nd	
4-6	49.8	3.95	17.3	84	nd	
6-8	47.5	-	17.5	96	nd	
8-10	45.2	4.44	15.7	115	nd	
10-14	42.8	4.01	14.8	112	nd	
14-18	43.0	3.56	15.5	112	nd	

Core nr.: WA6 Date: 15.08.84 Dose: 200 gC/m

Depth (cm)	Water content (Wt %)	Alkal-inity (meq)	PO4	NH4	NO2	NO3
			ugat/l			
OW	-	-	2.2	-	.2	
0-1	78.6	4.01	32.9	166	1.8	
1-2	63.4	3.39	21.1	123	.7	
2-4	58.1	3.74	30.9	178	.4	
4-6	52.8	4.14	29.0	176	.1	
6-8	48.2	4.65	19.5	114	-	
8-10	46.9	4.66	21.7	126	-	
10-14	45.7	3.91	20.1	144	-	

Table 5 . Number of individuals (retained on a 1mm siev) from boxes dosed with 50 g carbon/m

Sampling date	30.04.84	24.05.84		04.0984	
Box nr.	VA3	ØA3	VA1	ØA6	VA8
Species					
Turbellaria indet.				2	3
Trematoda ?	1				
Nemertinea indet.	1	3	2	3	2
MOLUSCA					
Gastropoda indet					
Chaetoderma nitidium		4			
Leucon nasica	1				
Musculus cf.marmoratus		1			
Mya truncata	2		1	1	
Nudibranchia indet.					5
Nuculoma tenuis	12	7	9	8	6
Techibranchia indet.				4	1
Thyasira sp.	26	1	3	4	1
Saxicava sp.				1	
Mytilacea juv.				1	1
Spisula eliptica		2	1		3
Bivalvia E	2				
ANNELIDS					
Amphicteis gunneri					1
Anaitides maculata					
Capitella capitata				1	
Capitella sp.					
Cirratulus cirratus	1	2			3
Chaetozone setosa	11	1	13	7	8
Chone sp.		1			
Dipolcirrus glaucus	4		2		1
Diplocirrus setosa					
Dorvellidae ?					
Eteone longa					
Glycera alba	6	7	4	4	5
Goniada maculata	7	6	1	6	4
Harmothoe sp.				1	3
Harmothoe cf. imbricata		1			
Hetromastus filiformis	135	261	162	79	74
Lumbrineris fragilis	2	26	18	19	7
Lumbrineris sp.				1	
Maldane sarsi	1				
Mediomastus fragilis	8		3	6	65
Melinna cristata		2	1	1	2
Nereimyra punctata					
Nereis cf. virens ?					
Ophelina acuminata					3
Ophiodromus flexuosus				2	1

Paraonis fulgens				2	2
Pholoe minuta			2	3	11
Polycirrus sp.					1
Polydora caulleryi					
Polydora socialis					1
Polyphysia crassa				2	
Praxillella sp.				4	
Prionospio malmgreni			1	1	3
Prionospio cirrifera				2	2
Sabella peneicillus					
Sabella sp.					3
Scoloplos armiger					1
Scalibregma inflatum	4	7	2	3	8
Sosane gracilis	13	5	16	29	33
Spiochaetopterus typicus	1	2	2	2	1
Synelmis klatti			1		
Trochochaeta multisetosa		2	1	2	2
Trichobranchus sp.			1		
Typosyllis variegata	2	1	2		4
Oligochaeta indet.					1
CRUSTACEA					
Copepoda (Calanoidea)		1			
Copepoda (parasitic)				1	
Cirripedia				1	
Euphausiacea indet.		1			
Palemonidae				3	
Eudorella truncatula					
Amphipod					
Amphipoda A			1		
Amphipoda B			1		
Amphipoda D					
Amphipoda E				1	
Amphipoda F				1	
Corophidae ?					
Priapulida			1		
Sipunculidae					1
Pycnogoidae					1
ECHINODERMATA					
Ophiura sp.				26	8
Ophiura filiformis			1		
Asteroidea indet. juv.				9	2
Acideacea indet	2	1			
Number of individualds	242	345	252	243	284
Number of species	20	23	26	36	39
Shannon-Weaver diversity	2.59	1.69	2.29	3.77	3.76
Pileou's index of evenness	0.59	0.37	0.49	0.78	0.71

Table 6 . Number of individuals (retained on a 1 mm siev) found in boxes dosed with 200 g carbon/m.

Sampling date	30.04.84	24.05.84		04.09.84	
Box nr.	VA4	VA7	QA5	ØA7	VA6
Species					
Turbellaria indet.					5
Trematoda ?		2			
Nemertinea indet.	1			2	3
MOLUSCA					
Gastropoda indet	1				
Chaetoderma nitidium					
Leucon nasica	1				
Musculus cf.marmoratus					
Mya truncata	4		1	1	
Nudibranchia indet.				1	7
Nuculoma tenuis	4	7	4	9	2
Techibranchia indet.					1
Thyasira sp.	3	1	1	1	6
Saxicava sp.					
Mytilacea juv.					2
Spisula sp.			1		1
Bivalvia E					
ANNELIDS					
Amphicteis gunneri		3			
Anaitides maculata				1	
Capitella capitata				91	148
Capitella sp.				1	
Cirratulus cirratus	1	2	5		
Chaetozone setosa	15	5		5	6
Chone sp.					
Dipolcirrus glaucus					1
Dorvellidae ?				1	
Eteone longa		1		1	
Glycera alba	6	1		5	
Goniada maculata	5	5	7	5	7
Harmothoe sp.					
Harmothoe cf. imbricata					
Hetromastus filiformis	213	217	84	121	276
Lumbrineris fragilis	9	3	4	3	2
Lumbrineris sp.					
Maldane sarsi			1		
Mediomastus fragilis	2	3		38	87
Melinna cristata	1				1
Nereimyra punctata			1		1
Nereis cf. virens ?					5
Ophelina acuminata	1	1			4
Ophiodromus flexuosus					
Paraonis fulgens				1	1

Pholoe minuta	1			4	2
Polycirrus sp.					
Polydora caulleryi	3				
Polydora socialis			1		
Polyphysia crassa					
Praxillella sp.				1	
xPrionospio malmgreni					
Prionospio cirrifera				1	
Sabella penicillus				2	
Sabella sp.					
Scoloplos armiger					
Scalibregma inflatum	2	1			
Sosane gracilis	18	1	4		1
Spiochaetopterus typicus		1	8	1	3
Synelmis klatti		1	1	1	
Trochochaeta multisetosa			1		
Trichobranchus sp.					
Typosyllis variegata	2	4	3	3	2
Oligochaeta indet.					
CRUSTACEA					
Copepoda	3	3	2		
Cirripedia					
Euphausiacea indet.					
Palemonidae					
Eudorella truncatula	2				
Amphipod					
Amphipoda A					
Amphipoda B					
Amphipoda C				3	
Amphipoda D				1	
Amphipoda E					
Amphipoda F					
Corophidae ?	1				2
Priapulida		1	1		
Sipunculidae					
Pycnogoidae					
ECHINODERMATA					
Ophiura sp.				1	1
Ophiura afinis	4				
Amphiura filiformis				2	1
Asteroidea indet. juv.					
Acideacea indet					
Tunicata	1				
Number of individuald	304	263	130	311	580
Number of species	25	20	18	29	28
Shannon-Weaver diversity	2.05	1.32	2.23	2.89	2.30
Pileou's index of evenness	0.44	0.31	0.53	0.59	0.47

Table 7 . Number of individuals from 4 cores (total surface area 113 cm^2) down to a depth of 6 cm retained on a 500 µm siev . Samles from 19.01 showes mean values from a total of 12 cores.

Sampling date	19.01	30.04		24.05.84		04.09.84	
Dosing (gC/m^2)	0	50	200	50	200	50	0
Box nr.		ØA8	ØA4	ØA3	ØA5	ØA6	WC2
Species							
Pelosina sp.						1	
Turbellaria indet.							
Trematoda ?							
Nemertinea indet.			1				
MOLUSCA							
Gastropoda indet							
Chaetoderma nitidium							
Leucon nasica							
Musculus cf.marmoratus							
Mya truncata							
Nudibranchia indet.							
Nuculoma tenuis							
Techibranchia indet.							
Thyasira sp.	0.3						
Saxicava sp.							
Mytilacea juv.							
Spisula sp.							3
Bivalvia E							
ANNELIDS							
Amphicteis gunneri							
Anaitides maculata							
Capitella capitata	0.3						
eCapitella sp.							
Cirratulus cirratus		1					
Chaetozone setosa	18.3	12	8	18	3	31	15
Chone sp.							
Cossura longocirrata	4.3	2	14	6	2	4	3
Dipolcirrus glaucus				1			
Dorvellidae ?							
Eteone longa	0.6						
Eumida punctifera						1	
Glycera alba	0.3	1		2			
Goniada maculata							
Harmothoe sp.							
Harmothoe cf. imbricata							
Hesionidae indet	0.3					1	
Hetromastus filiformis	20	19	15	16	8	1	22
Lumbrineris fragilis	1		1	2		2	
Lumbrineris sp.							
Maldane sarsi							
Mediomastus fragilis	28.3	15	1	19	4	11	33
Melinna cristata							

Nereimyra punctata							
Nereis cf. virens ?							
Ophelina acuminata						2	
Ophiodromus flexuosus							1
Paraonis fulgens	2.6	1	4	2	2	2	6
Pholoe minuta	0.6			1	1	1	2
Phyllodocidae indet			1				
Polycirrus sp.							
Polydora caulleryi	0.6			1			
Polydora socialis	0.3						
Polyphysia crassa							
Praxillella sp.							
Prionospio cirrifera	1.3			4			2
Prionospio malmgreni	2		1	1		1	
Pseudopolydora paucibranchiata	1.6	1					
Sabella penicillus							
Sabella sp.							
Schistomeringos sp.	5.6	4	2	13	3	3	5
Scoloplos armiger							
Scalibregma inflatum	0.3						
Sosane gracilis	1.6		2	1	1	2	6
Spiochaetopterus typicus							
Sphaerodorum peripatus			1				
Synelmis klatti							
Trochochaeta multisetosa							1
Trichobranchus sp.							
Typosyllis varigata							1
Oligochaeta indet.							1
CRUSTACEA							
Copepoda (Calanoidea)	0.6			3	4		
Harpacticoidea		3		14	3	1	3
Cirripedia							
Euphausiacea indet.							
Palemonidae							
Eudorella truncatula							
Leptognathia filiformis				2			
Amphipod							
Amphipoda A							
Amphipoda B							
Amphipoda C							
Amphipoda D							
Amphipoda E							
Amphipoda F							
Corophidae ?							
Priapulida							
Sipunculidae							
Pycnogoidae							
ECHINODERMATA							
Ophiura sp.	0.3						
Ophiura afinis							
Amphiura filiformis						1	

Asteroidea indet. juv.							
Acideacea indet							
Tunicata							
Chaetognatha						1	
Number of individualds	91.1	59	51	106	31	66	104
Number of species	22	10	12	17	10	17	15
Shannon-Weaver diversity	3.24	2.54	2.77	3.36	3.07	2.83	3.01
Pileou's index of evenness	0.72	0.77	0.77	0.82	0.92	0.69	0.77

Table 8 . Number of animals found dead on the surface of the sediment in boxes 30.04.84.Twelve boxes are sampled , 6 dosed with 50 gC/m and 6 dosed with 200 gC/m.

	High dose							Lowe dose						
Species	1	2	3	4	5	6	T	1	2	3	4	5	6	T
Nemertinea indet.	1	1	1	1			4							
Spisula eliptica									2				1	3
Amphicteis gunneri					1		1							
Amphitrite cirrata	1						1							
Anaitides grøenlandica			1				1							
Chaetozone setosa		1		2			3							
Diplocirrus glaucus				1			1							
Dorvellidae ?			1				1							
Flabelligeridae					1		1							
Glycera alba	3	1	2		4	4	14				1			1
Lumbrineris fragilis	2	7	1	4	1		15							
Ophiodromus flexuosus				1			1							
Pistsa cristata		1		1			2					1	1	2
Polycirrus sp.		1					1			1	1		3	5
Prionospio malmgreni		1	3				5					1		1
Prionospio sp.									2					2
Sabella penicillus											1			1
Scoloplos armiger				2			2							
Scalibregma inflatum	1			3	1		5							
Sosane gracilis			1				1							
Syllidae indet														
Terrebelidae indet				1			1							
Trochochaeta multisetosa			1				1							
Number of species	5	8	8	9	5	2	20	0	5	1	3	2	5	8
Number of individuals	8	15	11	16	8	5	64	0	5	1	3	2	5	16

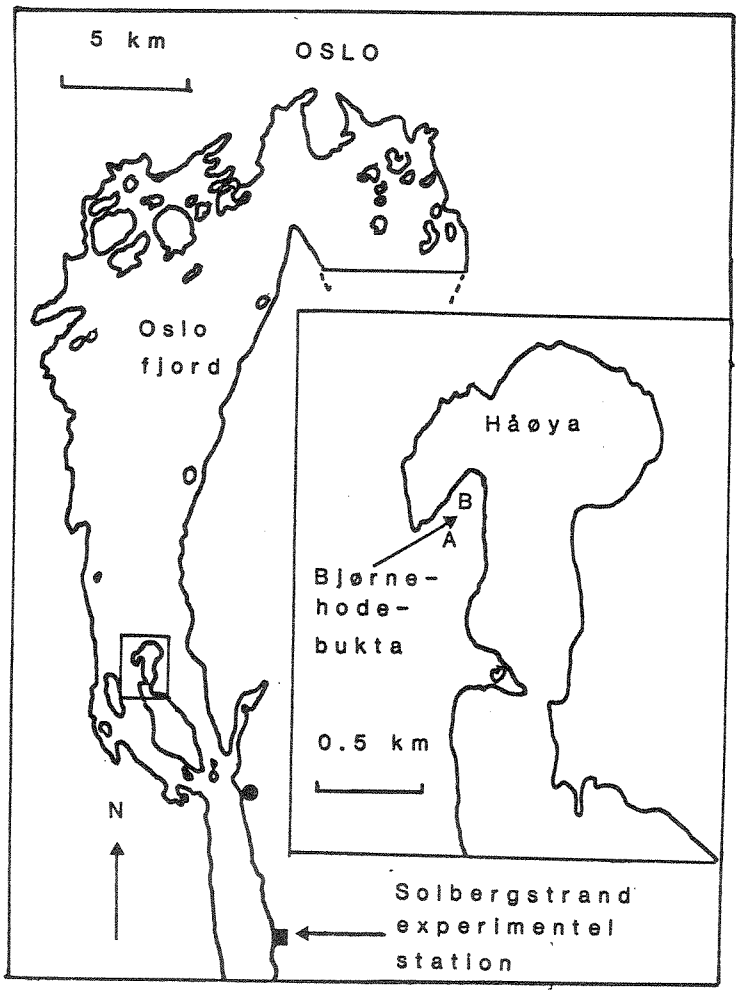

Figure 1. Map of the inner part of the Oslofjord. A more detailed map of the sampling area in Bjørnehodebukta is inserted. Sediment brought into the mesocosm were collected between A and B. The location of Drøbak is indicated by ●.

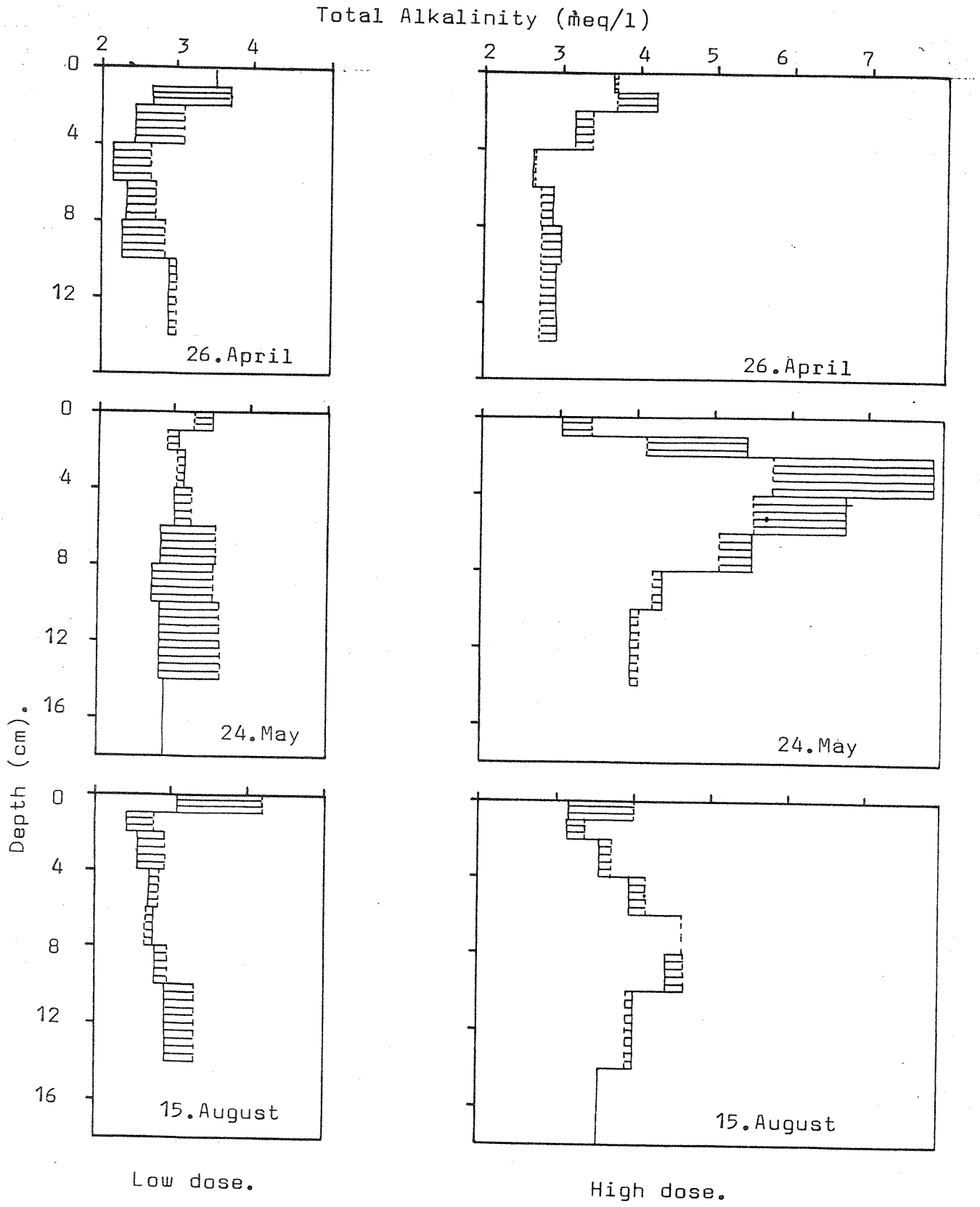

Fig. 2a. Vertical profiles of total alkalinity. Full and broken vertical bars represent the boxes from the eastern and western basin, respectively. Thus the shaded areas show the range of concentrations in parallell samples.

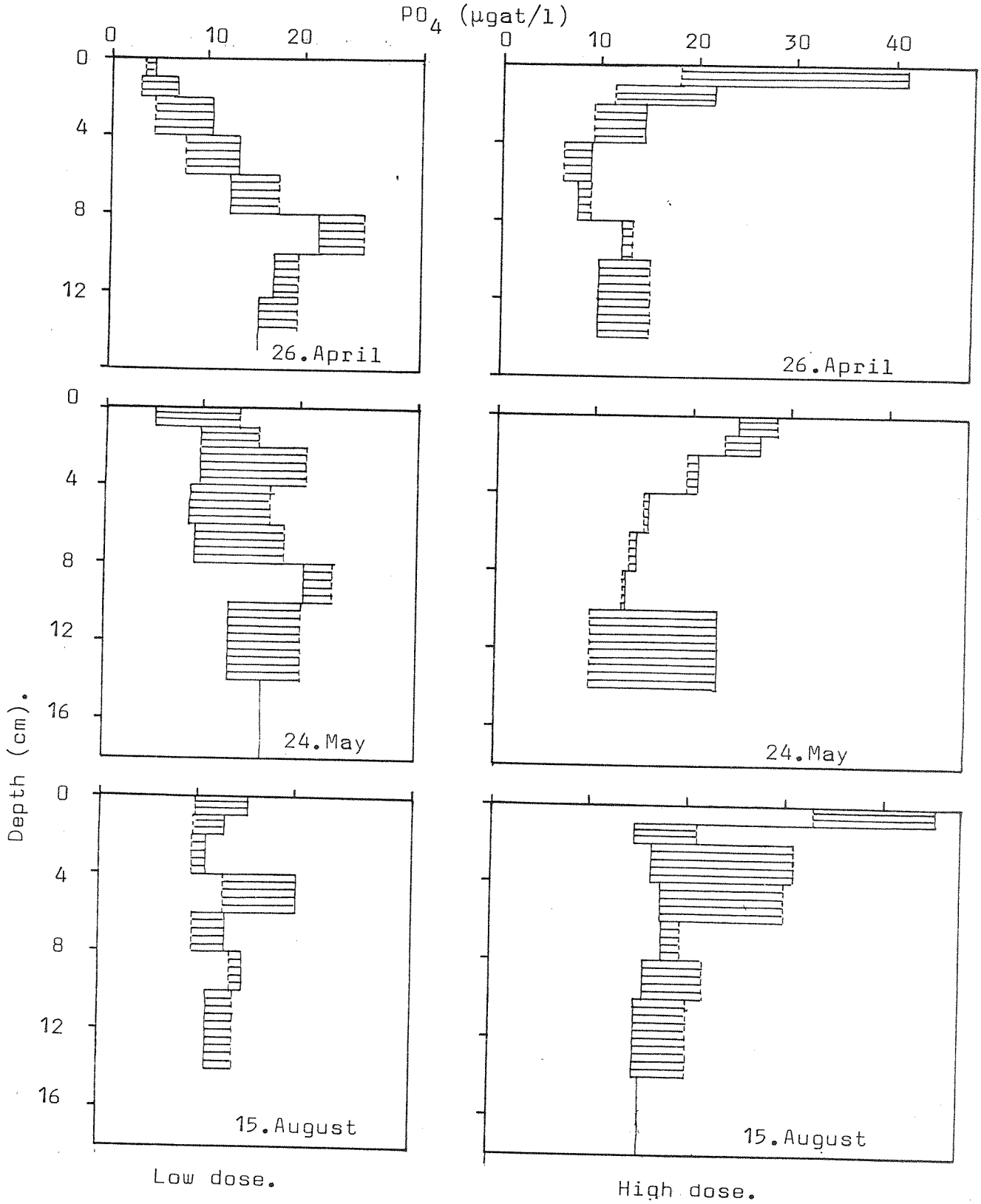

Fig. 2b. Vertical profiles of dissolved phosphate. See text of fig. 2a.

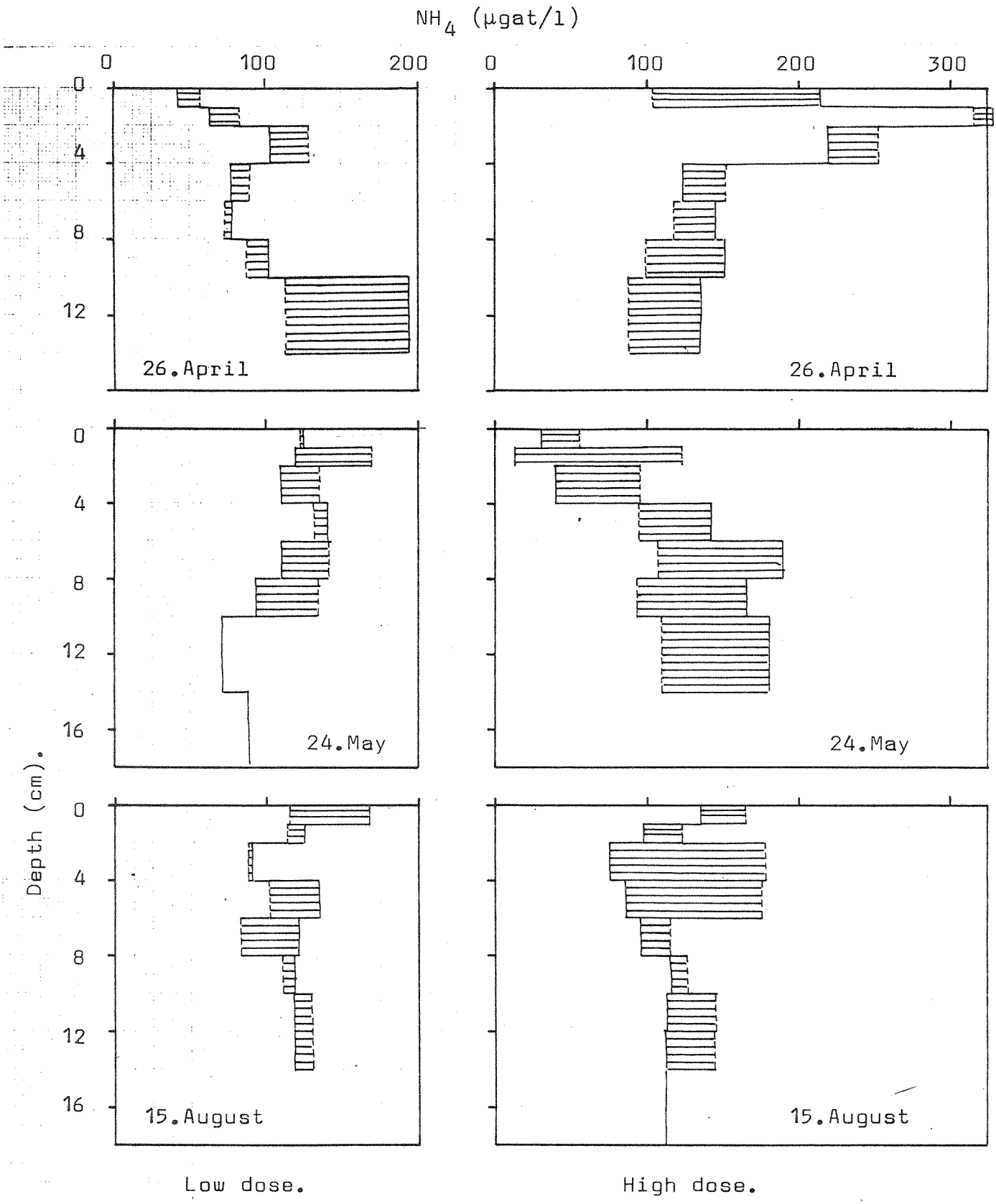

Fig. 2c. Vertical profiles of dissolved ammonia. See text of fig. 2a.

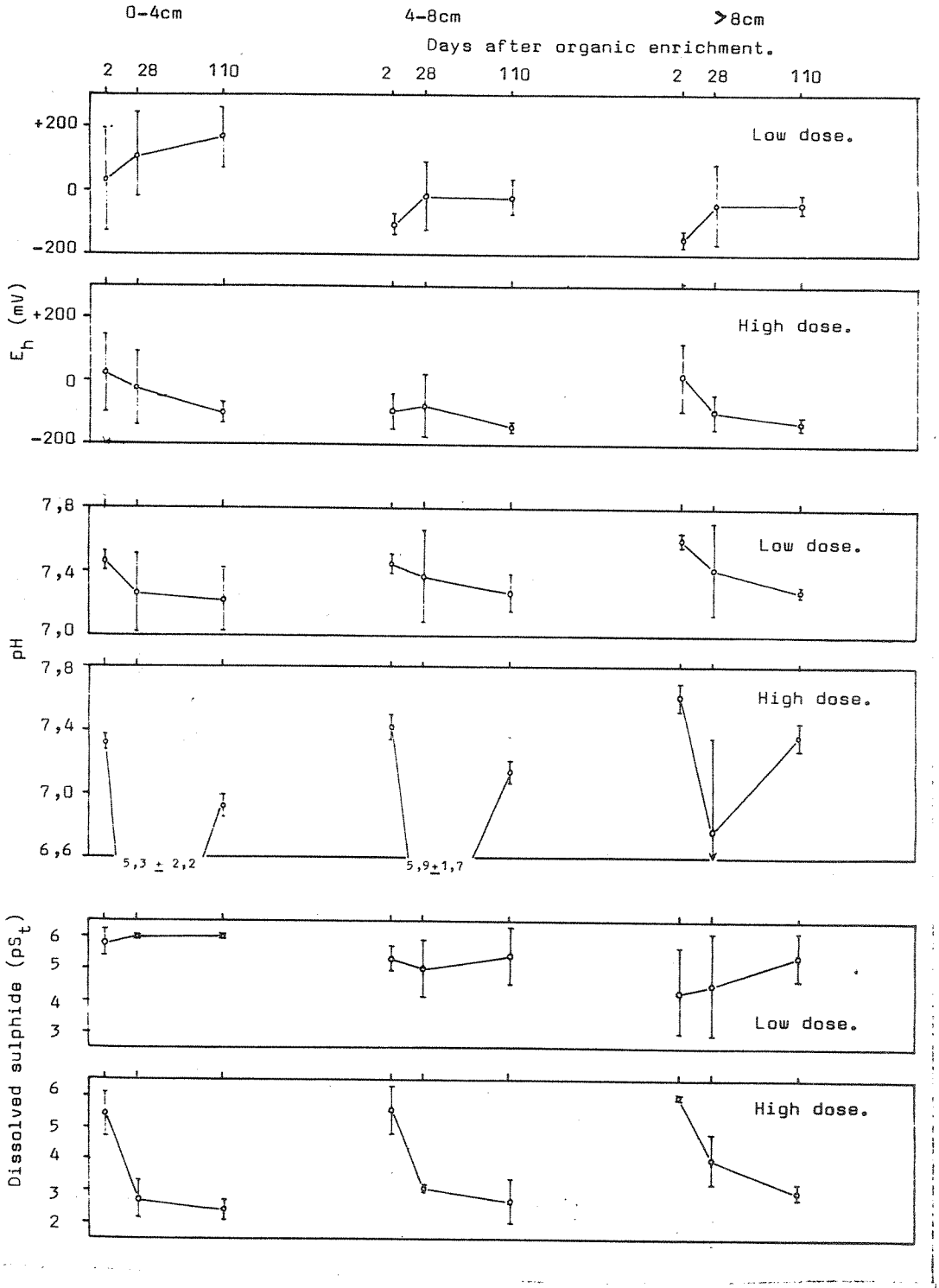

Fig. 3a. Variations with time of pore water properties within the 0-4cm layer(left), 4-8cm layer (middle) and below 8cm (right). The points represent mean values of two parallell boxes (n=4-8), and the vertical bars show the standard deviation ($2\sigma_{n-1}$). Off-scale points for pH in high dose boxes are replaced with figures.

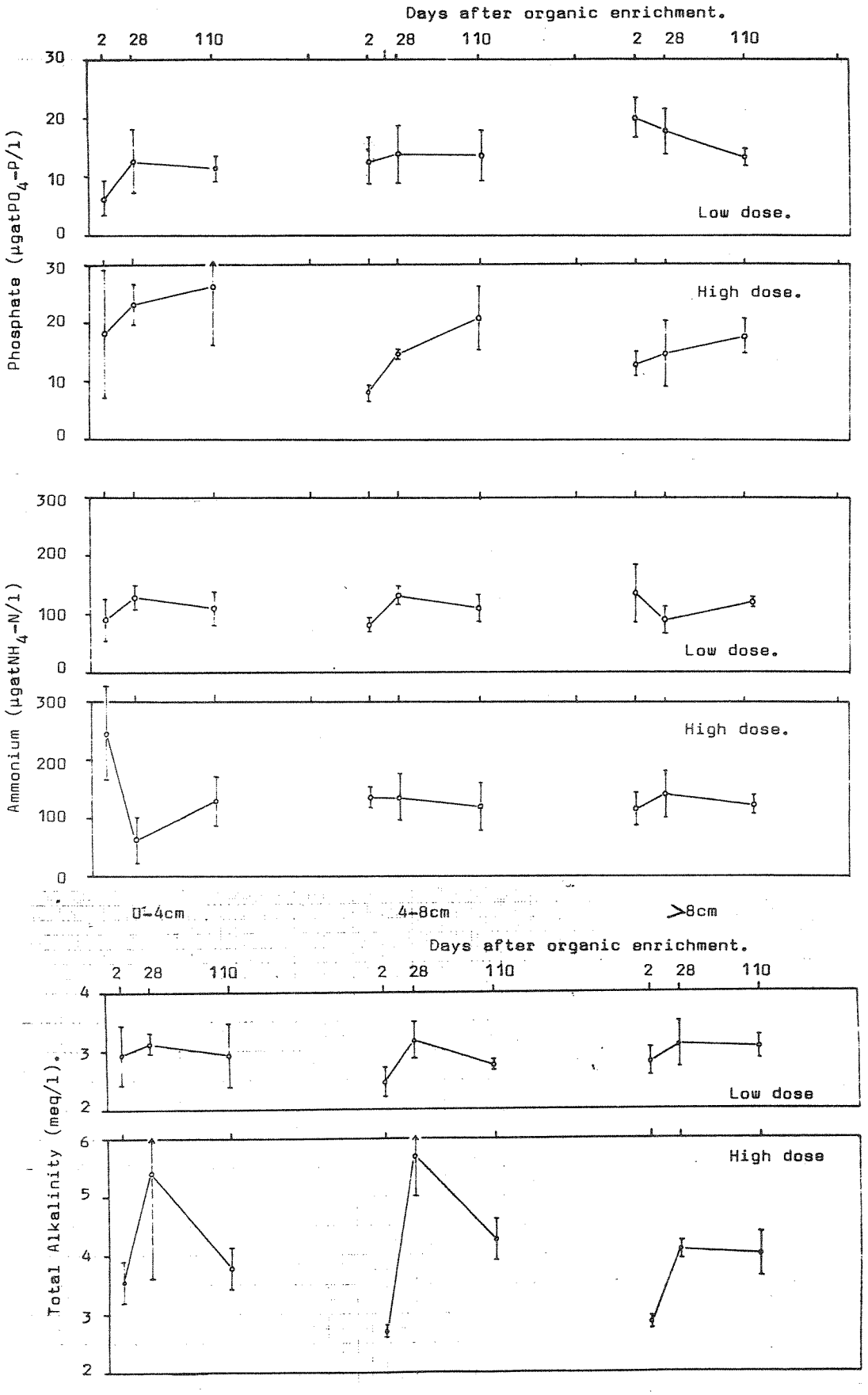

Fig. 3b. See text of fig.3a.

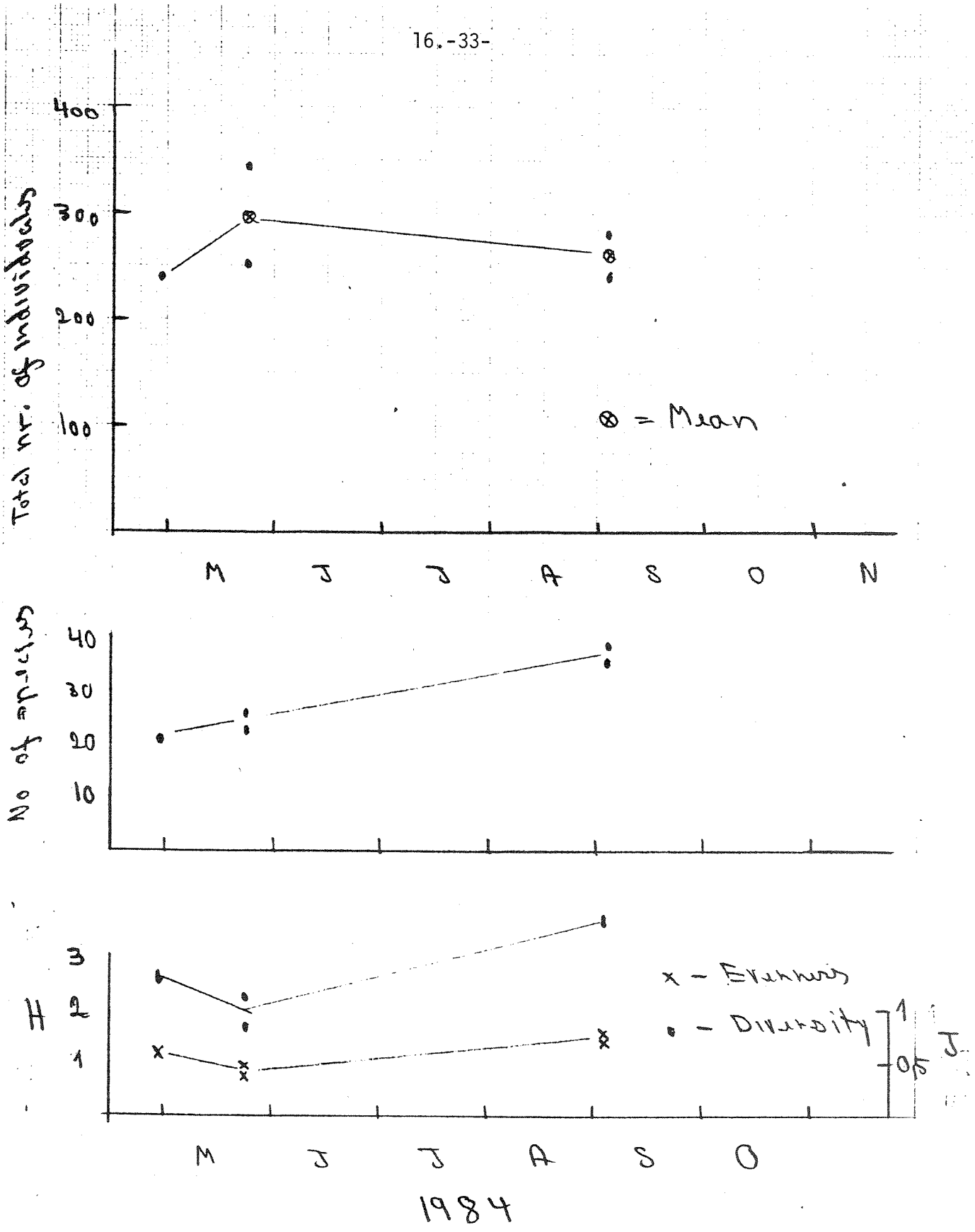

Figure 4. Community parameters (1mm siev) in boxes dosed with 50 gC/m^2 of Aschophyllum nodosum on 24/4-84.
A: Number of individuals in each box. B: Number of species in each box. C: Evenness (J) and Diversity (H) in each box.

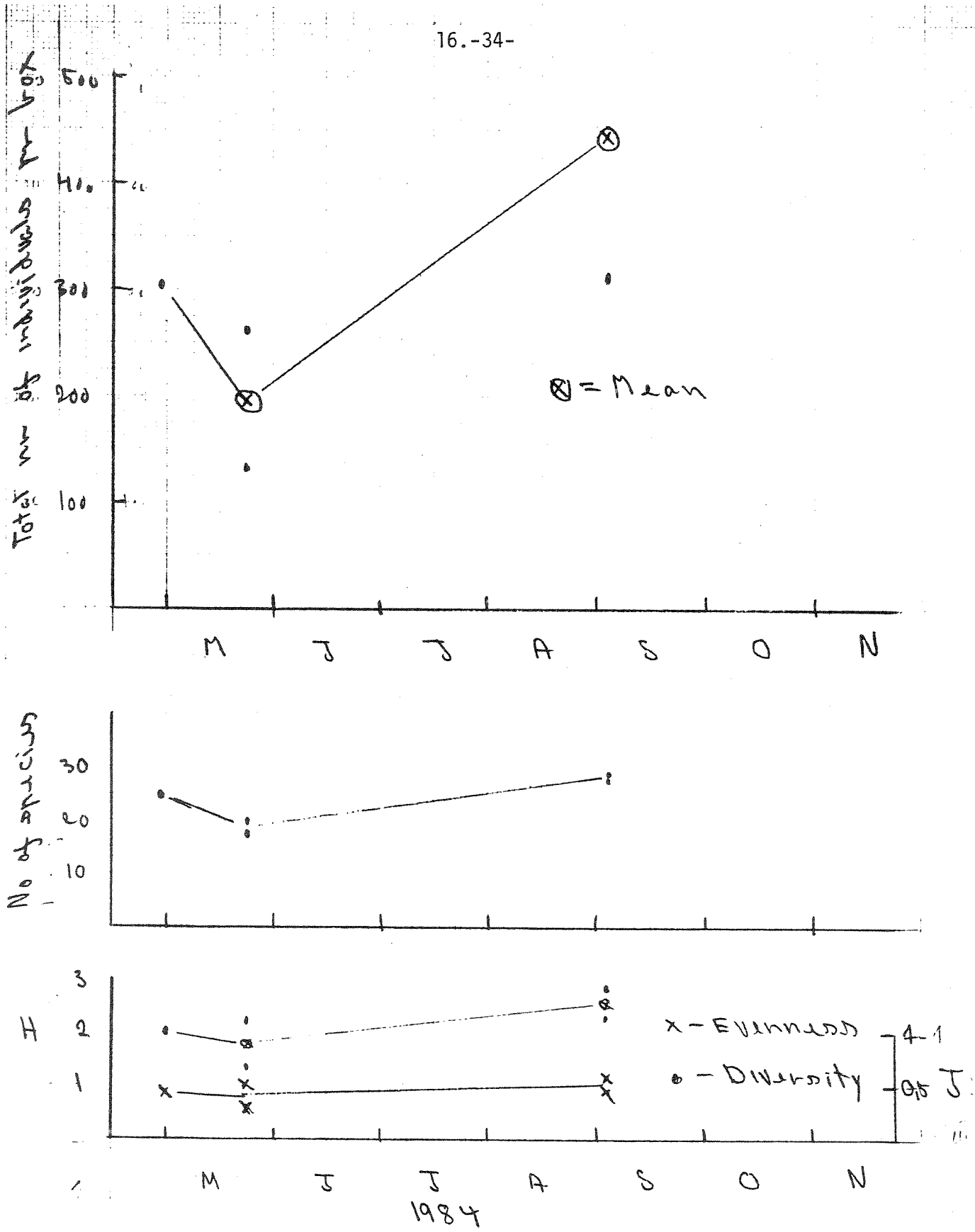

Figure 5. Community parameters (1 mm siev) in boxes dosed with 200 gC/m^2 of Aschophyllum nodosum on 24/4-84.

A: Number of individuals in each box. B: Number of species in each box. C: Evenness (J) and diversity (H) in each box.

17. Effects of the feeding activity of the polychaete Streblosoma bairdi on sediment composition, meiofaunal abundance and community structure.

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Abstract

Streblosoma bairdi forms prominent faecal mounds in the sublittoral mud mesocosm established at Solbergstrand, Oslofjord, Norway. The sediment in the mounds were visually diffrent from the sediment outside the mounds. The water content in the sediment in the mounds (mean=66.8%) was significantly higher than in the sediment outside the mounds (mean=62.5%). Sediment particles in the size range 5 - 16 um were significantly more abundant inside the mounds than outside the mounds, whereas particles in the range 250 - 500 um were significantly more abundant outside the mounds. The mean total carbon and nitrogen contens in the sediment were higher inside the mounds (3.45%; 0.18%) than outside the mounds (2.92%; 0.15%) but not significantly. The number of bacteria pr. g dryweight sediment and the percentage of silt/clay in the sediment were higher inside the mounds ($23.40 \cdot 10^8$ /g; 77.5%) than outside the mounds ($20.0 \cdot 10^8$ /g;70.8) but not significantly.

The abundance of meiofauna (nematodes and copepodes) is highest in the mounds and very reduced in the surrounding feeding area, with intermediate densities in unaffected areas. Species diversity is highest in the feeding area, with a more equitable distribution of species, while the mounds and unaffected areas have higher dominance and lower diversity. It is suggested that these differences arise partly because of varying levels of predation, disturbance and sediment modification resulting from the feeding activity of the polychaete. However, differences in trophic structure of the nematode component of the meiofauna between the three areas suggests that diversity profiles are also modified indirectly by the influence of the polychaete on the nature of the primary food resources available to the meiofauna. The importance of meiofauna/macrofauna interactions in controlling meiofauna community structure is emphasized.

18. The solitary bryozoa Monobryozoon limicola (Ctenostomata), a comparison of mesocosm and field samples from the Oslofjord.

J.A.Berge, H.P.Leinaas and K.Sandøy

ABSTRACT J.A.Berge, H.P.Leinaas and K.Sandøy. Subtidal sediment from the Oslofjoird was sampled and transported in boxes into an indoor mesocosm to establish a soft bottom community for experimental studies. After 7 months 662 individuals of the solitatrian bryozoa Monoibryozoon limicola was found inside the mesocosm in 6 of 24 cores (inner diameter 6 cm). This is the first record of M.limicola in Norway. The mean number of this species per corer was 27.5 with a higly aggregated distribution.The number of other organisms found in the cores where M.limicola were found was significantly lower than in cores were M.limicola was absent.

M.limicola was not found at the site from were the sediment in the mesocosm originated. It is proposed that the high density of this species in the mesocosm is a function of reduced competition, predation or disturbance from other organisms which were more vulnerable to the environmental conditions in the mesocosm and/or the method of sampling and transportation.

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Introduction

This paper reports on the first record of the solitary bryozoan Monobryozoon limicola in Norway.

The discovery of a solitary bryozoan (Monobryozoon ambulans) created a sensation when it was described by Remane in 1936, since all previously known bryozoans were regarded as colonial organisms (Franzen,1960). Since the discovery of Remane three additional species of this genus have been described. In 1960 Franzen described Monobryozoon limicola from the detritus layer on soft muddy sediment at 55 m in the Gullmar fjord,Sweden. Ott(1972) described M.bulbosum from the Atlantic coast off North Carolina,USA, and in 1981 d'Hondt and Hayward described Monobryozoon sandersi from 800 meters depth in the Atlantic. Based on the mode of bud formation Ott(1972) proposed the posibility of the genus Monobryozoon being polyphyletic. In 1975 d'Hondt sugested that M. limicola should be included in the genus Nolella which thereby contain 14 species of which two others also are described as solitary (d'Hondt 1983).

At the present we will not take part in the discussion about the taxonomic status of Monobryozoon limicola and therefore use the original scientific name.

Solitary bryozoans are seldom reported from benthic

community studies. This may be a function of rarity, but might equally be attributed to these species' relative small size (M.limicola is 0.5 - 2.5 mm).They have been regarded as part of the meiofauna (Gray 1971) which is often neglected in benthic surveys. More over, bryozoans in the Order Ctenostostomata have no hard parts or prominent features which are easily recognized, and in a fixed state the crown of tentacles is retracted leaving few characteristics pointing to a bryozoa. Thus it is easy to confuse the presence of a few M.limicola with the fragments of other organisms.

Because of the sparse recordings of solitary bryozoans their distribution and biology are not well known. The aim of this study is to contribute to the present limited knowledge of ecology and zoogeography of this species.

Material and Methods

The material was found during the establishment of a subtidal soft-bottom community in a mesocosm at Solbergstrand Experimental Station, 5.4 km south of Drøbak on the east side of the Oslofjord ,Norway (Figure 1).

The soft bottom-mesocosm consists of two basins each with a surface area of 120 m with a maximum water depth of 120 cm . The basins are supplied with seawater from 42 m depth in the fjord outside the station.

The sediment brought into the mesocosm was collected at the end of April, 1983, at Bjørnehodebukta 59° 42.8' N and 10° 32.2' E (see Figure 1) approximatly 12.2 km north of the mesocosm. The macrofauna community and environmental conditions at Bjørnehodebukta have been described by Valderhaug and Gray (1984). The sediment was obtained with a Day grab (0.1 m^2) and then placed into two sizes of boxes (0.5 m^2 and 1 m^2) which were filled with sediment from 8 and 16 grabs respectively . The boxes were placed on the bottom inside the mesocosm. During 1983 there were some initial problems in establishing an adequate waterflow in the mesocosm. This resulted in black patches of ironsulfid on the sediment surface in some of the boxes.Futhermore, in September oxygen concentrations as low as 1.4 ppm was observed in the water 5 cm above the sediment in some of the boxes. A new water flow system has recently been constructed and

has in 1984 proved to be satisfactory for maintaining a natural subtidal sediment community in the mesocosm. Salinity and temperature during the period April - November, 1983 were in the range 30.5-33.4‰ S and 6.8-13.0 °C respectively.

The sediment in the boxes was sampled with hand operated corers (6 cm inner diameter) on 8. November 1983.

A total of 24 cores were taken inside the mesocosm. In June 1984 a gravity corer (inner diameter 6 cm) was used to sample the community in Bjørnehodebukta ; again 24 cores were taken. This field sampling was performed at two locations in Bjørnehodebukta (12 corers at each station) both at 30-32 meters depth. The outer station (station A) was at the mouth of the bay (Figure 1) and corresponds to the station sampeld by Valderhaug and Gray 1984, the inner station (B) is located 250 m futher into the bay.

Only the top 3 cm of each corer was used. All samples were processed in the same manner, preserved in 10 % neutralized formalin, stained with Rose Bengal and washed through a double set of 500 and 250 µm sieves. The fauna (excluding nematodes)was sorted and identified under a binocular microscope.All 500µm samples from the field were sorted in order to find if M.limicola were present, but only in 8 were all other organisms identified.The material retained on the 250 µm sieve was only sorted and analyzed for M.limicola if this species had been found on the 500

µm sieve. Statistical treatment was performd by using student's t-test on log-transformed data for stabelization of varians.

Results and Discussion

The animals found agree with the description of M.limicola (Franzen 1960) and our identification has been verified by Franzen. Figure 2 shows a drawing of the species. The largest individual observed measured 2.25 cm in length (stolons not included), which is within the range described by Franzen. However, while Franzen mention 2 or 3 as the normal number of stolons (with 7 as the maximum), we found a mean number of 4.6 (S.D.=1.5, N=25) and a maximum of 8 stolons .

M.limicola had a very patchy distribution in the mesocosm. A total of 662 specimens were found in 6 out of a total of 24 cores collected on 8 November,1983, (Mean=27.5, S.D.=98.6, N=24). The number of M.limicola in the six cores ranged from 1 (in two samples) to 490. Totally 64 % of the specimens were found on the 500 µm sieve.

The highly aggregated distribution of M.limicola in the boxes reflects their ability to reproduce asexually by bud formation from the stolons. A large number of specimens in the process of bud formation were observed. This probably indicates a healthy population with a high growth rate that originated from a limited number of founding animals. In contrast, the rest of the infauna in the boxes showed clear signs of environmental stress indicated by the appearence of dead polychaetes and bivalves on the surface

of the sediment after the establishment of the mesocosm community. Consequently only few animals were found in the cores from the mesocosm in November 1983 (Table 2). The dominating species was Heteromastus filiformis ; however other polychaetes such as Paraonis fulgens and Sosane gracilis and the bivalve Nuculoma tenuis were also found.

The mean number of other organisms found in in cores where M.limicola were present (mean=1) was significantly lower (t-test, p=0.05) than in cores were this species were not found (Mean=5.4). Thus there seems to be an inverse relationship between the appearans of M.limicola and the rest of the fauna.

M.limicola was not found in the 24 cores from Bjørnehodebukta. The species composition at the two sites in Bjørnehodebukta can be seen in Table 1 .These two stations represent the innermost and outermost area of Bjørnehodebukta from which the sediment in the mesocosm was collected. The fauna was significantly richer on the innermost station in the bay, both in terms of density and in number of species (see Table 1). The densities and number of species at both sites were, however, several times higher than what was found in the samples from the mesocosm (Table 2).

The organisms with higher densities at the inner site compared to the outer site were Chaetozone setosa, Hetromastus filiformis, Mediomastus fragilis,

Pseudopolydora paucibranchiata, Shistomeringos sp. and harpacticoid copepods. Notably the polychaetes represents species which may increase in density after organic enrichment of the sediment and may be explained by considerable amount of wood particles on the sediment at station B.

Because of the differences in faunal composition within Bjørnehodebukta, the starting point of the fauna in the different boxes may have varied somewhat, reflecting where in the bay the sediment was collected. However, even at the mouth of the bay, the infauna denseties were significantly higher (t-test, $p=0.05$) than in the mesocosm (cf. Table 1 and 2). These findings reflect the seriousness of how most of the infauna were affected by the establishment of the mesocosm. Most probably the mortality has been partly caused by disturbance of the sediment when it was collected by the grab and lumped into the experimental boxes (This procedure has recently been improved by using an USNEL box -corer) . However, the anoxic or nearly anoxic conditions occuring in the sediment apparently have also been an importante factor affecting mortality. The striking difference between the high density and successful reproduction of M.limicola and the serious reduction in the numbers of other members of the infauna suggests not only that M.limicola is robust against the special conditions encountered in the mesocosm

in 1983, but also that this species has been directly favoured in the mesocosm. This can be caused by the environmental conditions directly or through a reduction of (1) competition for food,(2) predation or (3) mechanical disturbance of the sediment by larger animals.

Our results from the field indicate that if M.limicola were present at Bjørnehodebukta in June 84 then they must be so rare that they were not effectively sampled with the sample size used. Valderhaug and Gray (1984) sampled the macrofauna at Bjørnehodebukta 14 times during the period June 1981 - June 1983 without finding M.limicola thus it seems that if M.limicola have been present at Bjørnehodebukta they must have been extremly rare. We do not know if M.limicola was in the sediment when it was brought into the the mesocosm or if it recruited into the sediment via the water supply system. Irrespectivly of how the recruitment of M.limicola has occurred, they have proliferated because of the conditions in the basin have favored them.

It is possible that this species usually occurs in low densities with a small chance of being discovered, and that high denseties only occur under very special conditions.

Our investigation extends the range M.limicola into the Oslo fjord.Previously this species has been found in Sweden;England,France, and in Mexico (d'Hondt

1983).Results of quantitative samples of M.limicola are to our knowledge not published. These geographically highly separated records of M.limicola suggest that it has a wide distribution along the Atlantic coasts of Europe and America. The known recordings of M.limicola probably reflect the activity centers of marinebiologists more than the zoogeographic distribution of this organism.

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References

Franzen,A.,1960. Monobryozoon limicola n.sp., a ctenostomatous Bryozoan from the detritus layer of soft sediment Zoologiska bidrag från Uppsala.33:135-148.

Gray,J.S.,1971. The Meiobenthic Bryozoa. Pp. 37-39 in :N.C.Hulings(ed.), Proceedings of the First International Conference on Meiofauna. Smithsonian Contributions to Zoology.76.

d'Hondt,J.L.,1975. Bryozoaires.Ctenostomes bathyaux et abyssaux de l Atlantique Nord. Docum.Lab.Geol.Fac.Sci.Lyon,(H.S.).3(2):311-333.

d'Hondt,J.L.,1983. Tabular Key for Identification of the Recent Ctenostomatous Bryozoa. Memoir de l'Institut oceanographique,Monaco. 14,:1-134.

d'Hondt,J.L. and Hayward,P.J.,1981. Nouvelles recoltes de Bryozoaires Ctenostomes bathyaux et abyssaux. Cah.Biol.mar.. 20(3):361-369.

Ott,J.A.,1972. Monobryozoon bulbosum n.sp., a new solytary interstitial Bryozoon from the West Alantic coast. Cah. Biol.mar..13(4):421-428.

Valderhaug,V.A. and Gray,J.S., 1984. Stable macrofauna community despite fluctuating food supply in subtidal soft sediments of Oslofjord Norway.

Mar.biol..82,:307-322.

Table 1. Mean number of individuals in sediment samples from the mesocosn retained on a 500 um sieve in November 1983 . The sediment was brought into the mesocosm in March 1983 from Bjørnehodebukta where it was collecte using a grab and lumped into boxes .

Polychaeta	4.3
Bivalvia	o.4
Harpacticoid copepods	0.5
Monobryozoon limicola	17.8*

* Mean = 27.5 when results from 250 um siev are also included.

Table nr.2. List of species found at two stations in Bjørnehodebukta 4.June,1984. Number of organisms per species in a total of 5 cores (inner diameter 6 cm)at each station for the top 3 cm of the sediment retained on a 500 um siev are shown.

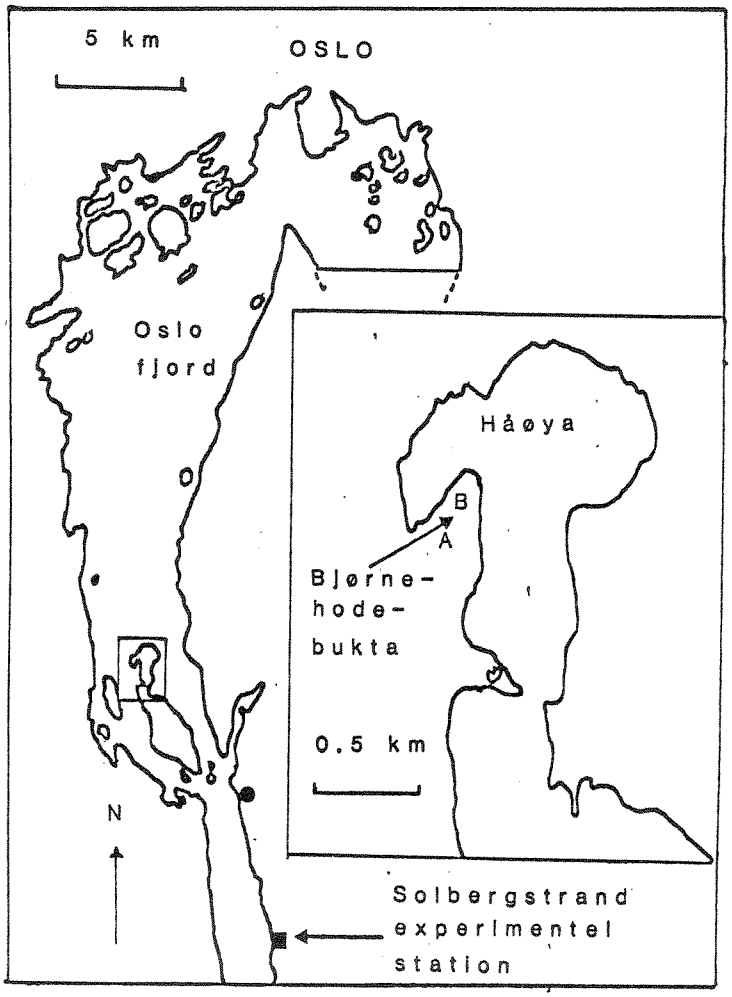
Name of species	Station A	Station B
Nemertinea indet	1	2
Polychaetes		
Harmothoe indet	2	
Pholoe minuta (Fabricius)	11	11
Eteone longa (Fabricius)		1
Phyllodocidae indet		7
Ophiodromus flexosus (Delle Chiaje)	1	
Hesionidae indet		1
Syllidae indet	3	2
Glycera alba (Müller)	1	3
Goniada maculata Oersted		1
Lumbrinewris fragilis (Müller)	1	
Schistomeringos sp	7	66
Scoloplos armiger (Muller)		1
Paraonis fulgens (Levins)		4
Prionospio malmgreni Claparède	14	2
Prionospio cirrifera Wiren	8	2
Polydora socialis (Schmarda)		1
Polydora caulleryi Mensil		1
Spionidae indet	1	1
Chetozone setosa Malmgren	5	26
Cirratulus cirratus (Müller)		1
Cossura longosirrata Webster & Benedict	7	18
Ctenodrilidae indet		2
Scalibregma inflatum Rathke		6
Ophelina acuminata Oersted		1
Mediomastus fragilis Rasmussen	5	102
Hetromastus filiformis (Claparede)	1	27
Maldanidae indet	1	
Ampharetidae indet		3
Sosane gracilis (Malmgren)	12	10
Melinna cristata (M.Sars)	1	
Polycirrus sp		2
Polychaeta indet ,juv.		2
Oligochaeta indet		3
Bivalvia		
Nucoloma tenuis Montagu	1	
Bivalvia juv.		6
Crustacea		
Philomedes brenda (Braid)	27	1
Harpacticoidea indet	3	28
Leptognathia filiformis Lilljeborg	4	1
Isopoda indet		1
Cumacea indet		2

Gammaridae indet	1	8
Insecta		
Halacaridae indet	2	1
Sipuncula		
Sipuncula indet		2
Echinodermata		
Ophiura affinis Lütken	1	
Ophiuroidea indet	8	
Total number of species	26	39
Total number of specimens	129	378
Shannon-Wiener diversity index	3.95	3.77
Pileous's index of eveness	0.84	0.71

Legend to figures

Figure 1. Map of the inner part of the Oslofjord. A more detailed map of the sampling area in Bjørnehodebukta is inserted. A and B indicate the outer and inner station respectivly. Sediment brought into the mesocosm at Solbergstrand were collected between A and B. The location of Drøbak is indicated by ●.

Figure 2. Monobryozoon limicola drawn from a photographc material of animals found in the mesocosm at Solbergstrand.


5 km
OSLO
Oslo
fjord
Håøya
B
A
Bjørne-
hode-
bukta
0.5 km
N
Solbergstrand
experimentel
station

Figur 1.

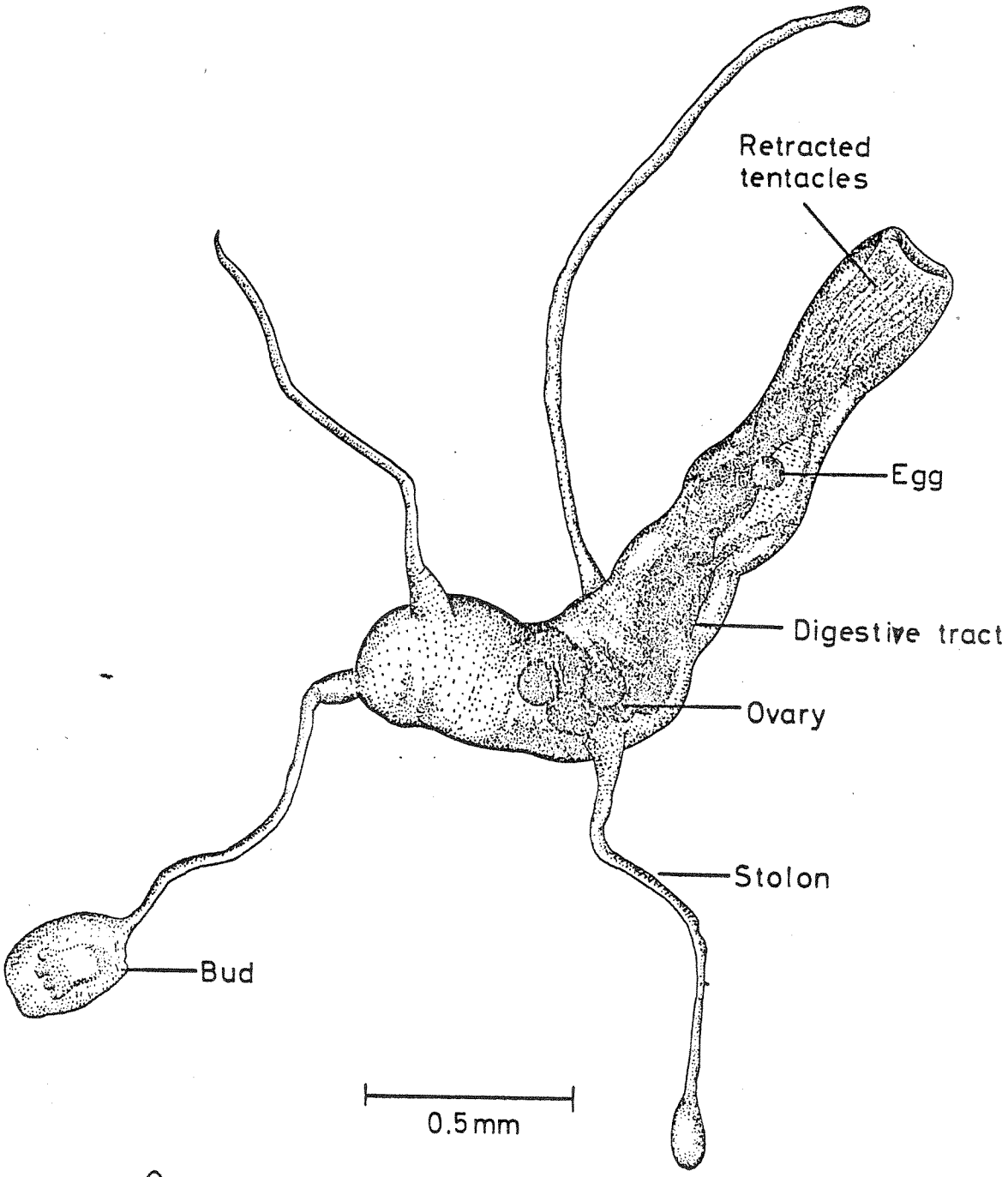

Retracted
tentacles
Egg
Digestive tract
Ovary
Stolon
Bud
0.5 mm

Figura 2.