Long term effects of oil on marine benthic communities in enclosures

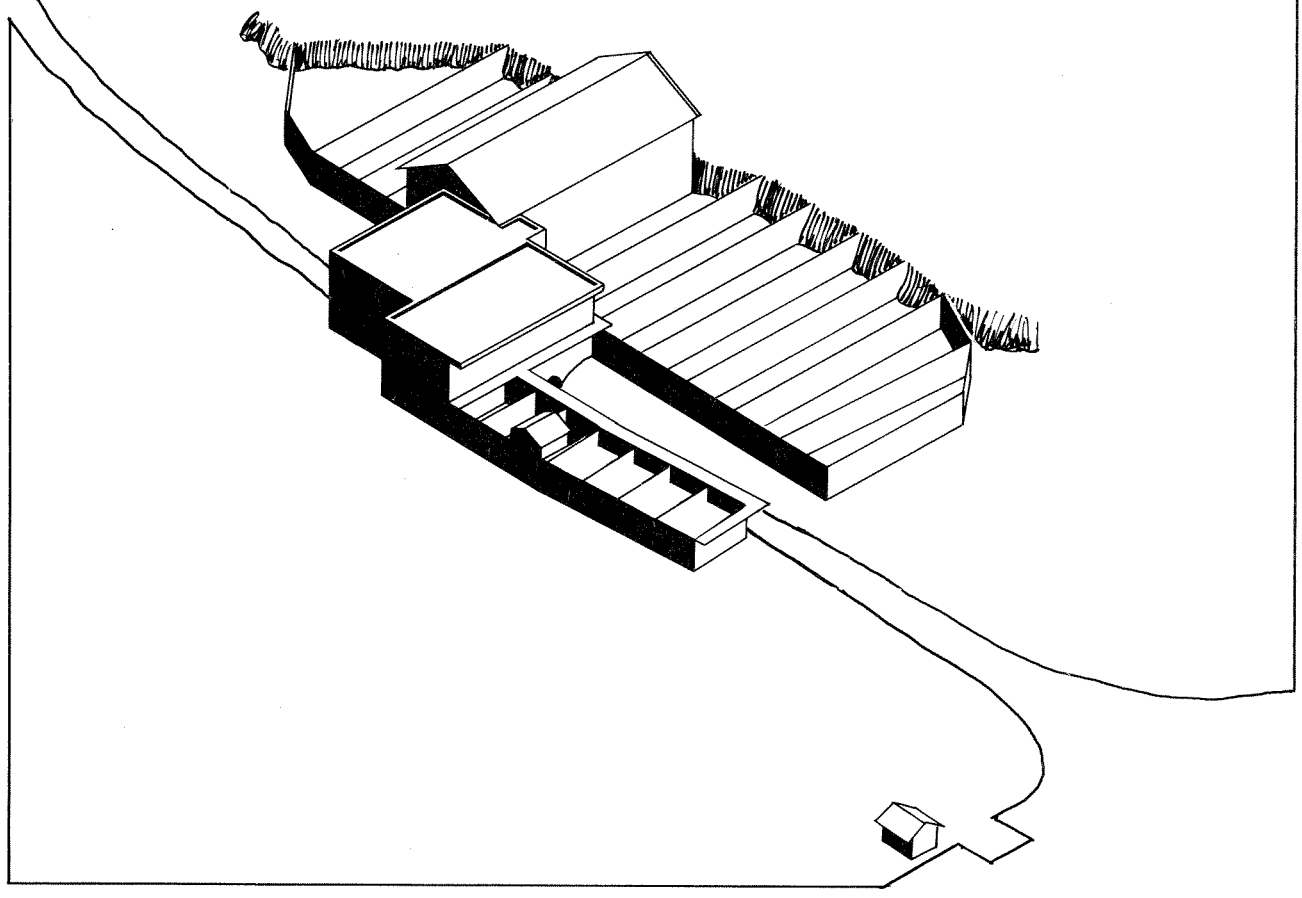
Progress Report 1985

BP Petroleum Development (Norway) Limited

Norwegian Institute for Water Research

University of Oslo

Marine Research Station
Solbergstrand



Norwegian Institute for Water Research NIVA

NIVA – REPORT

Norwegian Institute for Water Research NIVA

Report No.:	0-82007
Sub-No.:	VI
Serial No.:	1809
Limited distribution:	

Main Office
P.O.Box 333 Blindern
N-0314 Oslo 3
Norway
Phone (47 2)23 52 80

Regional Office, Sørlandet
Grooseveien 36
N-4890 Grimstad,
Norway
Phone (47 41)43 033

Regional Office, Østlandet
Rute 866
N-2312 Ottestad
Norway
Phone (47 65)76 752

Regional Office, Vestlandet
Breiviken 2
N-5035 Bergen - Sandviken
Norway
Phone (47 5)25 53 20

Report Title: LONG TERM EFFECTS OF OIL ON MARINE BENTHIC COMMUNITIES IN ENCLOSURES. PROGRESS REPORT 1985

Section I: Littoral Rock Community Project
Progress report No 6
Section II: Sublittoral Soft Bottom Project
Progress Report No 3

Date: December 6th 1985

Editor: Torgeir Bakke

Authors:

W.G. Ambrose	UiO	M. Moore	IMER
T. Bakke	NIVA	F. Moy	UiO
J.A. Berge	UiO	F. Oreld	SI
T. Bokn	NIVA	A. Pedersen	NIVA
P. Donkin	IMER	K. Sandøy	UiO
O-A Follum	UiO	M. Schaanning	UiO
M. Gee	IMER	O.I. Sigurdsson	UiO
J.S. Gray	UiO	G. Skeie	UiO
D. Livingstone	IMER	S. Sporstøl	SI
D. Lowe	IMER	K. Sørensen	NIVA
K. Moe	UiO	R.M. Warwick	IMER
		J. Widdows	IMER

Topic group: Marine

Geographical area: Akershus (Norway)

Number of pages (incl. app.) 69

Contractors ref. (or NTNF - No) BP Petroleum Development (Norway) Ltd.

Abstract:

The report summarizes the results of the subprojects under the BP/NIVA/UiO Research Programme on benthic mesocosms at the Marine Research Station Solbergstrand, for the period 1982-1985.

4 keywords, Norwegian
1. Marint modellsamfunn
2. Oljeforurensning
3. Organisk anrikning
4. Svabergseksperimenter
5. Bløtbunnseksperimenter

4 keywords, English
1. Marine Benthic Mesocosm
2. Oil Pollution
3. Organic Enrichments
4. Rocky Shore Experiments
5. Soft Bottom Experiments

Project leader

Torgeir Bakke

Torgeir Bakke

For the Administration

Tor Bokn

ISBN 82-577-1009-1

NIVAs hustrykkeri

C O N T E N T S:

PREFACE

The BP/NIVA/UIO Joint Research Programme at Solbergstrand was started in 1981 and will run until 1986. The aim of the programme is to investigate the effects of low level pollution by oil and related chemicals on two types of marine communites 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 and students. The Programme is funded by BP Petroleum Development (Norway) Limited.

The purpose of this report is to summarize the results from the subprojects of the two main projects at the present stage. The report compiles contributions prepared by the researchers responsible for each of the subprojects. Some of the results have already been published, and the others will be prepared for publication during 1986.

Oslo, 6 December 1985

Torgeir Bakke
Project coordinator

SECTION I

ROCK LITTORAL PROJECT

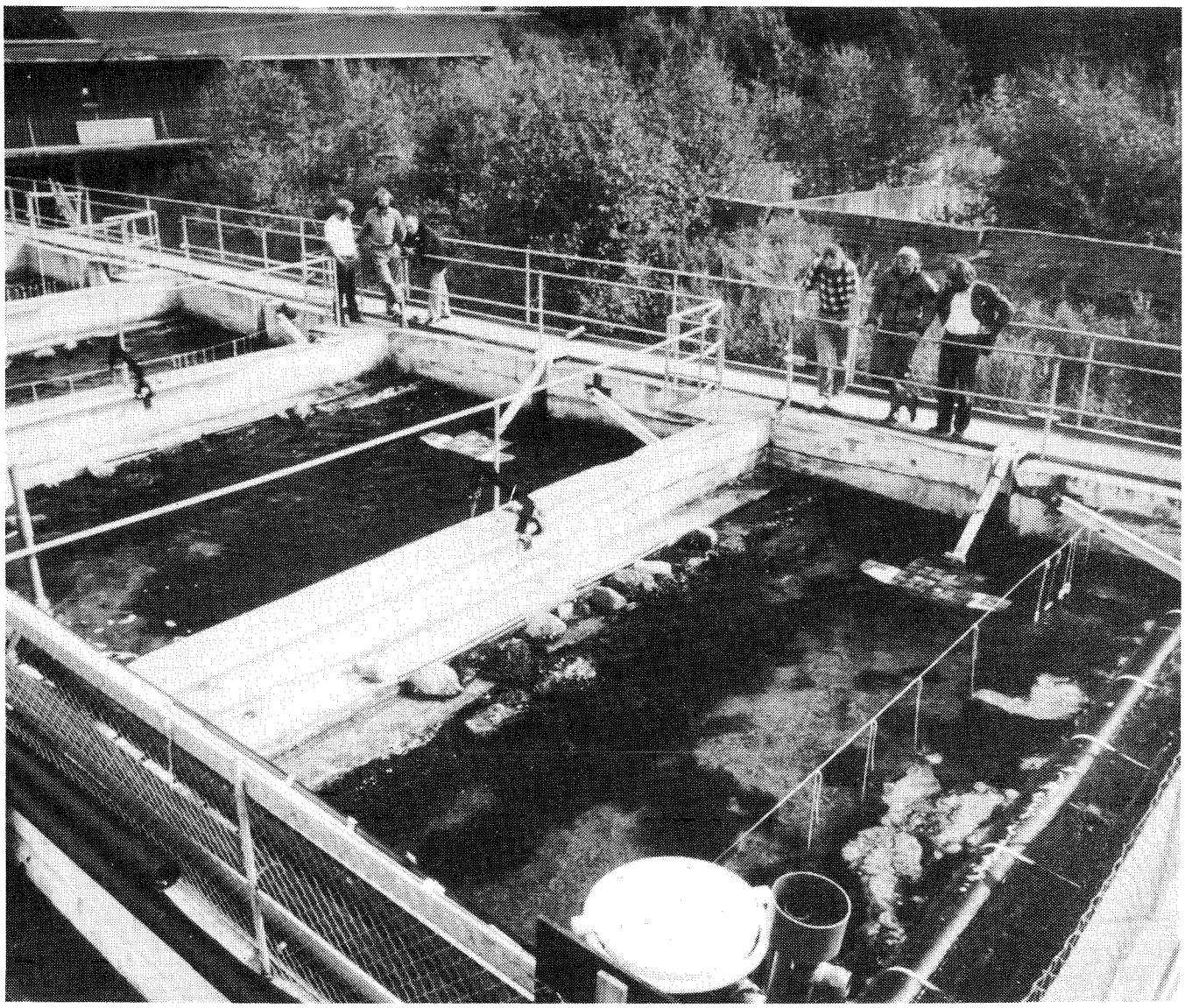
Introduction

There is substantial empirical basis for appraising effects of massive oil spills on shore communities. However, these communities are also exposed to chronic pollution stress, especially by oil hydrocarbons and degradation products, in the vicinity of harbours, and other areas of heavy boat traffic, around petrochemical refineries and other coastal industry. Although the littoral community is an important element in several coastal monitoring programmes, very few experiments on long term effects of sublethal oil pollution have been carried out.

The aim of the BP/NIVA/UiO Rock Littoral Project performed at the NIVA Marine Research Station Solbergstrand in the period 1982-1985 has been to investigate the effects of long term, low level exposure to diesel oil on natural sheltered experimental rocky shore communities.

Four rocky shore communities have been established in large concrete basins through transplantation of shoreline rocks with algae and animals (in 1979) followed by 3 years of external recruitment and self propagation. During a two years period (September 1982 to September 1984) two of these communities were exposed to different levels of a water accomodated fraction of diesel oil mixed continuously into the sea water entering the basins. Two communities were used as controls. The exposure period was followed by one year recovery time before the experiment was terminated.

Under this basic project design a series of partially independent subprojects were conducted to cover the effects of the oil on the various ecosystem elements. The subprojects covered all levels of biological organization from community structure to the individual and cellular responses. The emphasis was put on the key species of the community, and a multidisiplinary attack on these was also expected to give results which were mutually beneficial when each subproject was worked up. The project also expected to single out the sensitive elements of the community towards chronic oil pollution. Such information is highly valuable when designing cost-effective shoreline environmental monitoring programmes.



Overview of the four Rocky Shore Mesocosms. The wave generator bar is seen on the right side of the basin, the steps with the main littoral community on the left side.

Photo: A. Pedersen

Chemical analysis of hydrocarbon content in water

Torgeir Bakke and Kai Sørensen

Purpose

The purpose of the hydrocarbon analysis within the Rock Littoral project was to determine the levels of hydrocarbons in the dosing system, the basin water, organisms, bottom sediment, and the substrate on the walls. This report presents results from the analysis of the hydrocarbons in the water.

Work performed

Two types of water analysis were involved: Fluorescence spectroscopy was used to perform frequent and inexpensive estimates of the concentrations of oil hydrocarbons in the water/oil emulsions (water accomodated fraction, WAF) for routine adjustment of the dosing system and in the basin waters. The aim was also to improve the fluorecence technique for routine monitoring of oil concentrations in experimental set-ups. High resolution gas chromatography was used to determine the more realistic concentrations of oil hydrocarbons in WAF and basin water samples and the relative composition of the different oil components in these samples.

During the 24 months exposure period the following analytical scheme was adopted.The fluorescence sampling points were the outlet of the separation unit of the dosing system, and the inlets to the header tanks of the two exposed basins. Samples were taken every Monday, Wednesday and Friday. Samples were also taken 10-30 cm below the surface in the south end of each basin. GC samples have been taken from the same positions once every week during most of 1983/84, but the budget has only allowed a fraction of the samples to be analysed.

The fluorescence samples were analysed immediately. the GC samples were extracted on site and the extracts transferred to SI for

analysis.

Results and discussion

WAF analysis

The fluorescence analysis showed quite large fluctuation in the concentration of oil in the WAF even between samples taken the same day. Most of this variation can be explained by fluctuation in the mixing efficiency of the propellers. The range in values throughout the 25 months was (in mg/liter = ppm, based on means of duplicate samples):

Source	Maximum	Minimum	mean + 1 st.dev.	samples
Oil mix unit outlet	43.4	6.3	18.4 + 5.9	255
HO header tank inlet	39.1	4.2	15.8 + 5.6	255
LO header tank inlet	39.1	3.5	13.6 + 5.4	255

The GC extracts of WAF samples analysed to date cover the period from October 82 to June 83:

Source	Maximum	Minimum	Mean + 1 st.dev.	samples
HO header tank inlet	37.0	13.5	24.0 + 11.3	5

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

Basin waters

Direct fluorescence analysis of the basin water was done routineously after August 1983. Based on the regressions between these analyses and corresponding WAF analysis, basin concentrations for the whole 2 years period was estimated. This showed that the communities were exposed to the following mean concentrations of total oil hydrocarbons during the period September 1982 to September 1984 when background fluorescence (oil and other components, 5.6 mg/liter) was adjusted for:

HO basin: 129.4 + 33.3 mg/liter (st.dev.)
LO basin: 30.1 + 10.3 mg/liter (st.dev.)

The results showed that the mean exposure was highest in 1982, just

after the dosing system was started. The ratio between the concentration of the HO and the LO basin became gradually higher during this period: HO concentrations were 3.7 times higher than the LO concentrations in 1982 and 4.7 times higher in 1984.

The corresponding gas chromatographic analysis showed lower mean values, but only 6 reliable sets of samples have been analysed to date:

HO basin: 79.1 + 54.1 mg/liter (st.dev.)
LO basin: 24.9 + 21.1 mg/liter (st.dev.)

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 75-100% higher concentration of hydrocarbons than the water below.

Note: The fluorescence and GC analysis is based on different properties of the hydrocarbons present. When referring to the exposure levels presented here, one must therefore stress which series of analysis the values have derived from.

Work remaining to be done :

A series of frozen extracts will be analyzed by GC and GC/MS to obtain more information on the composition of the hydrocarbons in the basin water and to improve the comparison between the fluorescence and GC analysis series.

Chemical analysis of hydrocarbon content in marine organisms

Sigve Sporstøl and Frøydis Oreld
Center for Industrial Research (SI), Oslo

Purpose

The purpose of the project is to study the hydrocarbon load and dynamics of different species of marine organisms continuously exposed to low concentrations of diesel oil.

Work performed

Four different species were selected for tissue analysis: Fucus serratus, Ascophyllum nodosum, Mytilus edulis and Littorina littorea. Samples were collected from HO, LO and C_2 basins at different dates during the 2 years of exposure. Sediment samples and scrape-off from the wave generators were also analysed, and these samples were collected after 14 months of exposure.

The samples were analysed for total hydrocarbon content (THC) using capillary gas chromatography and for selected aromatic hydrocarbons (NPD) using computerized gas chromatography/mass spectrometry (GC/MS). The aromatic compounds were naphthalene, phenanthrene/anthracene, dibenzothiophene and their C_1, C_2 and C_3 alkyl homologs. These compounds are among the most abundant aromatics in diesel oil.

Results and discussion

A significant accumulation of aromatic hydrocarbons (NPD) was found for all samples collected from the HO as well as the LO basins. There might be a correlation between tissue concentrations and life cycles for some of the species. Highest concentrations were found in Mytilus and Littorina. The THC analysis is less sensitive than the NPD analysis for detecting accumulation. The main reason is high and variable amounts of biogenic hydrocarbons which cannot be distinguished from the petrogenic hydrocarbons.

Mytilus edulis

The average concentration of NPD for all samples collected from the HO basin was about 10 times higher than the average concentrations of samples from the control basins. Samples were only collected during the first year of exposure. There is a trend of increasing accumulation during this period. The concentration of NPD in the HO mussels was 9,3 mg/kg after 2 months of exposure and 25,4 mg/kg after 24 months of exposure.

Littorina littorea

The average concentration of NPD in samples collected from the HO basin was about 8 times higher than the average concentration in the control samples. The average concentration in the LO basin was about 3 times higher than in the control basins. There was a trend that the tissue concentrations were lower during the summer season than in the winter season.

Fucus serratus

The average concentrations of NPD in samples from the HO and LO basins was about 7 times higher and 3 times higher, respectively, than the average NPD concentrations of the control samples. The tissue concentrations remained quite stable during the exposure.

Ascophyllum nodosum

The average concentration of NPD in the HO and LO basins was about 9 times higher and 4 times higher, respectively, than the concentration of the control samples. There was a trend in the HO basin of increasing content of NPD during the exposure period. The same trend was not found in the LO basin, where the concentrations remained quite constant during the exposure.

Scrape-off from wave generators

Samples collected after 14 months of exposure showed a 6 times higher concentration of NPD in the HO basin compared with the control basin.

Sediments

The concentration in the 0-2 cm top layer of the HO basin sediment was about 3 times higher than that measured in the control basin, after 14

months of exposure.

Work to be done

Samples of Littorina littorea were also collected one year after the termination of the dosing. These samples will be analysed in December 1985.

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	01.05.83	182	4.11
HO	22.09.83	175	3.20
HO	02.12.83	467	3.46
LO	20.12.82	191	1.13
LO	01.05.83	225	1.43
LO	22.09.83	292	1.02
LO	02.12.83	196	1.80
C_2	20.12.82	267	0.60
C_2	01.05.83	198	0.23
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
HO	16.08.84	159	4.48
LO	20.12.82	152	0.86
LO	01.05.83	113	1.53
LO	16.08.84	37	1.10
C_2	20.12.82	35	0.52
C_2	01.05.83	73	0.26
C_2	16.08.84	35	0.12
Mytilus edilus:			
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	25.03.83	97	1.65
C_2	09.12.82	59	1.15
C_4	Nov.83	78	0.82
Fjord	09.12.82	93	2.83
Fjord	25.03.83	183	1,26
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
Scrape off from wave generator			
HO	23.11.83	842	8.33
C_2	23.11.83	709	1.34
Sediments			
HO	23.11.83	21	0.17
C_2	23.11.83	2	0.05

COMMUNITY STRUCTURE

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

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. Lately recovery succeeding the termination of the oil exposure has been studied.

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. Six parallel quadrats from each step/bottom are investigated.

15 characterizations of the community structure have been performed during the period of investigation June 1982 - September 1985. Data of the monitoring have been processed for about 20 species from the basins. The community structure is also documented by photographs in connection with the monitoring.

During 3 years about 50 different species of algae and benthic animals have been recorded. During the 15 monitoring periods some organisms have shown normal annual changes, while other species have been reduced or have increased in density.

Ascophyllum nodosum and Fucus serratus have been very stable during the entire period. Reduced populations of F. vesiculosus are observed with exception of C4. The two oil exposed basins were the only basins to experience continuous decrease of F. distichus spp. edentatus. Regarding fucoid germlings the growth was greater in the two controls than in HO. During 1985 the Laminaria digitata population has increased in the two oil exposed basins, while Ulva lactuca is reduced during the same period.

Mytilus edulis has remained "not observed" in LO, while HO experienced settlement during the fall 1985. Balanus balanoides has been stable (LO) or increased (HO) in the two oil exposed basins while the controls have showed some decrease. Littorina littorea has been reduced in LO and HO, while Asterias rubens showed an increase since 1984.

Processing of the 1985-data is not started, but will be worked out during 1986.

The preliminary conclusions until the end of diesel oil exposure are:

1. 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 of the communities in the basins.

2. The cluster analysis did indicate an evolution from four basins with minor differences to basins grouped in two as controls (control two, control four) and oil exposed (high oil, low oil).

3. The grouping of canopy species reflects the stability of the perennial brown algae in the basins, thus the stated difference between the basins are due to the understory species and the motile animals, of which the populations in the controls experienced small changes from 1983 to 1984 compared with the oil exposed basins, mostly due to the decrease in Mytilus edulis and Asterias rubens respectively.

4. Very low diesel oil concentration mixed into the sea water may favour the growth of some algae.

Recolonization and population structure of intertidal organisms

T. Bokn (NIVA)

Introduction

The aim of the subproject presented here was to see if low continuous exposure to diesel oil has any effects on colonization of intertidal organisms, and to study any recovery succeeding the termination of the oil exposure.

Material and methods

The growth [1) covering degree, 2) species composition, 3) dry weight and 4) ash free dry weight] in an oil exposed basin (HO) is compared with corresponding data from a control basin (C2). For this purpose chips of granite (10cm x 10cm) are used.

Originally 44 chips were located on steps in HO and C2 corresponding to the lower part ot the intertidal in the basins. To avoid grazing by littorinids two racks with 44 chips are mounted in free water, one in each basin. The sampling procedure is to remove three short-term chips every month and two long-term chips every second month from each rack and each step. The sampling has been carried out every month during three years (October 1982-September 1985). Only the data from the first year have been processed, but the remaining samples and data are expected to be worked out during 1986.

Conclusion

The present processed data of ash free dry weight are inconclusive as to whether the diesel oil improves or inhibits the growth on the chips. However, all the predominating species in the oil exposed basin during the first year have been diatoms, and none has played any important role in the flora in the control basin with very few

exceptions. Included in the predominating species of the control basin have been two annuals of Phaeo- and Chlorophyceae, which never showed the same appearance in the oil exposed basin.

For more datails:

Bokn, T., 1984: Effects of diesel oil on recolonization of benthic algae. Hydrobiologia, 116/117: 383-388. Contribution No 1 from the Marine Research Station, Solbergstrand, Oslo.

Community metabolism in fouling assemblages

Are Pedersen, NIVA.

1. Objectives

- The main objective is to study the impact of diesel oil on the metabolism of fouling assamblages found in the vicinity of marinas harbours and areas of fuel or diesel oil discharges
- The following elements are examined: Production, respiration, C-,N-, P-ratios, biomass and species composition.

2. Design

- Rocky shore communities have been established in four concrete basins.
- Size: 8x5x1.5m, 1.25m water depth.
  Basin water volume: $25m^3$ at mid tide.
  Water supply: from the fjord at 1 m depth.
  Exchange rate: $10m^3.h^{-1}$ through header tanks.
  Tidal range simulation: 0.3 meters amplitude. 12 hours sinusoidal cycle.
  Wave generator: Mechanical 18 strokes.min^{-1}.
- The substrare for fouling assenblages is GRANITE TILES which are exposed to permanenet wave actions in the eulittoral zone.
- The tiles are divided into two groups: NON-GRAZED and GRAZED.
- The grazed tiles are placed on the STEPS in the basins and the non-grazed tiles are situated on a RACK.
- Periwinkles (Littorina littorea) are the key grazers in the basins.
- Some tiles are replaced every month.

3. Methods

a) LABORATORY measurements of production and respiration
   - Oxygen method
   - Waterbath- constant tempeature (+ 3 ^{0}C from basin temp.)

- Light intensity (PAR) regulated by use of different grey filters to give 12,24,48,74,105,145 and 279 $\mu E.sec^{-1}.m^{-2}$.
- Oxygen produced and consumed at different light intensities is recorded on a X-Y recorder.

b) IN SITU oxygen and light are performed by use of a respirometer.
   - Oxygen tension is recorded simultaneously in three duplicate chambers every 5 minutes together with ambient light intensity (PAR).
   - All measurements are logged on a data recorder.
   - The chambers are automatically flushed for a 2 minutes period every hour.
   - Every 24 hours readings are made on a new set of tiles. Maximum 11 sets of tiles per. month.

c) Based on in situ and lab. experiments, production vs. light (P-max) and production vs. time curves are produced.
   - The SLOPE (α) of the P-max curve reflects affinity to light. The INFLECTION POINT (Ik) gives a relative measure of both reponse to light and magnitude of production. Ic is the COMPENSATION POINT ie. where production = consumption.

   - Differences in P-max, Ik and Ic between oil and control assamblages may reflect oil effects.

4. Conlusions for the dosing period.

- The NET and GROSS PRODUCTION, RESPIRATION and Ik of the assemblages on the MONTHLY REPLACED tiles are significantly different between the oiled basin and the control. This is most pronounced when refering to m^{-2} and not the biomass as Chl.a.

- Tiles successively collected from the start of the experiment-LT. (Sept-82), show very few significant differences in the above mentioned parameters between the oiled basin and the control.

- The SPECIES COMPOSITION in the oiled basin at times differs from that of the control basin.

Further work to be accomplished:

- Analyse the recorded data and the samples for taken for chl. and

CNP during the recovery phase.

- Analyse the recorded data and the samples for taken for chl. and CNP at the racks in HO, C2 and C4 during the recovery phase.
- Comparison with the result obtained from the subprojects concerning 1)Energy balance in Littorina littorea and 2) Dry weight and ash free dry weight on the tiles.
- Impovement on the 8 already existing computer programs developed to analyse the data recorded on the respirometer.
- It would be of great value to do some more identification of the species growing on the tiles to support the parameters calculated. The species-composition may itself show distinct differences between the oiled basin and the control as it did on oiled and non-oiled soft bottoms (Bakke et al. 1985).

Publication

ABSTRACT from a poster exhibition at the 2nd. International Phycological Symposium, Copenhagen. Denmark.

Pedersen, Are
Norwegian Institute for Water Research, P.O.Box 333, Blindern 0314 Oslo, Norway.

COMMUNITY METABOLISM ON FOULING ASSAMBLAGES

The project presented is incorporated in a large scale enclosure programme presently being performed at the Marine Research Station Solbergstrand in the Oslofjord. The aim is to study long term effects of diesel oil on the metabolism of fouling assamblages in the littoral zone.

Clean granite tiles were used as assamblages. The tiles, 10 x 10cm, were placed in the littoral zone in three large basins (50 m^3) Of which one was exposed continously to a diesel oil/sea water emulusion at a level of 100 ug/l. The tiles were divided into non-grazed tiles and tiles exposed to grazers. remaining two basins act as controls. In a one of the controls and the "oil basin", half of the chips were exposed to grazers ie. mainly the snail littorea and the other half of the chips were not exposed to grazers. Some tiles were replaced every month by new ones. Production measurement by use of the oxygen method were performed on the tiles to give P-max curves for the assemblages.

The mean Ik values during a period of one year found in the oiled basin were on the grazed tiles 93 $\mu E.sec^{-1}.m^{-2}$ and nongrazed tiles 97 $\mu E.sec^{-1}.m^{-2}$ In the control basin the values were 69 and 91 $\mu E.sec^{-1}.m^{-2}$ respectivly . The mean net. P-max at the same kind of tiles were for the oil basin found to be 74 and 114 mg $O_2.m^{-2}.h^{-1}$ on grazed and nongrazed tiles respectively. In the control the grazed tiles reaced a mean production of 203 and 153 mg$O_2.m^{-2}.h^{-1}$ resectively. Samples were also taken for Chl. a, ash free dry weight, C, N and P.

Settlement, growth and community structure on granite chips A comparison between four bottom basins at Solbergstrand Experimental Station and a sheltered locality in the Oslofjord.

Odd-Arne Follum, UiO.

Purpose

To try to clearify some of the growth patterns in the rock bottom basins, compared with each other and with a natural locality.

To try to elucidate factors depending on oil contamination or by internal differences beetween the basins.

Work performed

Granite chips (15 x 15 cm) were used as settling substrate. They were placed, vertically, four and four in aluminium frames, facing north-south. In each locality there was a "pelagic treatment", where the frame was out of reach from grazers and predators, and a "benthic treatment", standing on a stair about 25 cm below the surface. Two chips in each frame were changed with new ones every month. They were all analyzed every fourth week from March to December 1984. Per cent cover was counted, non destructively, under a transparent plastic sheet with 100 evenly distributed points.

The natural locality was located at Emmerstadbukta, a sheltered bay about 3 km south of Solbergstrand.

In addition to the settling experiment, monthly plankton hauls were taken from the fjord and compared with hauls taken from the inlet of every basin.

Results

Even if there were some indications of the first basin received the largest and heaviest particles like sand, algal fragments and other organic particles, no significant evidence was found for different input of organism to the different basins. Still, the large bulk of phytoplankton found in fjord hauls was absent in the basin hauls.

The most conspicuous group of organisms in the pelagic treatment was the diatoms. They dominated the whole period, and especially in the high oil basin. Basin 2 (C2) had the least cover of diatoms, and basin 3 (LO) and basin 4 (C4) were surprisingly similar, both in diatom cover and also in settlement of other primary growth and canopy.

Settlement and growth of organisms other than diatoms was negatively correlated with the diatom layer. It consisted mainly of the barnacle Balanus balanoides and the red alga Polysiphonia sp.

In the natural locality, Emmerstadbukta, the pelagic treatment was dominated by barnacles; B. balanoides from April to June until strong northerly winds removed most of them. This made free space for Balanus improvisus to settle. Red and green algae dominated in the autumn, and only in September were benthic diatoms observed.

As expected, the chips exposed to herbivore grazing had less algae than the pelagic treatment. Again, HO basin had most diatoms and LO and C4 had least. Only primary growth resistant to grazing survived, i.e. barnacles and the encrusting brown alga Ralfsia sp. In HO basin, the feeding rate seemed to be reduced, probably because of the oil contamination. In C2, the population of Carcinus maenas reduced periwincle grazing.

At Emmerstadbukta, the great natural population of the Littorina littorea cleared the surfaces except for some barnacles.

Conclusions

The different growth patterns in the four rock bottom basins are not caused by different plankton input.

Benthic diatoms dominated the HO basin bacause the contamination gave these organisms advantages as reduced grazing and apparently less growth from competitors. Obviously an "oil-effect".

The oil contamination in the LO basin seemed not to be strong enough to affect settlement and growth, because LO and C4 were very similar in this respect.

The two control basins (C2 and C4) seemed to offer the organisms a different environment, because the structure of settlement, growth, grazing and predation was quite different.

Even if Emmerstadbukta was very sheltered in the predominating southerly winds, it was still more exposed than the basins. This created a different growth pattern on the substrate, with a quicker change in dominating organisms. The structure of the grazer population also made this locality different from the basins

This research was terminated December 1984.

On settlement and growth of sessile organisms in the littoral zone.

A comparison of a natural Oslofjord locality and an artificial semi enclosed locality at Solbergstrand Experimental Station.

Odd-Arne Follum, UiO.

Purpose

To see if there were differences in settlement and growth between one control basin (C2) and a natural, comparable locality in the fjord, and eventually what factors could create such differences.

Work performed

Pavement stones with rough sides were placed at both localities; three replicates at three depth intervals. Per cent cover of every organism on them was counted every 4.th - 6.th week from September 1981 to December 1982.

A transparent sheet of plastic with 100 evenly distributed points was laid over each side of the stones for non destructive counting. The differences of per cent cover were recorded for localities, depths and orientation. The most important mobile animals like Asterias rubens and Littorina littorea were counted each time.

Incoming light was once registrated simultaneously on both localities. Nutrient samples and plankton hauls were taken on both localities from April to December 1982. Comparison of wave heights were done on several occasions in different wind strenghts and directions.

Results

The fjord locality was dominated by primary growth of the barnacle Balanus improvisus and the encrusting brown algae Ralfsia sp. The canopy, which was very scarce, was dominated by the green algae

Chaetomorpha linum, not very palatable to the many grazers.

The basin stones were totally dominated by the opportunistic green algae Ulva lactuca and Enteromorpha spp. On this substrate, primary growth was scarce.

The high amount of L. littorea at the fjord locality quickly cleaned the surface from settling algae, and made the substrate available for the barnacles, even if they were preyed upon from Asterias rubens in the autumn. Neither A. rubens nor L. littorea were recorded in great numbers in the basin. It is believed that the periwincles here were controlled effectively by the great bulk of Carcinus maenas. Because of less wave exposure in the basin, the shore crabs could move easily in the whole basin. This predator was not observed in the uppermost depth level in the fjord.

Although the basins received less light than the fjord locality, because of the walls and the dark wave generator, this had apparently no significance for algal growth.

The only organisms not found in plankton hauls in the basin were the most fragile ones. Medusae and Comb jellies were believed to be destroyed in the pump. The great differences of the plankton samples was the dominance of phytoplankton in the fjord hauls. In the basin, very few, and mostly large, specimens were observed.

Conclusion

A clear difference in settlement and growth could be observed between basin 2 (C2) and a natural fjord locality. One main explanation could be the difference in grazer and predator population, with the water movements as the trigger factor.

The pumping system of seawater in to the basin is not thoroughly investigated as a source of less input of settling organisms or food.

The basin worked as a sediment trap compared with the fjord. This affected the primary growth on the oversides of the stones, smothering the surfaces.

The great differences between the localities made it difficult to say if they were caused by the artificial semi-enclosed basin, or just the natural differences between two clearly differently exposed

localities.

The work performed is part of my Cand. real thesis and was terminated December 1982.

LENGTHWISE GROWTH OF BROWN SEAWEEDS

T. Bokn (NIVA)

The aim of the present study was to see if low continuous dosage of diesel oil has any effects on the length growth of two commercial species of seaweeds, and if so to study any recovery succeeding the termination of the oil exposure.

During June/July 1982-85 about a 100 tips of Ascophyllum nodosum were tagged each year. Growth (rate of elongation) was was followed by measuring from the upper end of the youngest vesicle to the very top of the tip. During March 1983-85 about 100 specimens of Laminaria digitata were also tagged.

No significant growth difference of A. nodosum tips was observed in the first year. However, during 1983 and 1984, the new tips of the year in both oil exposed basins were significantly shorter compared with the tips of the control. The growth difference. of L. digitata from March to July 1983 was found not to be of significance. However, during spring 1984, both oil exposed basins have become significantly different from the controls. Studies of recovery were performed during spring/summer 1985. The data has not been processed yet.

For results and discussion cf.:

Bokn, T., 1985: Effects of Diesel Oil on Commercial Benthic Algae in Norway. Proceedings 1985 Oil Spill Conference (Prevention, Behavior, Control, Clean up), February 25-28, 1985. Los Angeles pp. 651. Contribution No 2 from the Marine Research Station, Solbergstrand.

Long term effects of oil contamination on population dynamics of Littorina littorea in enclosures at Solbergstrand Experimental Station

Kjell Moe and Einar Lystad, University of Oslo.

The research on Littorina littorea population dynamics in the enclosures at NIVA Marine Research Station, Solbergsstrand during 1982-1983 formed the basis of our thesis (Lystad og Moe 1985) at the University of Oslo. Recruitment, growth, population densities, mortality and the breeding cycle were examined for both the oil exposed and control populations also throughout 1984, i.e. the obtained data covering the period of oil contamination.

Methods (work performed)

Details of the following methods are given in previous progress reports and in Lystad & Moe (1985).

Recruitment; plankton hauls from both inlet and outlet water were sampled during a three days period in spawning season 1983. Post-larvae and juveniles were recorded separately. Growth; accurate measurement of individually marked winkles every second month gave exact shell height increments. Tissue dry weight of ten individuals from each basin were measured every month. Estimations of population densities were based on capture-recapture of marked individuals. Survival rate and mortality were estimated on the basis of the population size parameters. These mortality data were accompanied by shell height measurements of dead winkles in sediment samples. Annual breeding cycle; gonads of ten individuals from each basin were sectioned and maturity stage examined each month.

Results :

Recruitment (1983-1984). No statistical significance was observed between the amount of eggcapsules received in each basin. There was

zero recruitment in HO and LO populations. Significantly higher number of post-larvae juveniles and pre-mature individuals were observed in C2 and especially in C4. A large number of the 1984 generation were found in all basins in Nov.-84.

Growth, shell height increment (1983). Individual growth rates within the populations varied significantly. Individual growth rates of mature winkles were ranked from high growth rate to low growth rate in the order C2 LO HO C4. The differences in growth rates were statistically significant ($p < 0.05$) between C2 and the other populations and between LO/HO and C4 individuals.

Growth described by the parameters L_∞ & k in von Bertalanffy`s growth equation was in each population:

	HO	C2	LO	C4
K	0.616	0.755	0.528	0.616
L_∞ (mm)	24.15	25.95	25.14	21.41

Significantly higher growth rates in all basins in 1984, except in C4.

Dry tissue weight (Dtw) (1983-1984). First year mature L. littorea in HO had statistically significant ($p < 0.05$) lower Dtws than winkles of the same size in the other populations (which were statistically equal ($p > 0.05$)). With size close to the population specific L_∞, winkles in C4 had the lowest Dtws (stat.sign $p < 0.05$). These Dtws were highest in LO (stat. sign. $p < 0.05$) and equal in HO and C2 (stat. equal $p > 0.05$). Calculated Dtws from monthly sampled winkles (applied to a winkle of "standard" length = 22.10 mm) were lowest in HO (not equal, stat. sign. $p < 0.05$) and equal (stat. equal $p > 0.05$) in C2, C4 and LO. These results were similar for both sexes. Dtw analysis were sensitive registering spawning periods.

Population size and mortality (1983). Population size (N) was highest in C4, N in HO higher than N in C2 and LO, where N in C2 was the lowest (differences in N, stat. sign. p 0.05). Taking into account the confidence intervals and inaccuracy built into the model (Chapman, modified Petersen estimates), the decline of N during 1983 in HO, was the only decline of N which was significant. Sediment samples documented very high mortality of post-larval juveniles in all basins.

About 90 % of the empty shells were smaller than 4 mm.

Annual breeding cycle (1983-1984). Examination of the gonads showed that annual breeding cycle was synchronized neither individually nor between the populations. There were indications of HO winkles having a shorter maturing stage and a somewhat shorter spawning period compared with C2, C4 and LO animals. Connective tissue build up in the spent stage seemed to be at a slower rate in HO winkles.

Conclusions

Lack of recruitment of L. littorea in the oil contaminated basins (and to a certain extent in C2) could be an effect of heavy predation pressure by Carcinus maenas on post-larval juveniles as well as oil contamination. In enclosures with little wave exposure, the effect of shelter on survival of the juveniles was demonstrated by the presence of a large number of juvenile winkles only in C4 (on the rough surface of the steps.) Sharing the "limited" food resources in an enclosure, the high population density might be the explanation why the C4 winkles had the slowest growth rates. This seemed to have a greater effect on the shell height increment than High Oil contamination. If the significant lower Dwts were an effect of the oil contamination in HO, as we suppose it was, tissue metabolism seemed to suffer more than shell height increment. The analysis of spent winkles support this speculation.

The present conclusion of our data recruitment, growth and Dtw must be that young L. littorea (which had lived a relatively longer period in oil contamination) suffered more than adult individuals. We argue that the decline in population size, slow growth rates, low Dtws, early spawning and the short spawning period could be explained as negative effects of HO oil concentration on adult L. littorea. However where LO is conserned, with a dense cover of Ulva lactuca at the bottom, we assert that availability of preferred food and low population density, to a certain extent compensate the negative effects of the oil contaminated environment. The higher growth rates in HO, LO and C2 (not in C4) in 1984 support this argumentation. We stress this assertion only for our results from LO and shell height increment in HO. Additionally, the higher growth rates registered in 1984 in HO and LO could indicate long term adaptation.

Remaining work

- Complete analysis of the 1984 data concerning individual growth

and population sizes in 1984.

- Complete size frequency analysis to elucidate L. littorea populations selection strategy.

- Fecundity analysis and dry weight analysis of shells and tissue of frozen winkles sampled in spring and autumn 1984.

Litterature

Lystad, E. and Moe, K. A., 1985: En sammenligning av sider ved populasjonsdynamikk, vekst og kjønnssyklus hos Littorina littorea (Linnè) på fire kunstig etablerte og en naturlig lokalitet på Solbergstrand, Oslofjorden, hvorav to av de kunstig etablerte lokalitetene var belastet med hydro- karboner. Hovedfagsoppgave ved Universitetet i Oslo.

Population genetics of three organisms in the hard bottom basins

Svein E. Fevolden and Oskar I. Sigurdsson, UiO

Introduction and aim

Strong evidence has been put forward to establish a link between the marine physical environment and frequencies of specific alleles, or genotypes occuring in populations of marine organisms. While a correlation between balancing selection and allozyme frequencies has been related to fluctuations in natural environmental parameters like salinity, temperature and degree of exposure, less attention has been paid to genotypically mediated responses to stress from oil pollution. This study, therefore, was aimed at investigating the possible correlation between genetic variation and survival of organisms in water with low concentration of oil.

Survey design

The survey design was to compare the genetic structure of basin populations of three selected organisms before and after a 1-2 year period of oil exposure, and to compare new recruits that successfully settled in the oil-exposed and in the control basins respectively.

Organisms

The organisms selected for the study were the mussel, Mytilus edulis, the periwinkle, Littorina Littorea, and the barnacle, Balanus balanoides. Communities of those were well established in the basins prior to oil dosing.

Population genetic methods

Genetic variation within and between samples of the different orgamisms was examined by means of enzyme electrophoretic techniques. Initially about 30 enzymes were studied for all organisms to detect polymorphic gene-loci, that is loci which code for iso-enzymes from at least two alleles. The genetic variation was determined by measuring frequencies of the different alleles occuring at each locus. Tests for deviations from Hardy/Weinberg equilibrium and contingency table tests for allelic heterogeneity among the samples were used to detect potential selection from the presence of oil.

Results

Mytilus edulis

Lack of significant deviations from Hardy/Weinberg expectations at 30 gene-loci indicate that the basin populations of Mytilus edulis are recruited from one single randomly mating stock. Six polymorphic loci were compared among groups of individuals living in the experimental basins, with and without oil, and in the adjacent fjord. Isolated indices indicated differences between the basins and the fjord, and even between oil and non-oil basins. Divergence between adults in fjord and basin populations were at different loci than for those between juveniles in the two environments. A significant difference in allelic proportions at the IDH locus between juveniles in basins with and without oil was only seen in one of the two years of oil exposure. The inconsistency in allele differences both in time and age could suggest that the differences were caused by yearly variations rather than selection.

While the oil dosing had a severe effect on the mussels` viability in the basins, as well as on the settling of new recruits, these two fitness components did not seem to be correlated to genotypic differences for those gene systems that were studied

Littorina littorea

Only two out of 31 loci were polymorphic after the more restrictive criterion for polymorphism (the frequency of the most common allele

should be less than .950). One of those, 6PGDH, showed a significant among basin heterogenity before oil dosing started. Selective factors in the basins may have been present at the time of settling, or chronically over time, causing this anomaly. The same locus showed divergence between fast and slow growers in the high-oil basin after one year of oil exposure. The second highly polymorphic locus, PGM-2, together with the remaining loci, showed no divergence among the different samples, Neither were deviations from Hardy/Weinberg equilibrium found. Two years of oil exposure seemed to have minor effect on adult L. littorea`s ability to survive. Settling of new recruits, however was low in all basins. The low number of juveniles that could be electrophoretically analyzed showed no difference between oil and non-oil basins.

Balanus balanoides

About 1250 newly settled individuals from the different basins together with 600 that survived one year of oil exposure have been analyzed electrophoretically for 20 different loci. Thirteen of those loci were polymorphic. The statistical analyses of the electrophoretic data are due finished early 1986.

On Carcinus maenas (L.) in the four rock bottom basins at Solbergstrand Experimental Station.

Kjell-Are Moe, Odd-Arne Follum and Einar Lystad, UiO.

Purpose

To estimate the population of shore crabs in the four basins in order to explain some of the interactions between the crabs, the periwinkles Littorina littorea and the algal growth in the basins.

Work performed

Crabs from each basin were collected by SCUBA divers in July, August, September and October 1984. Two divers in each basin sampled for 30 min. In October, the sampling was divided into 10 min. intervals and continued until less than five crabs were found in an interval.

The net weight and carapace width were measured and the sex recorded before they were put back into their basins.

Results

Fig. 1 gives estimates of total number of crabs in October 1984. Calculating the relationship between observed number and estimated number in October gave estimates for total number also for the previous samples. We expected the number first to rise, due to growth in newly settled individuals, and then to decline due to death of older ones.

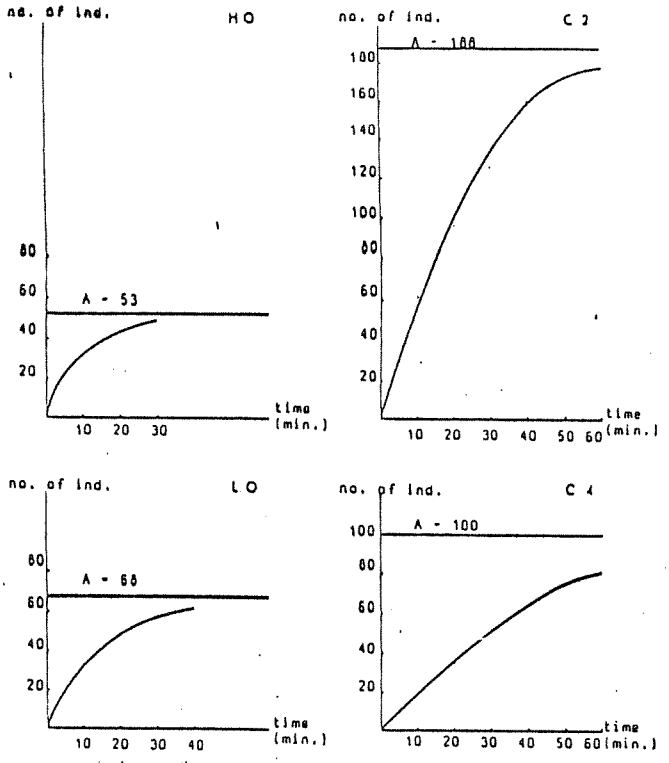


Fig. 1. Accumulated numbers of Carcinus maenas sampled in 10 minutes intervals in October(se Tab. 2.). The graphs are plotted using equation $Y = A - B\ t^x$, and A is the estimated total population at this time.

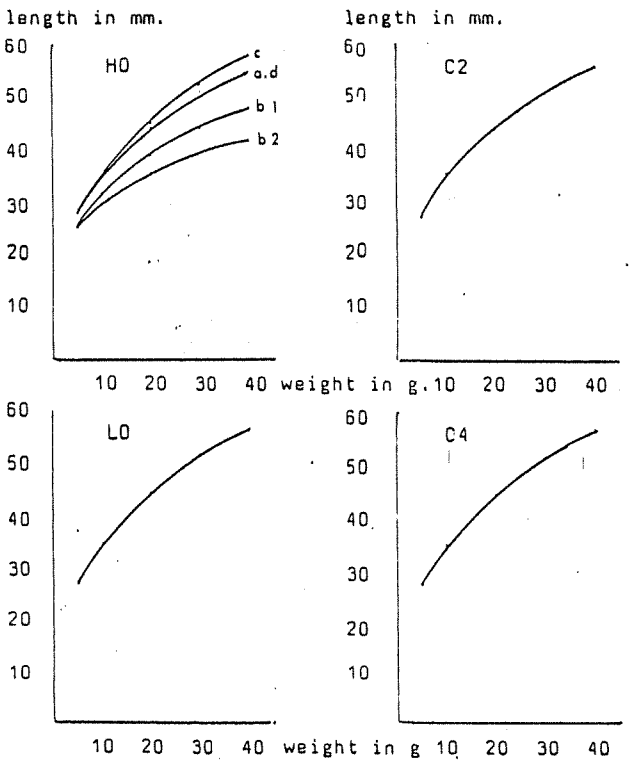


Fig. 2. The length-weight relation expressed by the equation length - a weight^b. The differentiation of the curves for each sex and sampling period are not visible on this scale except for HO, where a-20/7 both sexes, b1-females 20/8, b2-males 20/8, c-21/9 both sexes and d-16/10 both sexes.

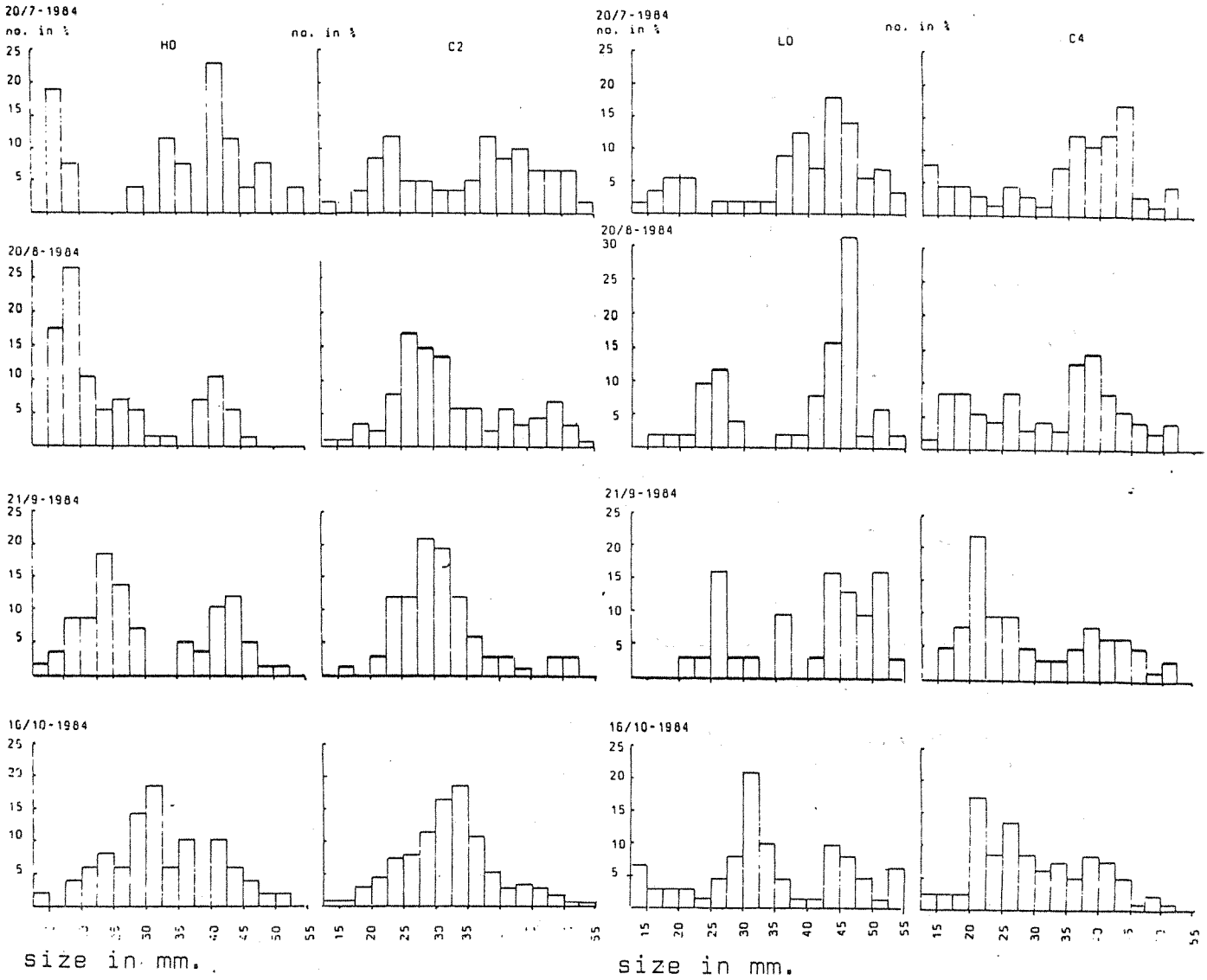


Fig. 3. Histograms of the size frequencies values of Carcinus maenas for each sampling and all basins.

The data in HO basin and in C4 fits this assumption. The variation in LO and C2 was probably due to the dense cover of algae and an artificial wall built in one of the basins (C2).

Size-frequency histograms were made for each sample (Fig. 3). Because moulting and spawning are beleived to take place over a long period of time and are dependent on basin conditions, the size-frequency analyses were very complex. Nevertheless, in the enclosed communities, the high number of adults has a great predatory influence on the Littorina population.

Conclusions

The C2 basin had a distinctly higher population of *Carcinus maenas* than the other basins. Then came C4 and LO with HO basin having least number of crabs.

This could explain the small population of *Littorina littorea* in C2 and the corresponding algal growth. The condition for *Littorina* recruits in C4 was very good, much shelter, so even a high predator population was not able to control the high number of periwinkles. In the oil contaminated basins, it was hard to say whether lack of food or the oil, or a combination of these, caused the small *Carcinus* population.

Since moulting in *Carcinus* depends on water quality, i.e. factors like temperature, Ph, Ca^{2+} ions and food resources, the ecdysis and water content in the crabs can vary a lot under abnormal conditions. Since oil contamination and food resources were variables, this can, and probably did, create unsynchronized moulting between the basins.

This research was terminated October 1984.

Physiological respones of Mytilus edulis during chronic oil exposure and recovery

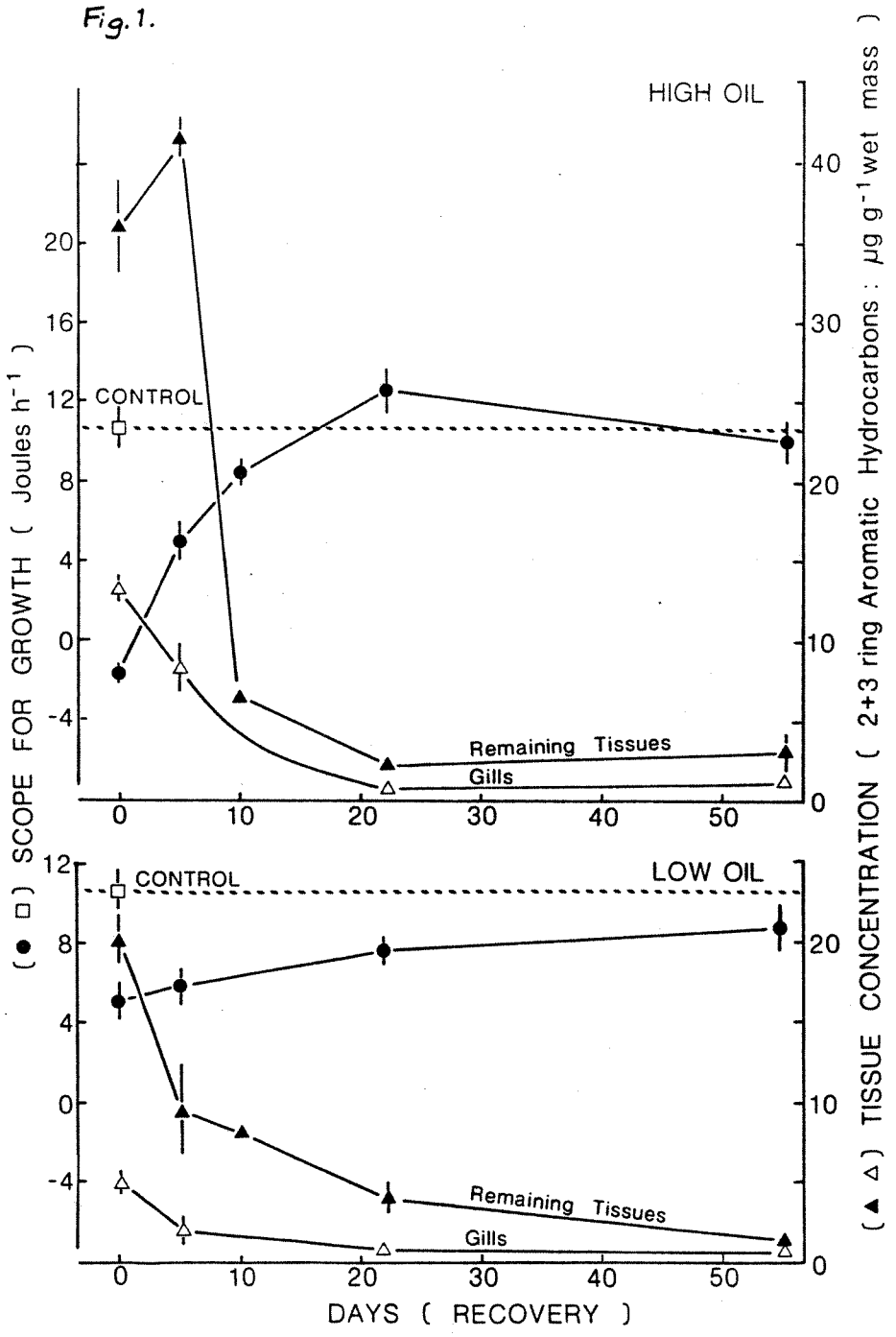
John Widdows and Peter Donkin, IMER, Plymouth

The objective of the study was to determine the physiological responses of Mytilus edulis to chronic oil exposure and the extent to which physiological recovery may be related to the depuration of hydrocarbons from the body tissues. The physiological responses (clearance rate, respiration, food absorption efficiency and ammonia excretion) of Mytilus edulis chronically exposed to two oil consentrations (30 and 130 $\mu g\ l^{-1}$) for 8 months were measured in May 1983 and May 1984. Mussels exposed to low and high oil conditions were transferred to the control basin for recovery and their physiological responses determind after 5, 10, 22 and 55 days. On each sampling occasion mussels were dissected into gills, digestive gland and remaining tissues, the hydrocarbons were extracted by steam distillation, analysed by HPLC and tissue consentrations expressed in terms of 2 and 3 ringed aromatic hydrocarbons.

The results showed an inverse relationship between scope for growth and hydrocarbon exposure. Mussels exposed to high oil ($130\ \mu g\ l^{-1}$) had a slightly negative scope for growth indicative of a severe stress condition, which accounted for the observed tissue degrowth. During recovery there was a close relationship between the recovery of physiological responses and the loss of hydrocarbons from different body tissues (Fig. 1). The gills depurated 40% of their accumulated hydrocarbons within 5 days and there was a comparable recovery of clearance rate. However, the digestive gland and remaining tissues of the "high oil" exposed mussels did not depurate significant amounts within 5 days and this could account for the maintenance of a reduced absorption efficiency. After 22 days in clean water the "high oil" exposed mussels not only had depurated a higher proportion of the accumulated hydrocarbons compared with the "low oil" mussels, but also their tissue hydrocarbon consentrations were lower. These observations correlated with a more rapid recovery and an "overshoot" in clearance (=feeding) rate and scope for growth by the "high oil" exposed mussels which gave rise to "catch-up" growth following tissue degrowth during "high oil" exposure. The rapid decline in tissue hydrocarbon

consentration of the "high oil" mussels within 22 days was probably the combination of an enhanced rate of hydrocarbon excretion/depuration, as suggested in a previous study (Widdows et. al., 1983) and a "dilution effect" due to enhanced tissue growth (catch-up-growth). The rate of recovery by "low-oil" exposed mussels was slower both in terms of tissue depuration and physiological performance, but both groups showed complete recovery after 55 days (i.e. not significantly different from controls). A synthesis of all measurements (oil exposed and recovery phases) showed a significant negative correlation (r^2=-0.84) between scope for growth and aromatic hydrocarbon concentration in the body tissues. This confirms that the degree of stress in mussels is a simple function of the tissue concentration of toxcicants.

Fig. 1.



Energy balance in Littorina littorea

Torgeir Bakke, NIVA

Purpose

To estimate the energy uptake and utilization of the four basin populations and one shore population of the common winkle Littorina littorea during the oil exposure period and for one year afterwards. To investigate if oil has an effect on the main pathways of energy conversion of the individual (food uptake, assimilation, respiration and excretion) and if sensitivity changes with season. To link the effects in the individuals to population responses (Lystad, Moe), biochemical and cytological responses (Moore, Lowe, Livingstone), to grazing effects (Bokn, Pedersen) and to tissue levels of hydrocarbons (Sporstøl, Bakke).

Work performed

Rates of food uptake, respiration, and ammonia excretion, and assimilation efficiency, have been estimated during the oil exposure (May, July and September 1983, April, June and August 1984) and during the recovery phase (October 1984, April/May, June and August 1985). Estimates of food uptake has been done by incubating single individuals for 1-3 days with either microalgae (1983) or Ulva lactuca (1984-85) as food, oxygen uptake of single individuals by closed respiration chambers, ammonia excretion of single individuals by 2 h incubation in filtered sea water, assimilation efficiency through the organic content of food and of faeces sampled from groups of 50 individuals. Rates have been related to individual size and weight.

Results and discussion

The feeding rate estimates from snails feeding on microalgae were considered unreliable. Estimates using Ulva showed that food uptake in the two exposed populations was less than in the three control populations during the dosing period (1984), but no

dose-response relationship could be defined (Figure 1). Feeding rate among the control populations varied considerably, the highest rates being found in the C4 and shore population. The C2 feeding rates lay between these and those of the exposed populations.

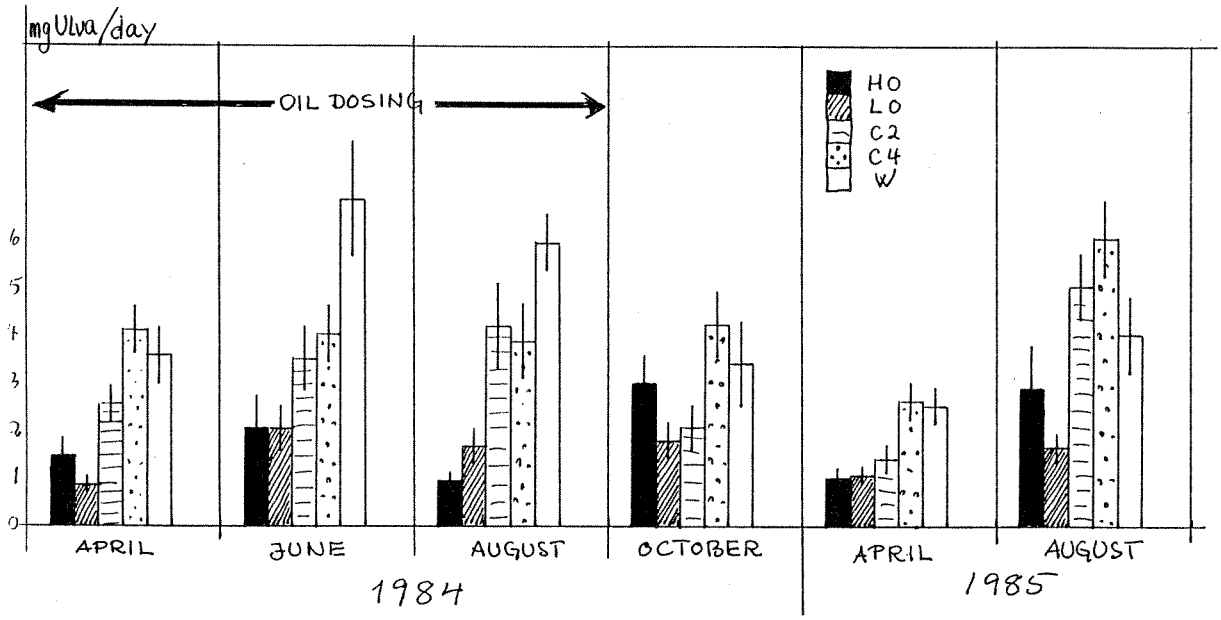
Within two weeks after termination of the oil exposure, feeding in the HO population had increased to the level of the fjord population, and higher than the C2 rates. No recovery was detected in the LO population. However, in April and August 1985, i.e., after nearly one year recovery, the two exposed populations were still feeding less intensively than the control populations, the LO population significantly less. This must either reflect an unexpectedly long-lasting effect of the oil or that the differences between exposed and control populations to a large extent were features intrinsic to the populations and not caused by the oil. It is anticipated that a more firm conclusion about this can be reached when biochemical and cytological recovery is fully analysed and the tissue levels of hydrocarbons one year after the end of dosing is determined.

During oil exposure the population differences in the other processes of energy conversion were small and rarely significant, but generally they strengthened the tendency of less favourable energy conditions in the exposed populations: respiration was slightly higher, and assimilation efficiency less than in the controls. Ammonia excretion was slightly higher at the beginning of the feeding season, but not for the rest of the summer or autumn. Analyses of the data for these processes during the recovery phase are not yet finished.

Work remaining to be done

- Analyse the data on respiration, ammonia excretion, and assimilation efficiency from the recovery phase, and relate all rates to individual size.
- Relate the effects on energy turnover to effects on bichemistry, cytochemistry and tissue damage, and to tissue levels of hydrocarbons.
- Relate feeding rates to grazing intensity estimates derived from the growth on granite chips, and energy conditions to the population growth estimates.

Figure 1. Estimated feeding rates (mg fresh Ulva/individual day) of exposed, control and wild populations of Littorina littorea during and after oil exposure. Each value is mean (± st. error) of 10 individuals from each population.



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

Michael Moore, David Livingstone and David Lowe.

Institute for Marine Environmental Research (IMER)
Plymouth

Responses of the cytochrome P-450 monooxygenase system of the common mussel, Mytilus edulis L., and the periwinkle, Littorina littorea L. - Progress report 1985.

D.R. Livingstone

Introduction

The cytochrome P-450 monooxygenase or mixed function oxidase (MFO) system is a multi-component enzyme system, membrane-bound in the endoplasmic reticulum of the cell, that is involved in the detoxication of foreign organic compounds (xenobiotics). The concentrations of its components and the activities of the system can be increased by exposure to the xenobiotic or pollutant, and such responses in marine organisms in the field have been proposed as a means of identifying biological impact by organic pollution (Lee et al., 1980). Bivalve and gastropod molluscs possess MFO systems and, in the case of M. edulis, it is primarily localized in the microsomal fraction (endoplasmic reticulum) of the digestive gland (hepatopancreas) (Livingstone, 1985).

Objectives

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

2. To investigate if the MFO responses are dependent on season, sex (M. edulis only), and oil dosage.

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

4. To assess the results in relation to the potential of the MFO system as a specific indicator of biological impact by hydrocarbon pollution.

Methods and Experiments

Methodology was as described in Livingstone et al (1985). The response of the MFO system was characterized mainly in terms of cytochromes P-450 and b_5 and activities of benzo(a)pyrene hydroxylase and NADPH-cytochrome c ($P^2$450) (NADPH-CYTCRED), NADH-cytochrome c and NADH-ferricyanide reductase activities. Visits were made in January, June, September and November of 1984 and June of 1985, and long-term exposure and recovery material collected and short-term experiments carried out.

Main Results

1. Cytochrome P-450 and b_5 and BPH and reductase activities occur in digestive gland microsomes of L. littorea.

2. Exposure to diesel oil results in increases in cytochromes P-450 and b_5 and NADPH-CYTCRED activity but not in BPH activity. Responses in L. littorea are more marked in the high oil than the low oil condition.

3. Responses in L. littorea were similar at all times of the year but seasonal variation is evident in M. edulis with an impaired or no response in June 1984 when the mussels were reproductively mature. Sex differences occurred in M. edulis.

4. Long-term recovery of the MFO system occurred which was more rapid in mussels than winkles.

5. Long-term exposure and recovery changes in the MFO system paralleled uptake and depuration of tissue hydrocarbons.

6. Short-term responses are indicated to occur within days but the analyses are not yet finished.

An example of the results is given for L. littorea in Table 1.

Main Conclusions

1. L. littorea, like a number of other molluscs examined (M. edulis, Cardium edule, Thais haemostoma), possesses an MFO system.

2. The molluscan MFO system is indicated to be responsive to organic xenobiotics (in particular to polynuclear aromatic hydrocarbons).

3. Recovery occurs and sex and seasonal interactions are evident.

4. NADPH-CYTCRED activity (particular in L. littorea) and cytochrome P-450 offer potential as specific stress indices.

Completed and Remaining Work

All hydrocarbon tissue analyses, all long-term and some short-term biochemical analyses are completed. Remaining work are some short-term samples from June and September 1984.

References

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

2. D.R. Livingstone (1985). Mar. Pollut. Bull. 16, 158-164.

3. D.R. Livingstone et al. (1985). Aquat. Toxicol. in press.

Table 1. Tissue hydrocarbon levels (2,3-dimethylnaphthalene equivalents - $\mu g\ g^{-1}$ wet wt.) and responses of digestive gland microsomal NADPH-CYTCRED activity (nmoles min^{-1} mg^{-1} protein) of *L. littorea* to diesel oil.

	NADPH-CYTCRED			Hydrocarbons		
Date	Control	30 ppb	125 ppb	Control	30 ppb	125 ppb
January 1984	7.5±0.4	11.3±0.7*	21.8±1.9**	0.25±0.01	5.99±0.76	12.50±0.60
June 1984	10.5±1.0	14.1±1.9	21.5±2.1**	0.14±0.01	5.42±0.71	27.50±0.10
September 1984	8.8±1.0	14.5±1.3**	20.5±1.2**	0.07±0.01	5.18±1.75	9.74±0.33
← Dosing stopped →						
November 1984	11.2±0.3	15.8±0.5**	16.5±0.9**	0.26±0.01	1.42±0.30	2.79±0.37
June 1985	8.4±0.6	7.4±0.2	7.4±0.1	0.07±0.01	0.18±0.01	0.65±0.23

Values are means ± SEM (NADPH-CYTCRED, n = 5) or ± range (hydrocarbons in total tissues, n = 2) (each sample is pooled tissues of 6 winkles).

*$P \leq 0.1$ **$P \leq 0.05$ (one-way analysis of variance, exposed or recovered versus control).

CEULLULAR REACTIONS (*Mytilus edulis* and *Littorina littorea*)

D.M. Lowe, IMER, Plymouth

Objectives

The purpose of these studies was to investigate and quantify using stereological and image analytical techniques the effects of exposure to diesel oil hydrocarbons on the digestive, nutrient storage and reproductive cell systems of *Mytilus edulis* and *Littorina littorea* and to measure the capacity of these cell systems for recovery.

Materials and Methods

In May 1983 three large stainless steel cages were placed in basins C, LO and HO and filled with mussels from the population in the Fjord adjacent to SES. These animals were later sampled following a period of exposure, the cages restocked and the sampling repeated after a further period of exposure; this procedure was repeated on several occasions between May 1983 and June 1985. *Littorina littorea* were sampled either directly from the basins for long term exposure and recovery studies or from a series of transplanted plastic containers, transplanted in basins C2, C4, LO and HO, for the short term exposure/recovery studies. No results are as yet available for the Littorinid work, however, all the samples have been cut and the stained sections are awaiting analysis. All samples of mussels taken have been cut and stained and all stereological analysis completed on the reproductive/storage tissues. The majority of the digestive gland material is also cut and stained and the image analysis program written.

Results and Conclusions

The results of the stereological analysis of reproductive and storage tissues in mussels can best be summarised as follows:

I exposure to hydrocarbons does not lead to a reduction in fecundity (gamete biomass) in mussels, however, the proportion of gametes which are atretic (degenerating) is significantly greater in animals exposed to both LO and HO, this infers that hydrocarbon exposure could have a population effect if it were to be continuous.

II there is significantly greater utilization of nutrient reserves in exposed animals and this cannot be attributed to gamete production. This increased demand on reserves is considered to represent the 'cost' of the buffering capacity in mussels, postulated by Bayne (1975), for the protection of gametes during periods of environmental change.

III following a period of depuration (53 days) mussels previously exposed for 143 days show a full recovery in both storage cell and gamete production.

IV mortalities are far greater in mussels first exposed in summer months than those first exposed during winter moths. This enhanced rate of fatalities is attributed to several factors including the combined effects of non-fatal spawning stress and hydrocarbon cytotoxicity. Mortalities are considerably greater in mussels exposed to HO than to LO.

V degenerating, non-viable, gametes resulting from hydrocarbon exposure are broken down and the metabolites recycled into the storage matrix to be used for basal metabolism. This additional source of energy is considered to be a further reason why winter exposed mussels, with their high levels of atretic (degenerating) gametes resulting from hydrocarbon toxicity, have a greater survival rate; summer exposed mussels which have undergone gametogenic development in a natural, unpolluted, environment do not experience gamete atresia to the same degree and do not therefore have the same level of recycled nutrients.

Outstanding work

1. Analysis of all mussel digestive gland material.

2. Analysis of all Littorinid material.

Responses of the digestive cell lysosomal-vacuolar system of the common mussel (Mytilus edulis) and the periwinkle (Littorina littorea).

M N Moore, IMER, Plymouth.

Introduction

The lysosomal-vacuolar system in the molluscan digestive cells provides the means for intracellular digestion of food in the midgut gland. It is also involved in the autophagic turnover of cellular components as part of the normal economy of the cell (Owen, 1972; Moore, 1976; Pipe and Moore, 1985).

The lysosomal system is a target for the toxic action of xenobiotics including oil-derived polycyclic aromatic hydrocarbons (PAH); these include increases in activities of lysosomal enzymes, increased lipofuscin pigment which is indicative of enhanced autophagy, increased membrane fusion events as evidenced by enlargment of secondary lysosomes due to fusion, and finally increased membrane permeability resulting in decreased lysosomal stability. These parameters have all been shown to be responsive to PAH in marine molluscs (Moore, 1985; Moore et al, 1986).

It is, however, with membrane permeability that we are concerned in this investigation. This parameter was used as an idicator of the state of the lysosomal system in response to diesel oil emulsion and as an indicator of recovery from exposure to PAH.

Materials and Methods

The effects of PAH on lysosomal stability in the mesocosm systems have been reported in previous Solbergstrand reports and by Livingstone et al, (1985). This report is concerned with visits in January, June and September 1984. Oil dosing ceased in September 1984 and samples were taken in November 1984 and June 1985 to test for evidence of recovery.

Lysosomal stability was determined as previously described by Moore (1976) and Moore et al, (1982).

Results

The data on lysosomal stability are summarized in Tables 1 and 2 for mussels and periwinkles respectively. It is clear that exposure to diesel oil-derived PAH increased the fragility of the lysosomal lipoprotein membranes at both exposure concentrations ($\sim$30 and $\sim$125 ppb).

Recovery of the lysosomal system in both species had occurred by November 1984 and was maintained in June 1985, indicating that the effects of exposure were reversible (Tables 1 and 2).

Discussion

The effects of diesel oil emulsion on lysosomal integrity are consistent with the data on tissue hydrocarbon concentrations (see report by Livingstone - Table 1).

Remaining work

Some short-term experimental exposures and recoveries require further analysis, as does data on the microsomal NADPH- neotetrazolium reductase.

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7 Livingstone, D.R. et al., 1985. Aquat. Toxicol., 7, 79 - 91.

Publication Plan

A Published primary publications

1 Livingstone, D.R., Moore, M.N., Lowe, D.M., Nasci, C, and Farrar, S.V. (1985). Aquat. Toxicol., 7, 79 - 91. (Contribution no. 5 from Solbergstrand).

B Referred to in other papers (reviews)

1 Benzo(a)pyrene hydroxylase and the monooxygenase system in marine molluscs by D. R. Livingstone and S.V. Farrar. In 6th. Int. Symp. on Microsomes and Drug Oxidations, Brighton, England, 1984. Published as Supplement no. 1 to Xenobiotica, Vol. 14 (1984).

2 Responses of the detoxication/toxication enzyme systems of molluscs to organic pollutants and xenobiotics by D.R. Livingstone. Mar. Poll. Bull., 16, 158 - 164 (1985).

3 Responses of the mixed function oxidase system of some bivalve and gastropod molluscs to exposure to polynuclear aromatic and other hydrocarbons by D.R. Livingstone and S.V. Farrar. Mar. Environ. Res. - In Press.

4 Molecular and cellular indices of pollutant effects and their use in environmental impact assessment by M.N. Moore, D.M. Lowe, D.R. Livingstone and D.R. Dixon. Water Research, In Press.

C Material in preparation

1 Lysosomal responses to diesel oil-derived hydrocarbons: destabilization and subsequent recovery of lysosomes in the digestive cells of marine molluscs. (Deadline: April 1986, Journal - Aquatic Toxicology).

2 Cytochemical responses of NADPH-neotetrazolium reductase to polynuclear aromatic hydrocarbons in marine molluscs exposed to diesel oil. (Deadline: June 1986, Journal - Histochemical Journal).

Table 1. Responses of digestive cell lysosomes to diesel oil in M. edulis

Sample Date	Lysosomal Stability (Labilization period of latent β-N-acetylhexosaminidase, minutes) Control	∿30 ppb	∿125 ppb
Exposure			
Jan. 1984	25(25,25) n=5	5(5,5)[a] n=5	2(2,2)[a] n=5
Jun. 1984	20.4(2,25) n=5	2.6(2,5)[b] n=5	2(2,2)[b] n=5
Sept. 1984	20(20,20) n=10	2(2,2)[a] n=10	2.3(2,5)[a] n=10
Recovery			
Nov. 1984	25(25,25) n=10	25(25,25) n=10	24.5(20,25) n=10
Jun. 1985	25(25,25) n=10	25(25,25) n=10	25(25,25) n=10

Values are means with data range in parentheses.

[a] $P \leq 0.01$, Mann-Whitney U-test comparing with control data

[b] $P \leq 0.05$

Table 2. Responses of digestive cell lysosomes to diesel oil in L. littorea.

Sample Date	Lysosomal Stability (Labilization period of latent β-glutonidase, minutes). Control	∿30 ppb	∿125 ppb
Exposure			
Jan. 1984	24(20,25) n=5	2.6(2,5)[a] n=5	2.0(2,2)[a] n=5
June. 1984	25(25,25) n=5	2.0(2,2)[a] n=5	2.6(2,5)[a] n=5
Sept. 1984	25(25,25) n=7	2.0(2,2)[a] n=7	2.9(2,5)[a] n=7
Recovery			
Nov. 1984	23.8(20.25) n=8	25(25,25) n=7	24.3(20,25) n=7
Jun. 1985	25(25,25) n=5	25(25,25) n=5	25(25,25) n=5

Values are means with data range in parentheses.

[a] $P \leq 0.01$, Mann-Whitney U-test comparing with control data.

SECTION II

SUBLITTORAL SOFT BOTTOM PROJECT

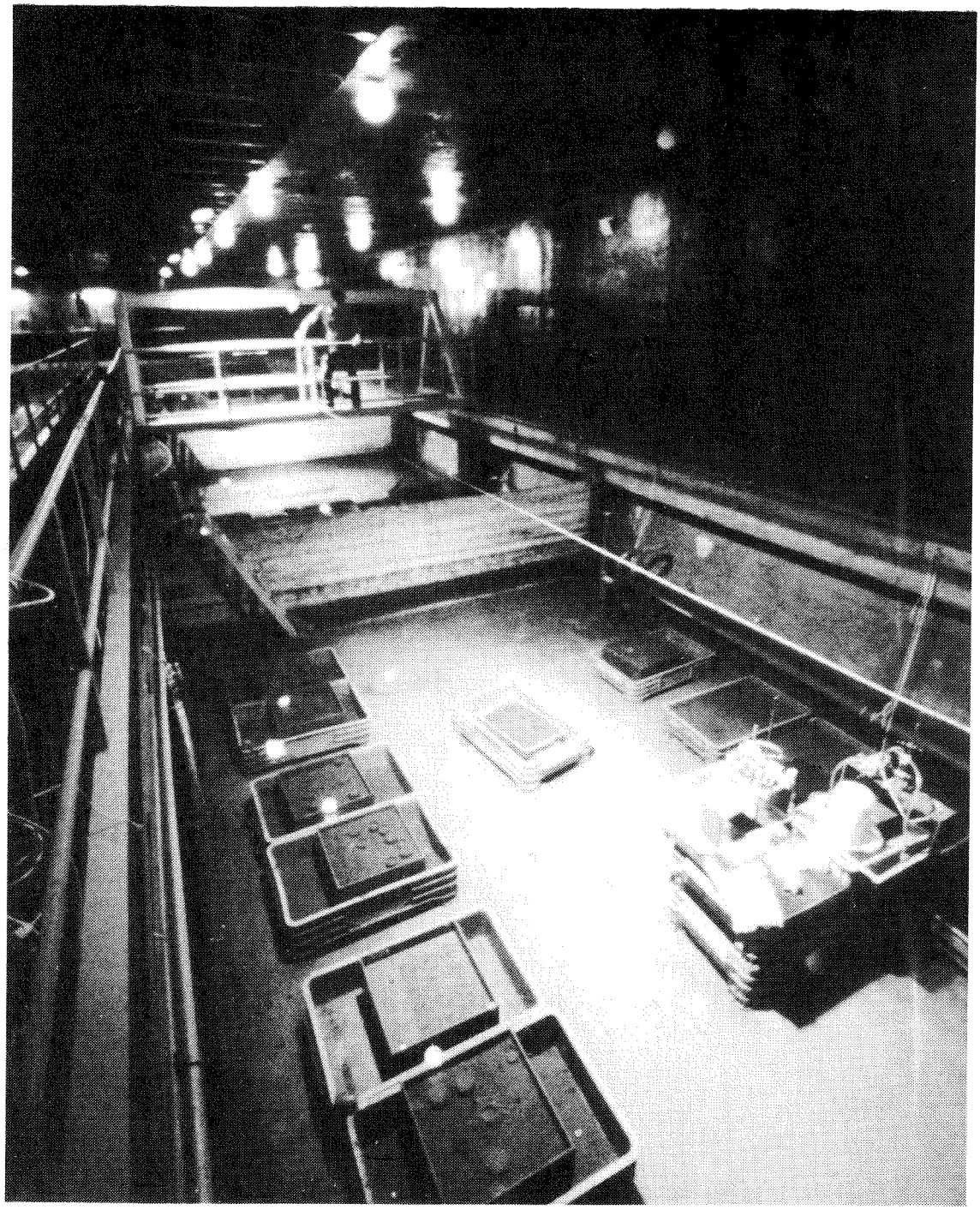
Introduction

Controlled experiments in the subtidal require diving, submersible vessels or instruments placed in situ. These methods often limit experimental design, and preclude designs that involve controlled exposure to pollutants. The use of large scale experimental ecosystems has provided an alternative approach to working in the field, and has generated results which could not be obtained in the field.

The aim of the Sublittoral Soft Bottom Project is to create a tool for performing highly controlled experiments and measurements on soft bottom communites from deeper water. The principle adopted has been to construct an indoor basin system (mesocosm) where light and water regime of deeper bottoms can be simulated, and a sampling procedure that enables transplantation of undisturbed sections of marine soft bottoms to these basins for manipulation and measurements. By this approach we have established a system not only for controlled pollution exposure, but also for applying a range of laboratory and shallow water research techniques on deep water communities.

The mesocosm was constructed during 1982 and 1983 and the first attempt to establish an experimental community was done in April 1983. The first series of experiments were aimed at finding the optimal sampling and transplantation procedures, and a large USNEL box-corer was purchased, which takes undisturbed sections of the bottom (0.5x0.5meter surface area, 0.4meter sediment depth). Included in these experiments were also manipulations with the water circulation of the mesocosm, and how to feed the communities. The main effort in 1984/85 has been an organic enrichment experiment performed on sediments from 35 meter depth, to which was tied experiments on the structuring effect of larger macrofauna on the sediment and reciprocal studies of the original field community. As with the Rock Littoral Project the Soft Bottom Project has a common basic experimental design which covers a series of partly independent subprojects.

Recently a pilot experiment on transplanting a community from 200 meters depth has been started, and this has been combined with experiment on the effects of oil based drill cuttings on this community. It is anticipated that we through this experiment will gain experience in experimenting on communities and pollution problems of high relevance to the North Sea and continental shelf oil exploration.



Overview of one of the six basins of the Soft Sediment Mesocosm. A row of 0.5 x 0.5 m sediment sections, transplanted from 35 m depth, are positioned along the left side of the basin.

Photo: Knut Kvalvågnæs

Subtidal sediment enriched with natural plankton

J.A. Berge (1) M. Gee (2) J.S. Gray (1) K. Sandøy (1)
M. Schaanning (1) G. Skeie (1) and R.M. Warwick (2)

(1) University of Oslo

(2) IMER, Plymouth

Abstract

Purpose: The sedimentation of marine detritus in subtidal coastal areas sets the ultimate limit for secondary production and mineralization. Few studies have focused on the sequential responses of sediment to a controlled amount of natural plankton. The facilities at Solbergstrand provide unique possebiletis for controlled dosage of suspended material to subtidal sediments. Thus, it was decided to investigate temporal responses in subtidal sediment to defined amounts of natural plankton.

Method: Sediment was obtained from the Oslofjord (30 m depth) using an 0.5 m^2 USNEL-boxcorer and placed in experimental boxes. The sediment was transplanted to the mesocosm at Solbergstrand. Plankton was obtained by filtering (25 um mesh) surface water from the fjord. Suspended plankton was dosed to the experimental boxes four times during a six weeks period, equaling total doses of approximatly 5 and 20 gC m^2. Three boxes were used for each tratment, untreated boxes were left as controls. Nutrient and oxygen fluxes over the sediment/water inerface were measured. Samples for bacterial production, bacterial numbers, sediment chemistry, porewater chemistry, meiofauna abundance and macrofauna abundance have been collected. Analysis of these samples are under progress.

Results: Results from the flux experiments are seen in Fig.1. Data on redox potential and concentration of H_2S in the sediment show that the oxygenconsumption in the sediment have not caused stagnation symptoms in the sediment. Preliminary data show that bacterial production increase 8-12 h after dosage. There is a correlation between bacterial production and the amount of plankton added. Macrofauna date have not yet been treated and meiofauna data are not yet available

Conclusion: Data treatment is in its initial phase,thus, we will not attempt to come with definit conclusions, However, the experimental system at Solbergstrand has proven ideal for the kind of experiments performed and preliminary data seems promising (Fig.1.).

Remaining work

Analysis of porwater profiles, content of carbon and nitrogen and number of bacteria in sediment. The material from macrofauna samples have been identified, however treatment of the data remain to be done. Identification of meiofauna samples are in progress.

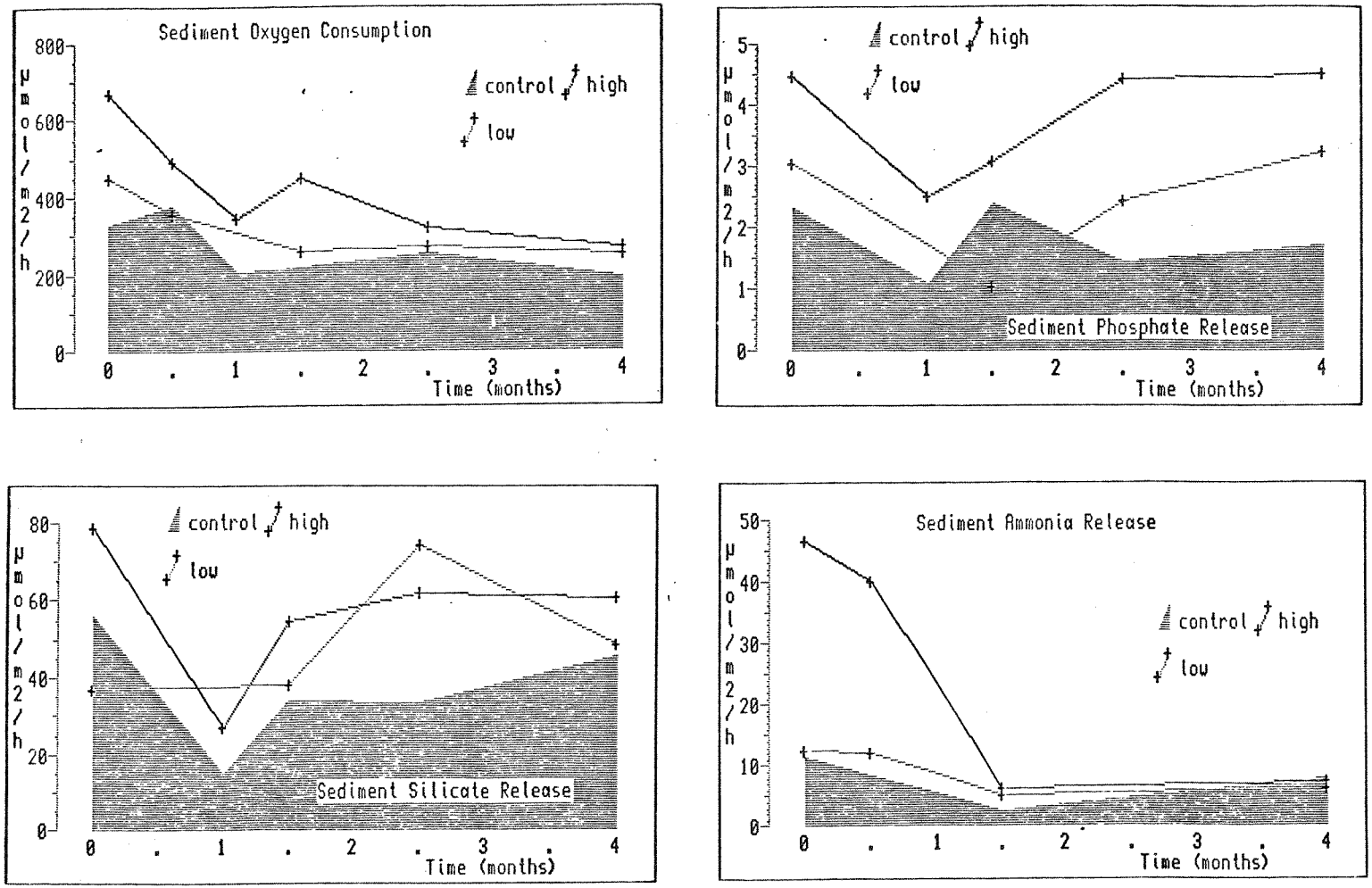


Fig.1. Fluxes (µmol $m^{-2}h^{-1}$) of dissolved, inorganic O_2, PO_4, SiO_2 and NH_4 across sediment/water interface. Natural plankton was added to the sediment surface one week before measurements at times 0, 0.5, 1 and 1.5 months. Low = 4x ca1.25gCm^{-2}. High = 4x ca5gCm^{-2}. Control = no addition.

Macrofaunal recolonization of oil contaminated sediments: dose response characteristics.

John A. Berge and Kirsti Sandøy, University of Oslo.

Abstract

Purpose: Test the potential for macrofauna recolonization in subtidal sediment contaminated with different concentrations of crude oil.

Method: The experiments were performed by placing oil contaminated sediment (0, 100, 400, 4000 ppm crude oil wet, weight) in experimental boxes (55 37 12 cm high) on the sea floor at 18 m depth in the fjord outside Solbergstrand in November 1983. The boxes were recoverd in January 1985. The following parameters have been measured in the sediment after retrivel of the boxes: pH, redox potential, pS_t (-log conc. H_2S), macrofauna densities, biomass and size frequency distribution of Mysella bidentata

Results: Important sediment parameters like redox potential, pH and pS_t were significantly effected by the oil (Fig.1.). The number of macrofauna species were nearly identical in all the boxes dosed with oil (50) whereas the control boxes 70 species were found (Fig.2A). The number of individuals decreased with increasing oil concentration (fig.2B). Biomass varied considerably with lower values in 4000ppm box than in the control boxes. The mean size of Mysella bidentata increased with the concentration of oil in the range 0-400 ppm.

Conclusion: It is concluded that both chemical and biological changes may be detected after 14 months in sediment initially dosed with 100-400 ppm crude oil. The increased growth of M. bidentata is probably a function of reduced competition or increased food availability in the oil contaminated boxes. This may be caused by the reduced density of macrofauna or increased number of bacterial oil degraders.

Remaining work: (1) measurement of the remaining concentration of oil, total carbon and nitrogen in the sediment, particle size distribution (?), (2) publication.

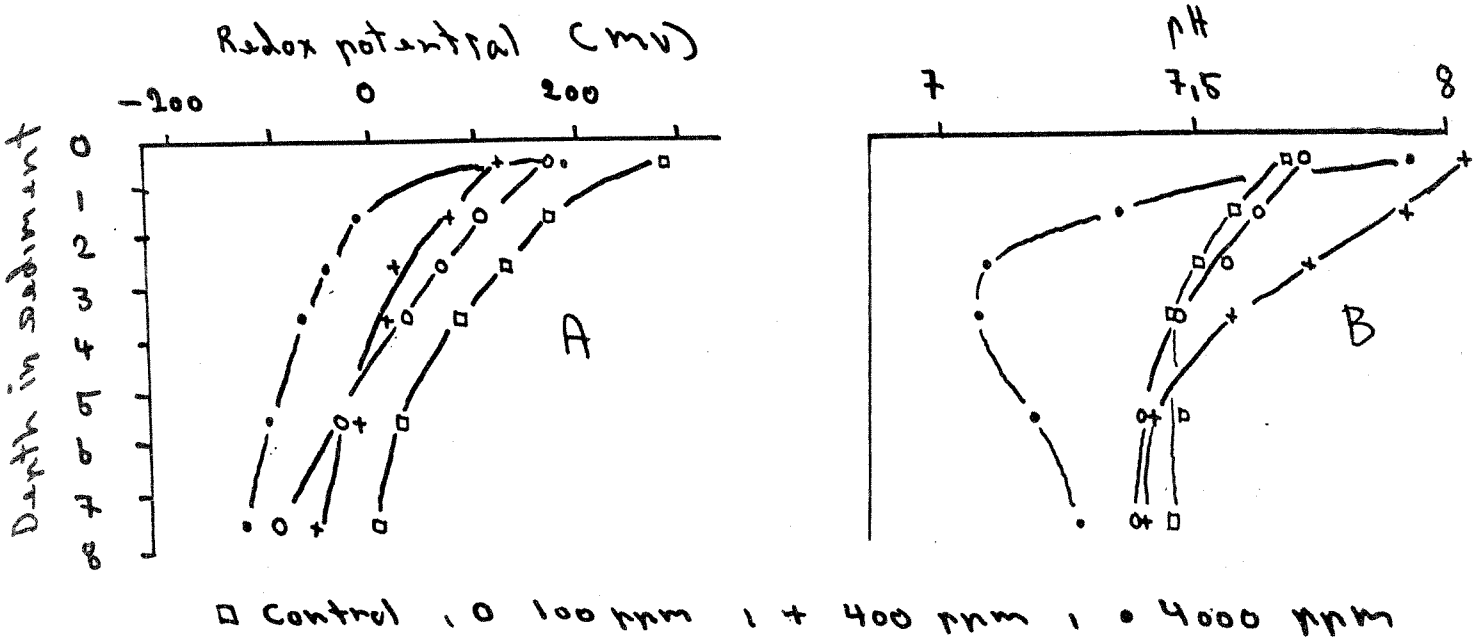


Fig.1. Redoxpotential (A) and pH (B) in sediment after exposure.

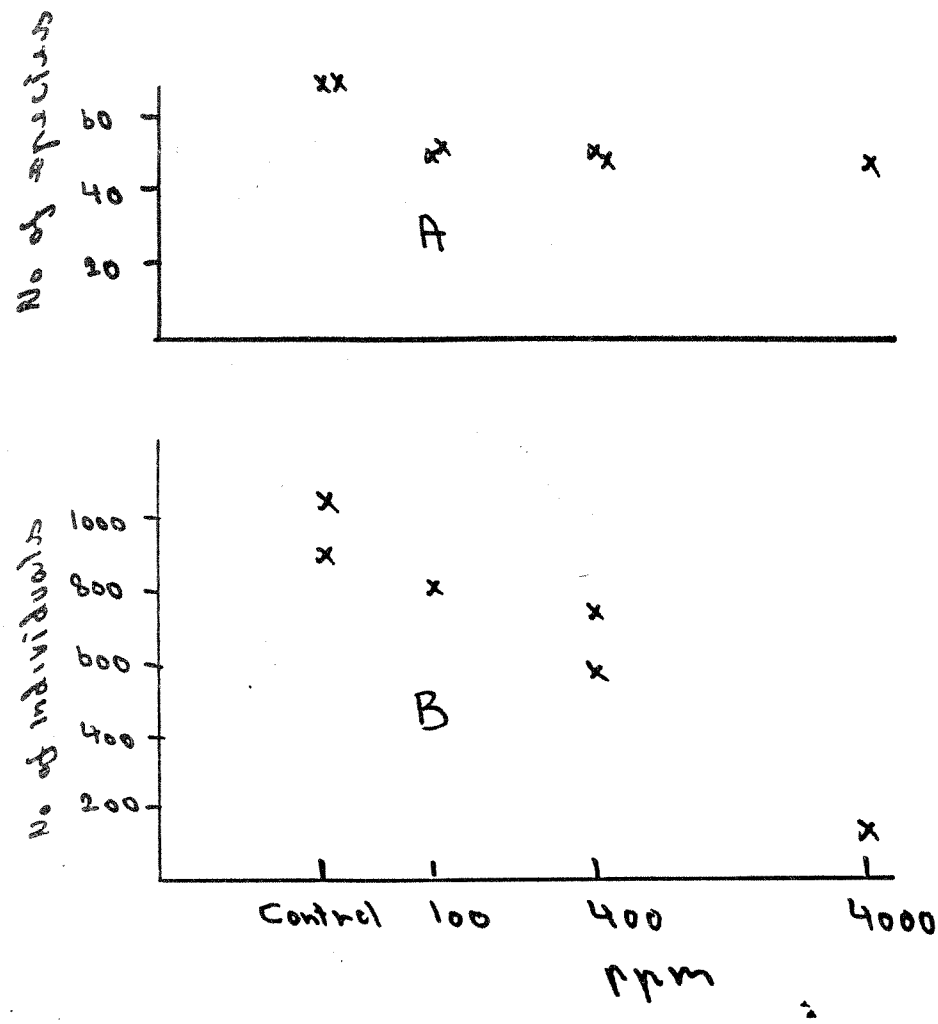


Fig.2. Number of species (A) and individuals (B) in boxes dosed with crude oil.

Meiofaunal community structure in soft-bottom mesocosm experiments.

G. M. Gee, IMER, Plymouth

1. Nutrient enrichment by natural phytoplankton.

The rationale and design of this experiment is as described by J. A. Berge.

For the meiofauna, at each sampling date (0, 2, 4, 8, 16 weeks after initial dosing), 5 sediment cores were taken from each box with a 16 mm internal diameter (2.0cm^2) sawn off, plastic syringe to a depth of 4 cm. All cores from each box were combined to form one sample and preserved in 4% formaldehyde for subsequent extraction and analysis of the nematodes and copepods.

So far the meiofauna in only the predosing samples (Week 0) have been extracted by a combination of elutriation in tap-water and flotation in LUDOX TM colloidal silica, using a 63 m sieve. The copepods have been picked out and the nematodes mounted on slides. The adult harpacticoid copepods in each box have been identified and the abundance and number of species are summarized below :

Harpacticoid copepods in Week 0 (predose) samples.

PROPOSED TREATMENT	CONTROL			LOW DOSE			HIGH DOSE			
BOX NUMBER	3	6	8	4	9	10	1	2	7	TOTAL
NUMBER OF INDIVIDUALS	3	15	14	31	30	41	34	33	20	221
NUMBER OF SPECIES	1	11	5	16	12	17	13	16	10	32

Theoretically the number of individuals and species in each box should be roughly the same (as they are all pretreatment samples). It is clear however that the boxes designated as the controls produced significantly lower numbers of individuals and species than those boxes which have been subsequently dosed. Comparisons of each treatment through the time scale of the experiment may therefore prove more valid than comparisons across treatments at any point in time.

The termination samples (16 weeks) will be analysed next and compared with those for Week 0. If significant differences between these two sets can be detected the intermediate two-week sets of samples will be analysed.

2. Ophiuroid feeding/disturbance.

Experimental design and rationale is as described by W. G. Ambrose. Meiofauna samples have been taken and preserved (in the manner described above) from each treatment at 6 and 12 weeks after the start of the experiment but none of the samples have been analysed so far.

The effect of predation and disturbance by ophiuroids on the structure of a subtidal community in Oslofjord

William G. Ambrose Jr., UiO

Purpose: Ophiuroids are conspicuous members of many subtidal soft-bottom communities and high densities of ophiuroids (greater than 1,000 m^2) have been recorded from communities ranging from the shallow subtidal to the deep sea. Many ophiuroids are predators and all species disturb the sediment when they move. Predation and disturbance by large, mobile epibenthic predators (crabs, fishes, and birds) and by predatory infauna (largely polychaete and nemertean worms) have been shown to be influential in structuring a number of soft-bottom communities, so ophiuroids have been predicted to be important in structuring subtidal communities. The logistics of conducting manipulative experiments in subtidal communities, however, have so far prevented a test of the importance of ophiuroids in structuring communities. The experiments I conducted were designed to test the effects of two ophiuriod species, Ophiura albida and O. affinis, on the structure of a soft-bottom community in Oslofjord.

Work Performed: In April, 16 boxes of sediment (0.25 m^2) were collected from Bjørdhudebukta where the density of O. affinis and O. albida combined can reach 400 per m^2 in patches. These boxes were arrayed in two rows in the mesocosm. From all but 4 of these boxes, which served as controls, all O. affinis and O. albida were removed. Removal was accomplished by lifting an individual ophiuroid off the sediment surface with a fine probe and catching it on plastic screen. This technique caused minimal disturbance to the sediment surface, but the removal was controlled for because ophiuroids were not removed from control boxes. Ophiuroids spend part of their time buried slightly below the sediment surface making them impossible to remove until they become active on the surface. Removals, however, were carried out until no ophiuroids were recovered on two consecutive occasions. The average density of ophiuroids per box was used as the ambient density for the experiment. Four replicates of the following treatments were assigned to the boxes using a stratified (by row of boxes) random design: 1) 0X (all O. affinis and O. albida removed), 2) 1X (ambient densities of ophiuriods returned, 68 per box) and 3) high density (250 ophiuroids added per box). Density treatments were maintained by returning ophiuroids which escaped the experimental boxes and remained in the outer surrounding box to the experimental boxes every 7 - 10 days. During the course of the experiments, sediment traps placed in each box for two separate periods of 3 and 8 days.

After 6 weeks, the experimental boxes were divided by pushing a plastic plate through the sediment and half of the box sampled for macrofauna (individuals retained on a 100 micron mesh sieve), meiofauna(63 micron mesh) and chemical parameters. The boxes were sampled a second time after another 6 weeks for macrofauna and meiofauna. At the the last sampling, a photograph was taken of each box and used to estimate ophiuroid density. The top 5 cm of each box was also sieved through 1 mm mesh to collect ophiuroids for size frequency analysis.

So far, only macrofauna cores from the last sampling period (12 weeks) have been sorted. Eight cores were taken from each box and 3 of these cores from 3 of the 4 replicates for control and Ox and high density treatments have been sorted. Unfortunatly, 1 replicate for each treatment was lost because half of the boxes in the back row went anoxic before the 6 week sampling. Between 4 and 8 hours are required to sort just the 0 -3 cm deep portion of one core so the processing time for the experiment is considerable and all cores will probably not be sorted.

Results and Conclusions: After 12 weeks ophiuroids were significantly more abundant in the high density treatment (mean= 107.3, SD=21.5) than in the control (mean= 42.3, SD= 2.5) or 1X treatment (mean= 45.6, SD= 1.5) based on the photoghraphs. No ophiuroids were observed in the OX treatment. Of the five most abundant taxa, only polychaetes were significantly affected ($p<0.05$, one-way ANOVA) by the presence of ophiuroids (Table 1). The effect on polychaetes was not great as the high density treatment had only 1.6 times as many polychaetes as the OX treatment. The sediment traps in the control and 1X and High treatments collected more sediment than the traps in the OX treatment which indicates that ophiuroids do disturb the sediment surface.

TABLE 1. Mean (standard deviation) number of individuals from control and OX and High ophiuroid treatments. Means are the average number per replicate of the total number of individuals collected in 3 cores (inside diameter of core= 4.3 cm).

Taxon	Control	OX	High	Sig. level
Polychaetes	52.3(9.0)	71.3(10.4)	43.6(10.3)	$p<0.05$
Bivalves	31.3(23.1)	29.3(5.5)	38.0(7.4)	ns
Copepods	206.3(129.4)	333.0(72.9)	393.7(113.2)	ns
Nematodes	1301.6(371.0)	1605.6(362.0)	1335.0(155.5)	ns
Foraminifera	354.0(183.2)	337.0(96.6)	445.6(131.1)	ns

Based on these preliminary results, ophiuroids appear to be having less affect on infaunal abundances than previously speculated. Previous studies of the effects of predation and disturbance on soft-bottom community structure indicate that juveniles are particularly vulnerable to disturbance. Newly settled individuals were found in all samples indicating recruitment had occured. Many of the species, however, have direct development which may allow them to escape predation and disturbance by the ophiuroids. Surface dwelling species should still be affected, but this analysis must wait until species identifications are completed.

Work Remaining to be Done: Most of the macrofaunal work remaining involves sorting samples and identifying individuals. This is very time consuming and is likely to take at least a year to complete because of my new position starting January 1986. All of the meiofauna work (R.M. Warwick) and the chemical analyses (M. Abdullah and M. Danielsen) must also be completed.

Measurement of bacterial production, numbers and biomass in a marine sediment

Geir Morten Skeie, UiO

Purpose

- Measuring fluctuations in bacterial production, numbers and biomass in sediments brought intact from the fjord into the basins in the soft bottom mesocosm.
- Measuring the effect of Chl a concentrations, C/N ratio and temperature on the parameters mentioned above.
- Evaluating the suitability of the methods applied under the conditions encountered in our sediment.

Work performed

16 samples were taken in the period December -84 to June -85 for measurement of :

1. Water content
2. Porosity
3. Organic matter content
4. C/N content
5. Chl a content
6. Bacterial production
7. Bacterial numbers
8. Bacterial biomass

On ten occasions samples were taken on the same day at the locality in the fjord from which the sediment was sampled.

In addition, samples have been taken for determining the effect of modifications of the method used for measuring bacterial production. The requirements for using the method have been tested.

Due to problems arising from non-specific staining of organic materials

in the sediment using the standard dye AO, I have tested an alternative dye, DAPI (4.6 -diamidino-2-phenylindole).

Results

The preliminary results are so far :

- Bacterial production ;
  The bacterial response to availability of organic matter is fast.

- The quality of the material regulates the response.

- Using the thymidine incorporation method as described by Moriarty and standard techniques for quench correction underestimates of bacterial production will be approximately 25%.

  Bacterial numbers ;

  The widely used AODC method is not suitable for enumerating bacteria from this kind of sediment, due to its non- specific staining of DNA, resulting in a strong background fluorescence from organic matter in the sediment.

Conclusions achieved

To measure bacterial response to sedimentation of the spring bloom, samples should be taken at intervals less than one week.

Care should be taken when correcting the observed values of thymidine incorporation for quenching. Quenched standars using the same isotope and origin of quenching should be made by the user of the method.

For enumeration of sediment bacteria, a more DNA -specific dye than Acridine Orange should be used. Prelimenary experiments using the dye DAPI have given promising results.

Work remaining to be done

Except the measurement of Chl a and C/N ratio in the sediments, all the laboratory work has been finished.

Calculations and statistical analysis of the data remain to be done.

In addition there will be some more laboratory work, depending on the results obtained by analysing the data and questions arising from these.

Effects of oil based drill cuttings on a sediment community from 200 meters depth. A pilot experiment

Torgeir Bakke, John Arthur Berge and Morten Schaanning

Purpose

The experiment has been initiated primarily to test the feasibility of transplanting soft bottom sections from 200 meters depth outside Solbergstrand to the mesocosm, by use of the same technique as has been developed during the sampling from 35 meters at Bjørnhodebukta. In addition a pilot experiment on these transplanted communitites has been started to test if the negative responses to small discharges of oil based drill cuttings as have been recorded in a recent field experiment (Bakke & al. 1985) could be reproduced by use of mesocosm communities.

Work performed :

The $0.25m^2$ box corer sections were sampled on 17 October 1985 from a level bottom at 190-200 meters depth. A total of 7 boxes were transplanted to the mesocosm of which 5 were considered acceptable as replicate test plots. The remaining boxes were kept for trying out various sampling details.

A 3 months exposure experiment was established, day 0 being 25 November 1985. At this day the following additions of cuttings from drilling with low aromatic mud were given:

Box 1: 500ml giving a layer of nominal thickness 2mm on the sediment
Box 2: 250ml giving a layer of nominal thickness 1mm on the sediment
Box 3: 12.5 ml giving a layer of nominal thickness 0.05mm on the sediment
Box 4: 75ml giving a layer of nominal thickness 0.3mm on the sediment
Box 5: No addition of cuttings (reference)

The cuttings were distributed by making a slurry with sea water which then was decanted off onto the water above the enclosed test plots.

The following program of analysis was adopted:

Hydrocarbon analysis. Triplicate core samples are taken from each box at day 2. The upper 10mm sections from each box are pooled and frozen for later analysis of toal hydrocarbons and selected aromatics.

Pore water characteristics. Single core samples are taken at days 2 and 90 from each box. Porosity, pH, redox potential, H_2S, alkalinity, NH_4, SiO_4, and PO_4 will be determined in the 0-1, 1-2, 2-4, 4-6, 6-9, and 9-12 cm sections. Material from these cores will also be used for determination of total carbon and nitrogen.

Fluxes across sediment surface. The boxes are incubated in flux chambers for 12-24 hours at days -4, 1, 10, 21, 40, 60, and 90. Fluxes of oxygen, alkalinity, ammonium, nitrate, silicate, and phosphate are determined.

Probe-measurements. Probes are used in situ at the same time as the flux determinations to measure pH, Eh and Es in 0, 1, 2, 4, and 6cm sediment depth.

Bioturbation. By use of glass bead tracer (50-100µm) the rate of vertical transport of particles will be estimated. The beads were distributed over all the test plots at day 0 (100ml beads/box). Triplicate core samples are taken at days 2, 30 and 90 from each box, and the concentration of beads in the 0-0.5, 0.5-2, 2-4, and 4-10cm sections of the cores will be determined.

Macrofauna. Five core samples are taken from each box at days 2 and 90. The characteristacs of fauna >0.5mm will be determined from the 0-3 and 3-6cm sections. At termination of the experiment the upper 20cm of each box will be sifted through 1mm sieve to determine total macrofauna.

Results and conclusions :

Logistically the sampling and transplantation from the 200m community was easier than transplanting from Bjørnhodebukta. The cruise distance was considerably less and the deeper clay layers of the

sediment made closing the bottom of the experiment boxes very easy. With the slight adjustments that were made of the sampling process, it is anticipated that one can sample at least 10-15 boxes during a normal working day from this community.

Prior to the addition of drill cuttings and glass beads, the pH and Eh was similarly distributed in all boxes (Table 1). Until day 21, the addition of drill cuttings and glass beads have had no clear effects on the redox potential distribution. The drill cuttings did, however, produce an immediate increase of the pH, resulting in the formation of pH-maxima at 5 cm depth. With time the maxima seemed to become reduced by vertical transport processes.

The consentration of hydrogen sulphide was below detection limits (less than 1μM) in all measurements performed so far.

The initial sediment oxygen consumption (SOC) in boxes 2-5 was 2551+028 μ molm^{-2} h^{-1}, but only 140 μ molm^{-2} h^{-1} in box 1. Because box 1 had a ca. 50% larger volume of incubated water flow velocity at the pumps of the incubation chambers, the low SOC might be a result of lower current velocity at the sediment surface. Therefore, at all later experiments the procedure has been altered from a fixed flow rate of 10 mls^{-1} to a fixed turnovertime of 45 min.

As evident from Table 2, high oil doses in box 1 and box 2 strongly stimulated SOC. The effect could be traced as soon as on day 1. Good correlation between dose and response was observed on day 1 and 10, less so on day 21. The retardation of the SOC between day 1 and day 10 may have several causes such as

1) adjustment of community metabolism to colder water
2) disturbance by sampling of cores on day 2, and
3) the addition of glass beads.

The decrease was, however, larger than would be expected by the moderate change of temperature alone. If the blanket of glass beads initially covering the sediment surface would reduce SOC by physical obstruction of the sediment/water exchange pathways, the effects should have been larger on day 1 when the blanket was least eroded. In a previous experiment core sampling was assumed to be responsible for a general decrease of exchange processes, and we suspect that this is the case also in the present experiment.

Remaining work :

The experiment will run for three months until 25 February 1986. Most of the samples taken have not yet been treated. They will be analysed during he first half of 1986.

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Chemical and macrofaunal response of subtidal sediments enriched with Ascophyllum nodosum

M. Schaanning, J.A. Berge, and K. Sandøy, UiO

Purpose. Eutrofication of subtidal sediments are generally controlled by the frequency of botttom water renewal and the rate of input of organic matter. Neither of these factors are easily quantified in the natural environment, and laboratory studies generally suffer from disturbance of sediment stratification and community structure. The mesocosm system at Solbergstrand appeared ideal for a quantitative investigation of the link between organic enrichment and the subsequent developement of eutrofication symptoms in pore water composition and macrofaunal community structure.

Experimental design. Undisturbed sections of subtidal sediment (.5mx.5mx.2m) were transfered to the mesocosm. In the mesocosm the sediment was exposed to subtidal light conditions and continuously flushed with well oxygenated fjord water from below the pycnocline. Series of sections were enriched with low and high doses of powdered Ascophyllum nodosum corresponding to 50 and 200gCm^{-2}, respectively. Subsequent changes of sediment and pore water composition and macrofaunal densities were observed after 2 days and 1, 4 and 7 months. Control sections were investigated by the end of the experiment.

Results. As shown in table 1, 1.1 mmolCcm^{-3} (65% of the added carbon), disappeared from the high dose sediment during the first month. Later on only minor variations in C-content occurred. Nitrogen showed no corresponding decrease. On the contrary, a 27% increase was observed between 1 and 4 months. The C/N ratio declined to a value corresponding to the mean ratio of 16.0±1.4 (n=48) observed within the 1-14cm layer. This

Table 1. Observed total carbon and nitrogen (mmol cm^{-3} wet sediment)in 0-1 and 1-2 cm sections during the experiment.

		2 days	1mnth	4mnth	7mnth
Carbon	0-1cm	5.17	4.08	4.27	3.93
	1-2cm	2.42	2.32	2.38	3.43
Nitrogen	0-1cm	.192	.199	.253	.254
	1-2cm	.123	.134	.138	.166
C/N ratio	0-1cm	27.0	20.2	16.9	15.5
	1-2cm	19.6	17.4	17.3	20.6

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trend of decreasing carbon, but conservation and even increase of nitrogen was observed also in the low dose series, less clear, however, than in high dose.

Increased pore water concentration of H_2S, alkalinity and nutrient species were observed in both series. In low dose the redoxcline remained at 2-4cm depth untill the end of the experiment and sulphide was hardly detectable. In the high dose boxes, however, negative redox potentials and H_2S concentrations at the millimolar level was observed throughout the top ten cm of the sediment at 1 and 3 months. White mats, probably from sulfur oxidizing Beggiatoa bacteria, became visible on the sediment surface, and extraordinary pH values down to 2.0 was observed in one high dose section.

Average alkalinity within the 0-7cm layer increased from initial values of ca 2.7 meq.kg^{-1} to 3.2 and 5.9 meq.kg^{-1} after 1 month and declined to 2.9 and 4.0 meq.kg^{-1} at 4 months in low and high dose boxes, respectively. Alkalinity remained at the latter levels for the last 3 months of the experiment. Ammonia (average 0-7cm) declined from 85uM in low dose and 190uM in high dose two days after enrichment to 10-20 uM at the end of the experiment. The final concentrations were similar to those observed in field and mesocosm control sediment. Phosphate tended to increase throughout the experiment from 9uM in low dose and 13uM in high dose two days after enrichment to 17 and 23uM, respectively, in the end. For comparison, 9uM PO_4 was observed in the fjord control and 19uM in the mesocosm control measured at the end of the experiment. The vertical profiles revealed two different sources for the release of phosphate. In the beginning of the experiment, PO_4 increased near the sediment surface, obviously due to mineralisation of the added substrate. During the last three months, however, the increase occured below 4cm depth, probably due to mobilisation of mineralogenic PO_4. The latter mechanism probably also accounts for the high levels observed in the mesocosm control box.

Hetromastus filiformis was the most abundant specie in both series. Only minor variations in the macrofaunal community was observed in the low dose boxes. In the high dose boxes number of individuals of Capitella capitata increased dramatically between 1 and 4 months, whereas for H.filiformis the densities in both series decreased between 4 and 7 months.

Conclusions. A single shock load of 200gCm^{-2} of Ascophyllum powder is beyond the decomposition capacity of the aerob benthic community, whereas a dose of 50gCm^{-2} could be added without the occurrence of H_2S accumulation in the interstitial water. The increase in total nitrogen , indicates that the benthic community, given a surplus of energy (organic carbon), is capable of nitrogen fixation from other sources, e.g. molecular nitrogen or nitrate from the overlying watermass.

ISBN 82-577-0494-6 0-82007 lnr 1379

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June 1983

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