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Norwegian Institute for Water Research NIVA

Royal Norwegian Council for Scientific and Industrial Research

Address:
Postbox 333, Blindern
Oslo 3
Norway

Telephone:
47 2 23 52 80

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Authors:

Torgeir Bakke	Programme Manager	NIVA
J.A. Berge		UiO
Tor Bokn		NIVA
S.E. Fevolden		UiO
O.A. Follum		UiO
O.A. Frydenberg		UiO
S.P. Garner		UiO
D.M. Lowe	Inst. for Marine Environm. Research	Plymouth
Einar Lystad		UiO
Kjell Moe		UiO
M. Moore	Inst. for Marine Environm. Research	Plymouth
Fr. Moy		UiO
Are Pedersen		NIVA
S. Sporstøl		SI
K. Sørensen		NIVA
Pål Thome		UiO
Mats Walday		UiO
J. Widdows	Inst. for Marine Environm. Research	Plymouth

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Abstract:

The report presents results achieved during spring/summer 1983 in the subprojects under the research programme "Long term effects of oil on marine benthic communities in enclosures".

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Project leader

Torgeir Bakke

For the Administration

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1. FOREWORD

This report contains the compiled contribution from all the subprojects within the Rock Littoral Project at the Marine Research Station, Solbergstrand. It also contains a short progress report on the Sublittoral Soft Bottom Project. Basis for the contribution is the material presented at a research seminar arranged 2 November 1983 at NIVA. The aim of this seminar was to present and discuss as much as possible of the results from the spring-summer investigations of the Rock Littoral Project in order to form the best possible basis for a later determination on when to stop the oil dosing and go for recovery.

The present report will form the basis for an Advisory Board/Participating Researchers seminar arranged at Tømte, Hurdal (north of Oslo) at 26 - 29 February 1984, where these matters will be discussed thoroughly.

Oslo, 2 January 1984

Torgeir Bakke
Program Manager

2. INTRODUCTION

During the Advisory Board seminar of 11 and 12 August 1983 much of the discussion on the Rock Littoral Project concerned how the recruitment had been to the basins during summer. The general impression was that recruitment, at least of animals, had been very low compared to the shore outside. However, in the very early state of treatment of the summer data, it was not possible to state if the basins and the way they are run prevent significant recruitment. During the seminar of 2 November it was generally stated that recruitment had occurred during summer, but differed from basin to basin and was much less than on the shore outside.

During the November seminar it was also decided to code the basins in a way which could be easily interpreted. The following code was adapted and is used in this report:

- HO: basin or community/population given the highest oil exposure (formerly Basin 1)
- LO: basin or community/population given the lowest oil exposure (formerly Basin 3)
- C2: control basin/community/population positioned between the two oiled basins (formerly Basin 2)
- C4: control basin neatest to the laboratory (formerly Basin 4)

A common code for the control populations outside the basins (on the shore) was not adapted and terms such as "shore", "wild" or "fjord" populations are applied.

Oslo 2 January 1984

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3. CHEMICAL ANALYSIS OF HYDROCARBON CONTENT IN WATERS AND ORGANISMS

Project participants: T. Bakke
S. Sporstøl (SI)
K. Sørensen
U. Efraimsen
H. Juelsen
B. Silde

INTRODUCTION

The Rock Littoral Project design is briefly aimed at exposing two enclosed littoral communities to two different chronic levels of diesel oil hydrocarbons. The levels we try to keep are 200 and 50 ug/l of total hydrocarbons (dissolved and as droplets).

The purpose of the hydrocarbon analysis within this project is to determine the levels of hydrocarbons in the dosing system, the basins, the organisms, the sedimented material, and the substrate on the walls.

This report presents results from the first 14 months of sampling and analysis.

Two types of analysis are involved in this project:

Fluorescence spectroscopy is used to

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

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

- improve the fluorecence technique for routine monitoring of oil concentrations in experimnental set-ups of the present type.

- correlate with monthly average analysis of the same types of water samples by high resolution GC or GC/MS.

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

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

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

DOSING SYSTEM DESCRIPTION

The system for continuous dosing of oil hydrocarbons into the basins

consists of:

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

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

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

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

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

Fig. 1 shows a schematic view of the system.

The prototype of the mixing and separation unit has been developed at the Bermuda Biological Station for Research. The dosing system has been improved in the course of the experiment but the basic design is the same as when the system was started. Main improvements have been installment of the preconditioning unit and of larger capacity pipelines and pumps. The sea water supply to the unit is taken from 40 m depth and the conditioning temperature has been 12.5°C since February.

SAMPLING AND ANALYSIS

Water

Samples of the WAF for fluorescence analysis are taken at three points in the dosing system by filling a volumetric sylinder to 50 ml. The samples are extracted immediately with 5 ml n-hexane (p.a. grade) in the sylinder and the UV fluorescence intensity of the extracts is measured with a Perkin-Elmer spectrofluorometer at set exitation and emission levels (265 and 322 nm respectively). The fluorescence intensity is converted to total hydrocarbon units (mg/liter) by use of a calibration curve made by analysing fluorescence intensity of a dilution series of the diesel oil in n-hexane.

The sampling points are the outlet of the separation unit, and the inlets to the header tanks of the two exposed basins. Two parallells from each sampling point are taken once every Monday, Wednesday and Friday. To get a better information on the daily fluctuation of the dosing unit we are testing an in vivo method for analysing the WAF. The method seems to work satisfactorily.

Samples for fluorescence analysis of the basin waters are taken with 1 liter brown glass bottles without collecting the surface film. The samples are taken 10-30 cm below the surface in the midst of each basin. The samples are extracted twice with 25 ml n-hexane in separation funnels. The pooled extracts are analysed for fluorescence intensity in the same manner as the WAF extracts.

Samples are taken once every week (Wednesday) from both exposed basins

and one control basin. Occational samples have been taken from the surface film of the high oil basin and one control basin and from the basin outlet.

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

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

RESULTS AND DISCUSSION

Dosing procedure

The dosing system has been running for about 16 months since 15 September 1982. With the exception of a two weeks break due to a fire in February this year the interruptions have lasted only a few hours in connection to regular maintenance or repairs. The main elements of maintenance have been regular cleaning (weekly or more often) of bacteria and fungi in the whole system, skimming off surplus surface oil in the separation unit, flow adjustments, and exchange of membranes in the pumps. Although the maintenance demands much man-power, it must be concluded that the dosing system has worked satisfactorily.

WAF analysis

The fluorescence analysis has shown quite large fluctuation in the WAF concentration, even between samples taken during the same day. The range in values for the 16 months (based on means of duplicate samples) has been 6.3 - 43.4 mg/l in the outlet of the separation unit, 4.2 - 39.1 mg/l in the header tank of the high oil (HO) basin, and 4.7 - 38.3 mg/l in the header tank of the low oil (LO) basin. Most of the values of all three sampling points have been in the range of 15 - 25 mg/l.

GC analysis of the WAF at entrance to the HO basin has been done on 6

occations (Table 1) covering 9 months of dosing. A typical gas chromatogram of a WAF sample is shown in Fig. 2_a. The consentration of petrogenic hydrocarbons ranged from 6.5 to 37.0 mg/l with an average of 21 mg/l. The gas chromatograms show that the diesel oil enters the basins mainly as oil droplets (oil in water emulsion).

The GC results and the corresponding fluorescence results show a very poor correlation when all data are put together (r-sq = 14.7 %). In 1982 the samples for the two types of analysis were taken one after the other within a short period of time, but the poor correlation could be due to the short term fluctuations in WAF concentration as has been shown by the fluorescence analysis alone. In 1983 therefore the samples for fluorescence (duplicates) were taken exactly at the same time as those for GC by frequent excange of samplers. Although we only have 3 sets of samples from 1983 yet analysed by both methods, these show a remarkably good correlation (r-sq = 100 %). A linear regression line (GC = 1.33F - 14.13) through the points indicates that a 0 value in the GC analysis corresponds to a fluorescence value of about 10 mg/l, and that the fluorescence and GC values would be the same at about 43 mg/l. The consistently higher fluorescence values between these limits could be due to the difference in what the two methods are measuring. The fluorescence measures mainly the aromatic (and possibly phenolic) fraction of the hydrocarbons, whereas the GC analysis quantitates the concentrations on basis of both aromatic and alifatic compounds within a certain range of molecular size. Both methods relate back to the original diesel oil, but if there is an enrichment of the more soluble aromatic compounds in the WAF, the fluorescence will overestimate the WAF concentration, as is suggested by the regression. As the concentration of the WAF increases the contribution of the dissolved hydrocarbons will be less important, hence a better agreement between the two types of analysis at the higher WAF concentrations. The basis for this discussion will be improved when more samples have been analysed by both methods, and when the GC samples have been separated into alifatic and aromatic fractions.

Theoretical hydrocarbon concentrations in the basins have been computed from the WAF concentrations at the entrance to the headertanks and the flow rates of WAF and sea water to the basins. The weekly mean concentrations in HO and LO basins from these calculations are presented in Fig. 3. They show considerable fluctuation especially in 1982 when the experience in running the dosing system was low. After the fire in February the system was totally rebuilt and after that the variation was greatly reduced. From February to September there has been an increase in theoretical load in the HO basin from about 180 µg/l to about 240 µg/l and with a possible slight decrease to November. The teoretical fluctuations in the LO basin have been much less and since February the values have been in the range of 40 to 50 µg/l. The corresponding monthly mean concentrations are presented in Fig. 4. These calculations are based on the fluorescence analysis and may, as discussed above, be overestimations of the real values.

Basin waters

The GC analysis of the hydrocarbon content of the basins covers the first year of dosing. Analysis has been done on 7 occations (Table 2 and 3). Typical gas chromatograms of the basin waters are presented in Fig. 2, b-d).

The concentrations in the HO basin ranged from 17 to 197 µg/l when the amount of biogenic hydrocarbons (as analysed in control basin C2) is subtracted. The average concentration was 73 µg/l. The mean value from two analyses of the surface water was 180 µg/l suggesting that the hydrocarbons accumulate in the surface film. The gas chromatograms show that most of the oil was present as droplets.

In the LO basin the concentration ranged from 9 to 71 µg/l, with an average of 23 µg/l. Also in this basin the oil was present as droplets mainly.

Theoretically the concentration in the HO basin should be 4 times higher than in the LO basin. The measured ratios are not too far from this, ranging from 1.9 to 6.0 with a mean of 3.4.

Fluorescence analysis of the hydrocarbons in the basin waters was not done until August 1983, but since then performed regularly once a week. The samples have been taken simultaneously with those for GC analysis.

Fig. 5 shows the gross concentrations in the waters of the HO, LO and C2 basins from August to October 1983 based on fluorescence. The fluctuations are considerable even within one day (dotted line) in both HO and LO basins. The fluctuations are synchronous and do also correspond very well to the fluctuations at the outlet of the separation unit. The C2 basin shows a stabile and low 'hydrocarbon' value presumably due to other compounds fluorescing at the same wavelengths as the oil. The mean concentration of oil during this period was 128.4 and and 29.4 µg/l respectively in the HO and LO basins when the C2 value is subtracted. This gives a mean ratio between the basins of 4.4. Also the fluorescence analysis indicate that the surface layer of the HO basin has elevated hydrocarbon concentrations compared to the water below (about 50 %), but this is based on one sample only.

The correlation between corresponding GC and fluorescence values from the basins are positive and significant ($r\text{-sq} = 96$ %, $p<0.5$), but so far only based on four sets of samples. A linear regression through the points has the equation $GC = 0.41F + 1.29$ reflecting the GC values as being less than half of the fluorescence values, and also that a zero value by GC gives about zero value by fluorescence. Here again the fluorescence values could be an overestimation when related back to the pure diesel oil.

Loss of hydrocarbons in dosing system

The fluorescence analysis of WAF and basin waters during August - October has been used to model the loss of hydrocarbons through the dosing system. The results are presented schematically in Fig. 6. If the concentration at the outlet of the separation unit is taken as being 100 %, the concentration has sunk to 89 % at the inlet to the HO header tank. This could be due to accumulation of hydrocarbons in the bacterial film of the pumps and pipelines. The regular and necessary cleaning of these prevents the establishment of an equilibrium between the WAF and this film. Some loss could also be caused by evaporation into air bubbles in the pipeline, which are not sampled. From the header tank to the HO basin water there is a furter loss of 43 % due

to accumulation to the basin walls, sediments and organisms, loss to the surface film and further on through evaporation. Hence the HO basin water will contain only 46 % of the oil originally pumped from the separation unit. Likevise the concentration at the inlet to the LO header tank is 75 % of the separation unit. The loss further on to the LO basin water is 37 % giving a content of oil corresponding to 38 % of what it would have been without any loss. Fig. 7 indicates how the teoretical monthly basin concentrations would be if the loss model is applied on the data of Fig. 4. Further discussion of this model should await a longer series of corresponding analyses of WAF and waters.

Real concentrations in the basins

To summarise the results on the oil exposure given to the two test basins during the first 12 to 14 months the mean concentrations found or estimated in the basins are:

GC analysis: HO basin: 73 µg/l, LO basin: 23 µg/l

Teoretical based on fluorescence analysis of WAF, flow rates, and losses in dosing system: HO basin: 101 µg/l, LO basin: 26 µg/l

Fluorescence analysis on basin waters (August - October 1973): HO basin: 128 µg/l, LO basin: 29 µg/l

We assume the real mean concentrations to lie somewhere between the GC values and the teoretical values, which is about half of the concentrations aimed at when the project was started.

Tissues, sediments, etc.

The samples of tissues, sediments, and scrape offs have been stored frosen and are presently being worked up for analysis. The following species have been selected for tissue sample analysis within the available budget:

Fucus serratus
Ascophyllum nodosum
Mytilus edulis
Littorina littorea

For each of these species 9 samples will be analysed (3 dates each with 3 basins).

The sediments and scrape-offs chosen for analysis were sampled after 14 months of dosing. The sediments were taken from the 0-2 cm top layer of the HO, LO, and C2 basins. The scrape-offs were taken from the wave generators of the same basins. Samples of air have been taken 10 cm above the surface in the same basins for analysis of evaporated hydrocarbons.

FUTURE STRATEGY OF ANALYSIS

Within the available budget for 1984 there is not room for much

analysis work. The necessary monitoring of WAF and basin waters will continue with emphasis on the fluorescence method. The GC analyses already planned or performed on WAF and water samples is assumed to give a reasonable "calibration" of the fluorescence method, but simultaneous sampling for both methods will be continued. The tissue samples mentioned above are less than desired to provide a picture of the hydocarbon load and dynamics of these populations, hence efforts shoud be made to provide addidional funding for such GC analyses. In the meantime samples will be taken and frozen to a desired extent.

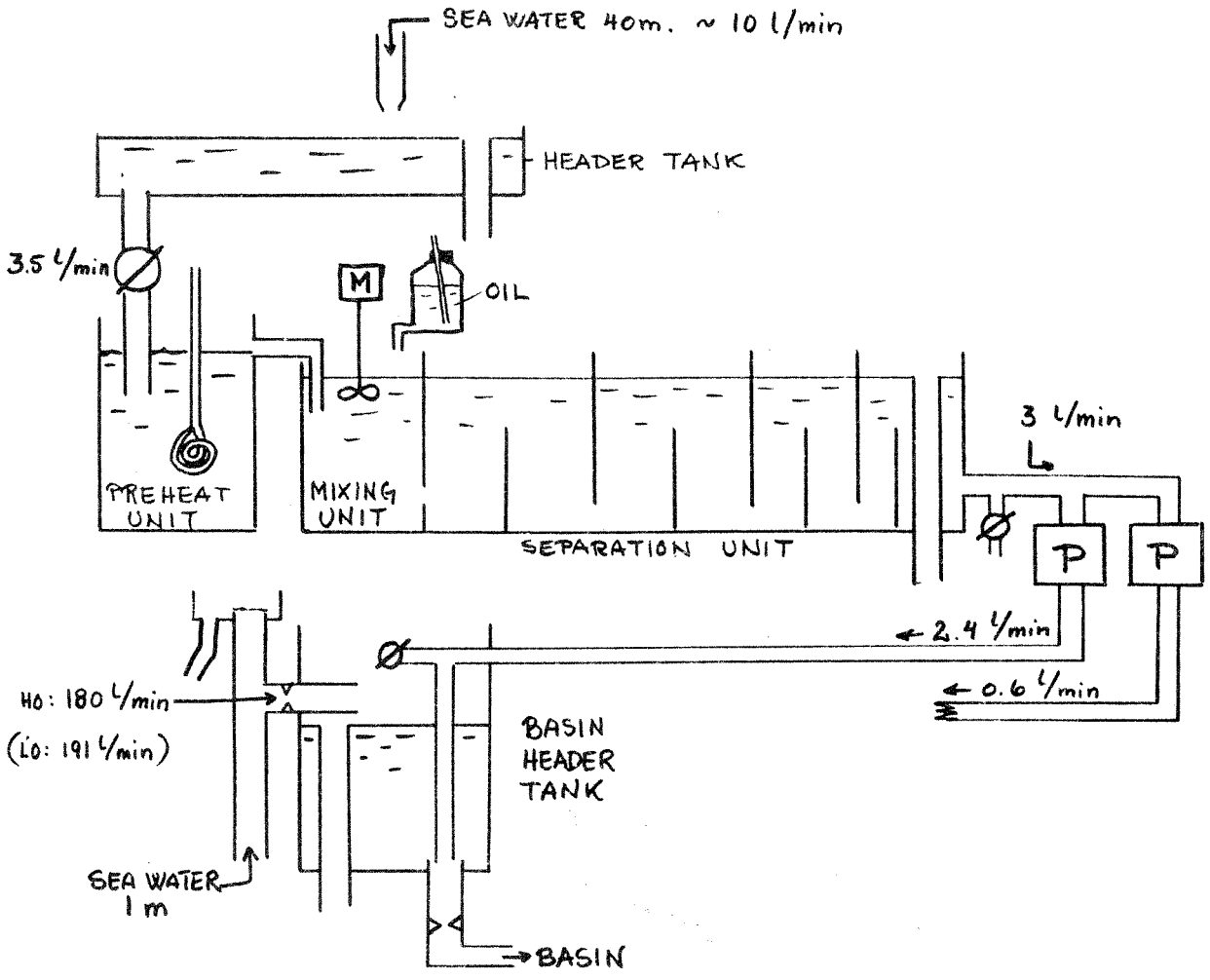

Fig. 1. Schematic view of the hydrocarbon dosing system of the Rock Littoral Project. Not drawn to scale. The flow rates indicated are the adjustment values.

Table 1. Amounts of hydrocarbons (THC) in the water accomodated fraction (mg/l) analysed by GC.
a) sampled from the inlet to the headertank of basin 1.
b) sampled from the outlet of the dosing unit.

Date of sampling		THC (mg/l)
5 October	1982 a)	18.2
4 November	1982 a)	35.5
6 December	1982 a)	37.0
19 January	1983 b)	6.5
24 May	1983 a)	13.5
22 June	1983 a)	15.7

Table 2. Amounts of hydrocarbons (THC) in basin 1 (µg/l) from analysis by GC.

Date of sampling	Sampled	THC µg/l)	Comments
4 November 1982	Sampled within	197	
6 December 1982	the basin at	35	
27 January 1983	20-30 cm depth	17 <-----	Service work per-
24 May 1983	and at 1-3 meters	68	formed on the
22 June 1983	distance from	84	dosing unit at the
29 September 1983	the inlet point.	60	time of sampling
12 October 1983		50	
5 October 1982	Surface water	100	
6 December 1982		260	
6 December 1982	Outlet water	80	Wave generator on
27 January 1983		70	" " "

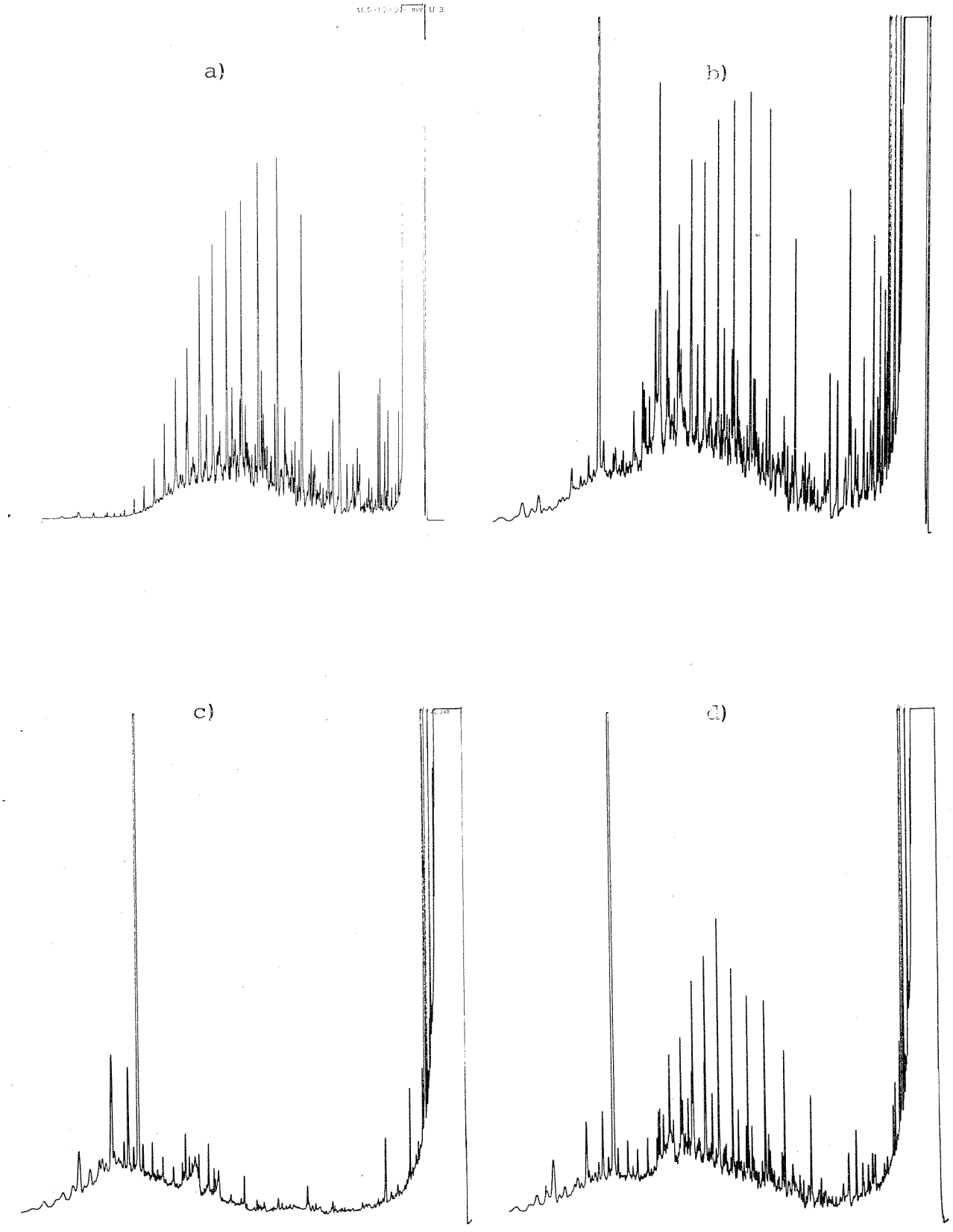

Figure 2. Typical gas chromatograms of samples from
a) water accomodated fraction b) HO basin 1
c) Control basin C2 d) LO basin 3

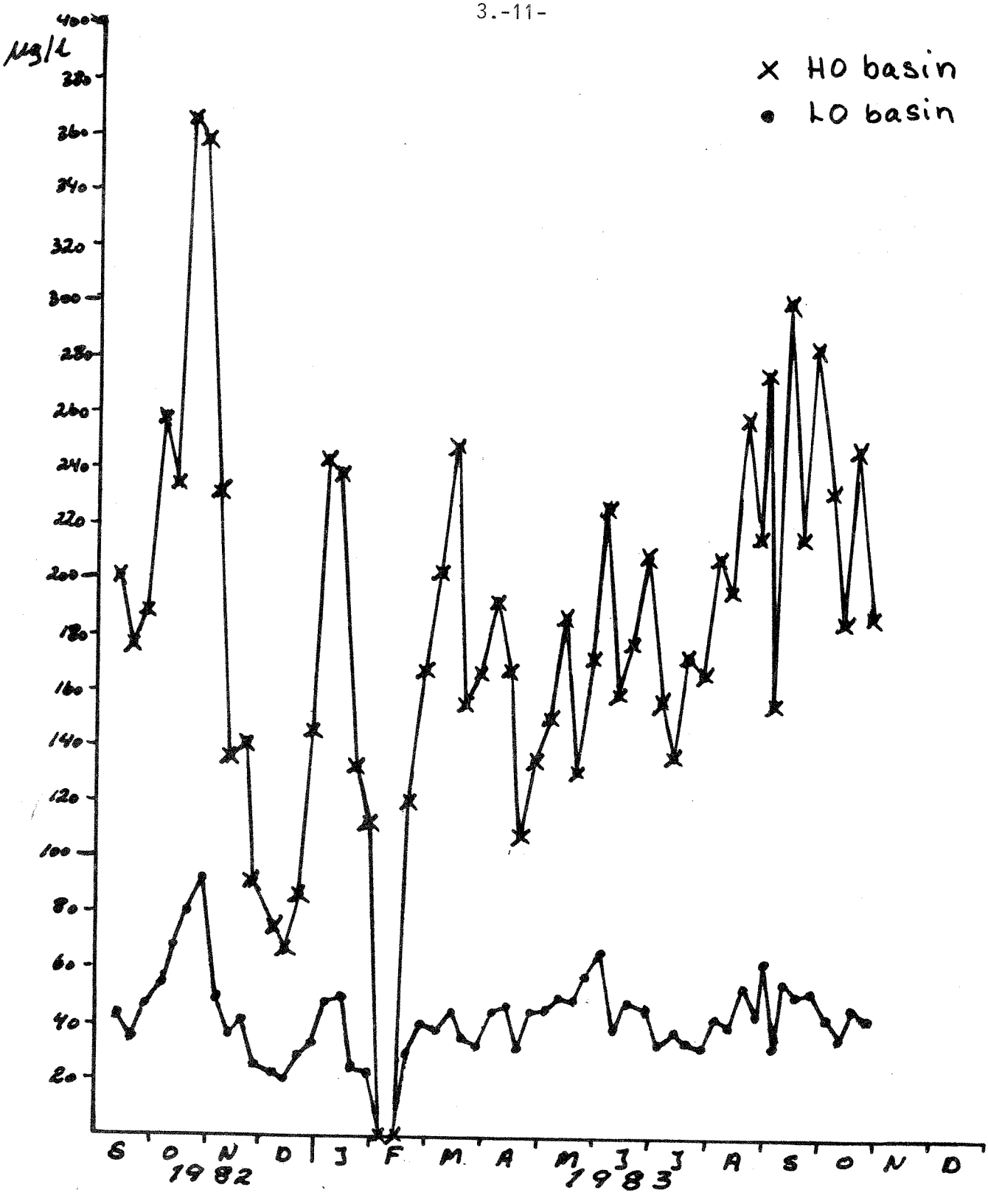

Fig. 3. Theoretical weekly mean concentration of hydrocarbons in the test basin, based on WAF concentrations and flow rates. Since the values are based on the input from the dosing system, control basin levels have not been substracted.

Table 3. Amounts of hydrocarbons (THC) in basin 3 (µg/l) from analysis by GC.

Date of sampling	THC (µg/l)		
4 November 1982	71		
6 December 1982	15		
27 January 1983	9	<-----	Service work performed on the dosing unit at the time of sampling
24 May 1983	26		
22 June 1983	22		
29 September 1983	10		
12 October 1983	12		

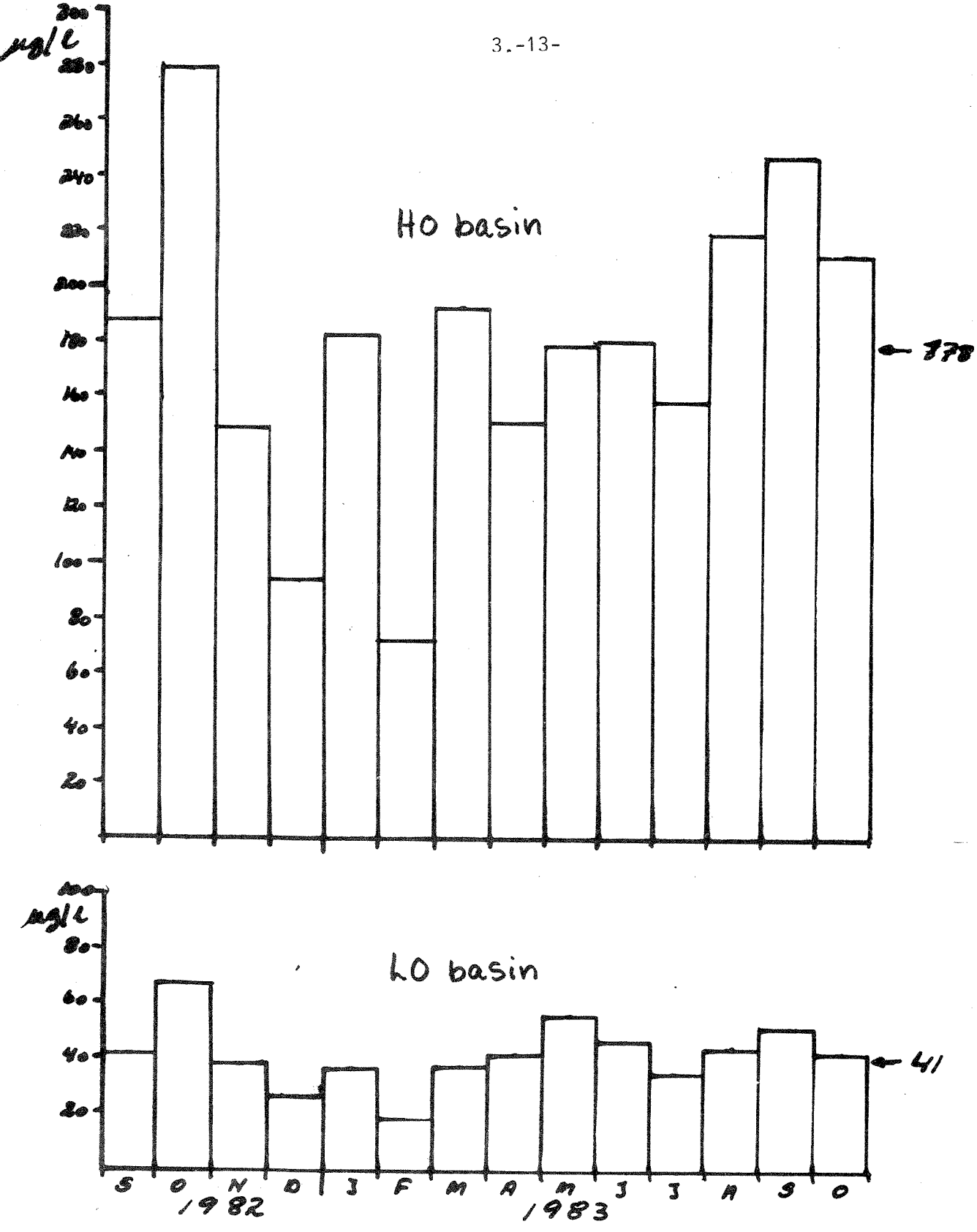

Fig. 4. Theoretical monthly mean concentration of hydrocarbons in the test basins based on WAF concentrations and flow rates. See also legend to Fig. 3.

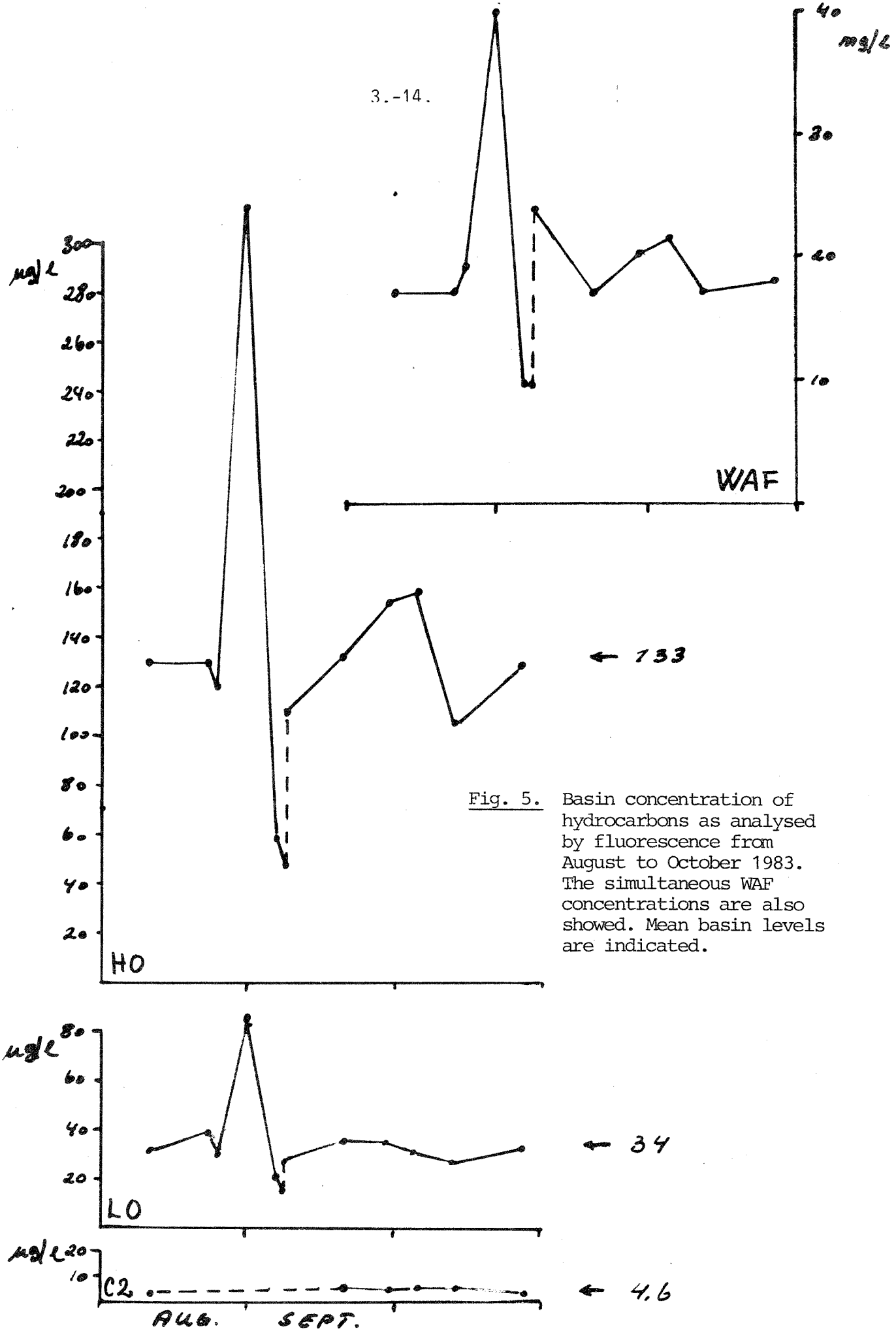

Fig. 5. Basin concentration of hydrocarbons as analysed by fluorescence from August to October 1983. The simultaneous WAF concentrations are also showed. Mean basin levels are indicated.

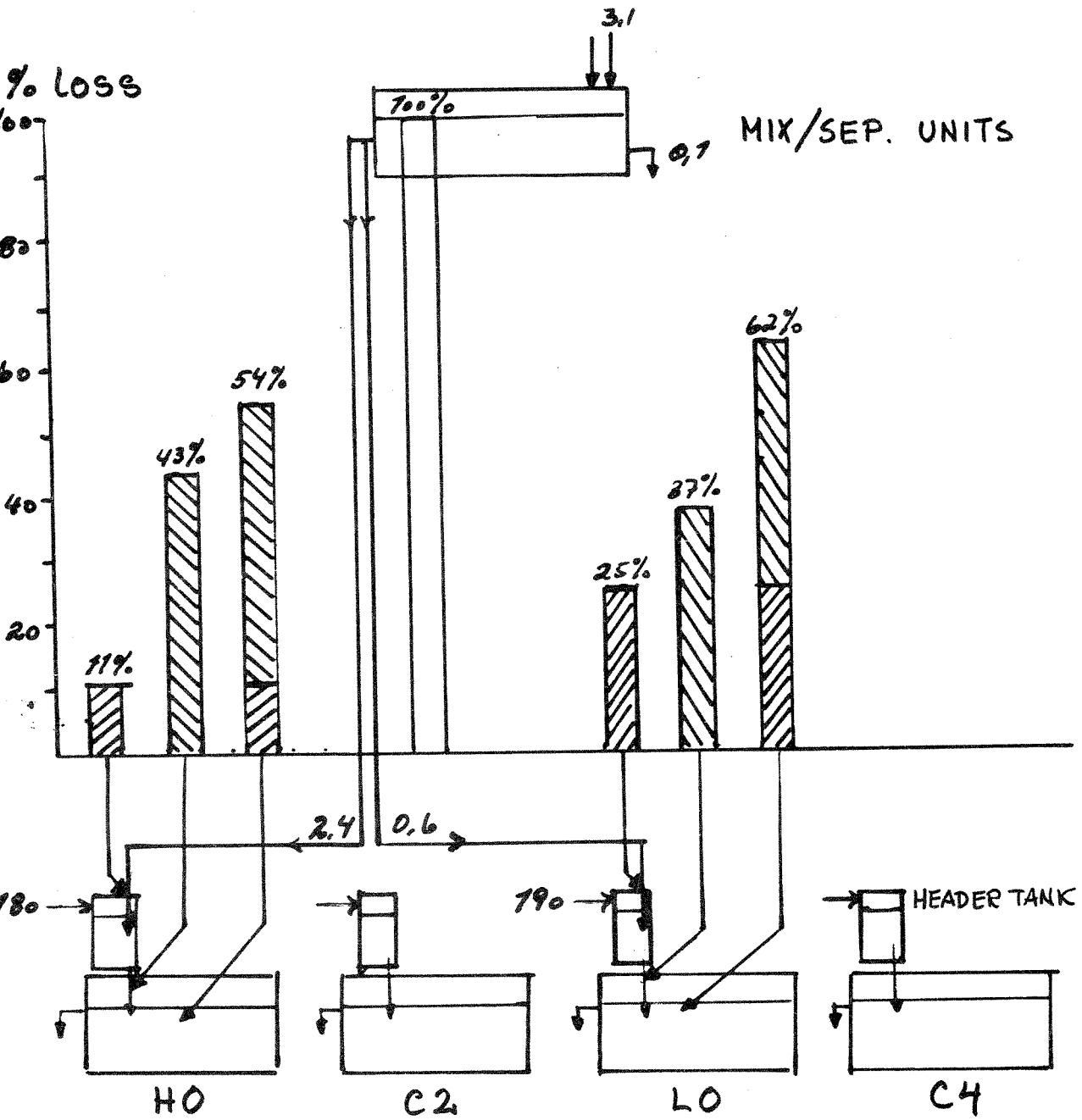

Fig. 6. Loss of hycrocarbons in the dosing system based on fluorescence analyses of WAF and basin waters from August to October 1983. See text for further explanation.

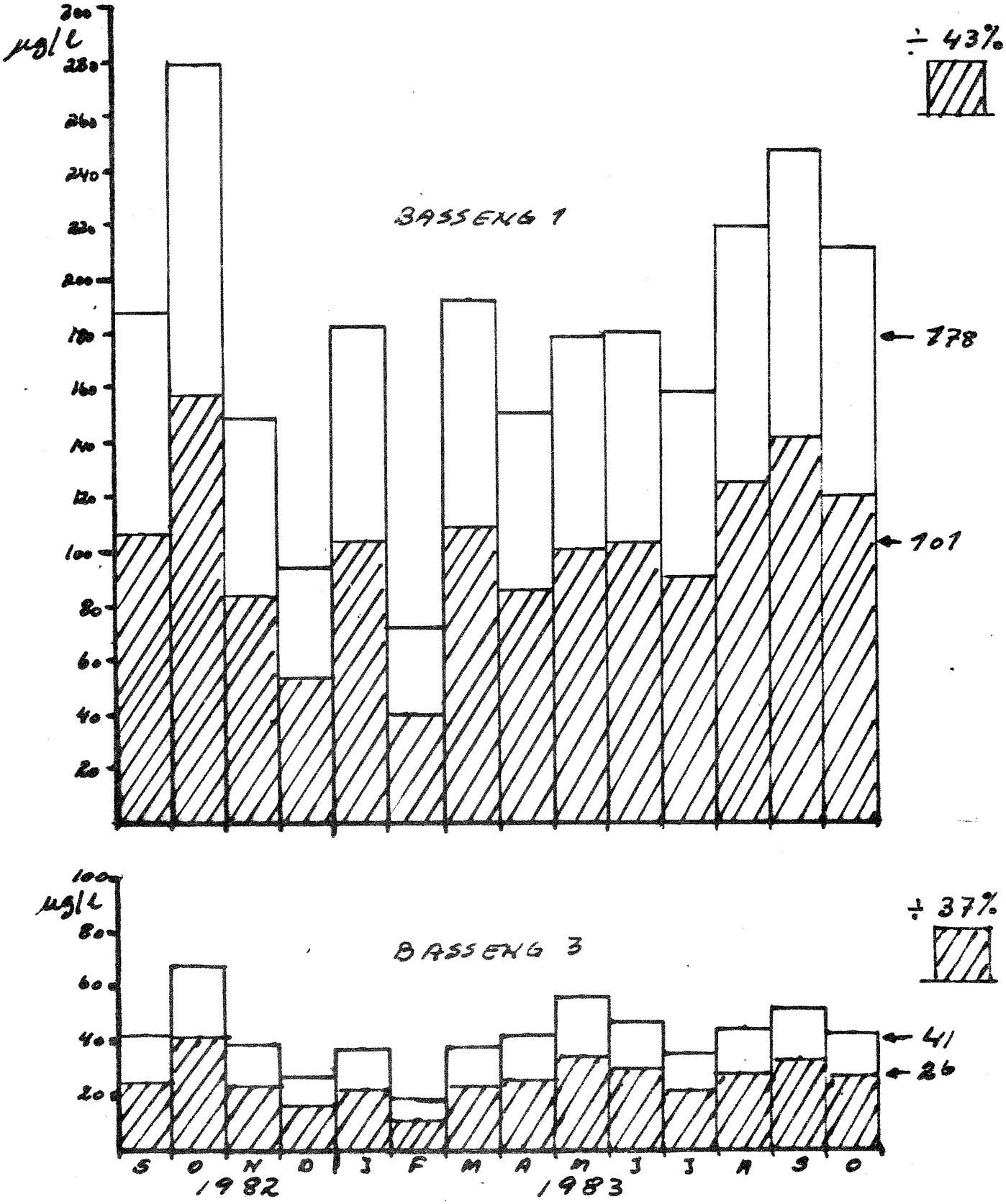

Fig. 7. Theoretical monthly mean concentration of hydrocarbons in the test basin based on WAF concentrations and flow rates (same as Fig. 4), and with application of the loss model shown in Fig. 6 (hatched histograms).

MARINE RESEARCH STATION SOLBERGSTRAND

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4. COMMUNITY STRUCTURE

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

Introduction

There have been conducted few experimental studies of the long term effects of chronic oil pollution on littoral communities, especially with relevance to East Atlantic boreal and arctic coasts.

At the Marine Research Station, Solbergstrand, 30 km south of Oslo, littoral communities have been established in four concrete basins during October 1979 with about 25 m^3 of running sea water. Two basins experience diesel oil exposure on a low continuous scale. The two others act as controls.

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

Materials and methods

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

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

Thus eight characterizations of the community structure have been performed during the period of investigation. Necessary equipment most of the year is a SCUBA-diving gear with a full face mask connected by cable to a radio and a tape recorder.

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

Results

During 1½ years about 50 different species of algae and benthic animals are registered, table 1. Covering degree or number of the different organisms observed in every square is filled into this standard schemes. The total number of every single species of 30 squares, i.e. all registrations from each basin are used for preparing density/time diagrams, out of which some of the most important organisms are presented here.

During the eight monitoring periods some organisms have shown normal annual changes, while other species have been reduced in density. In the Oslofjord five species of fucoids are growing of which four were introduced in 1979. During the summer 1981 some specimens of the fifth fucoid *Fucus spiralis* was established. However, this species has never come to be a common species in the four basins. *Ascophyllum nodosum* has been relatively stable in its occurence, fig. 2, while *F. serratus* has shown an annual variation, fig. 6. *F. vesiculosus* has been stable in one of the controls - C4. In the high oiled (HO), the low oiled (LO) and control 2 (C2) the populations have been somewhat reduced, fig. 3. *F. distichus* ssp. *edentatus* was also relatively stable in C4, somewhat reduced in LO and clearly declining in HO and C2, fig. 5. *Fucus* germlings have been recorded only for the two last periods. The results are presented in fig. 4.

Fig. 7 shows heavy losses of *Laminaria saccharina* in all basins. The red algae *Chondrus crispus* and the two green algae *Cladophora rupestris* and *Ulva lactuca* have grown in the same way, their density in the basins has increased. Exceptions to this are *C. crispus* in C2 and *U. lactuca* in C4, which are more stable throughout the investigation, figs. 8, 9 and 10.

Asterias rubens in all basins has shown a heavy reduction, fig. 11. *Mytilus edulis* has been relatively stable in basins C2, C4 and LO, while the mussels have nearly disappeared in HO. Only e few specimens were observed during the last monitoring period, fig. 12. *Littorina littorea* was very stable in C2 and LO. In HO a great variation of density was found through the whole period of registrations. The numbers in C4 have shown reduction since the start of the project, but some increase has been observed since the last two periods of monitoring, fig. 14. Seasonal variations are registered for *Balanus balanoides* in basins C2, LO and HO, while C4 shows a pronounced increase, fig. 13. *Spirorbis* sp. has shown a great increase in settlement in C4, while the three other basins have got few if any settlement during the entire monitoring period, fig. 15.

Discussion

Preliminary results indicate that only a few species of adult algae have shown any reduction of covering degree in oiled and control basins. Observed decreases in density of *F. vesiculosus* (fig. 3), *F. distichus* (fig. 5) and *L. saccharina* (fig. 7) in the oil basins can not be distinguished from one or both of the controls. In both the oiled basins (HO and LO) and at least one of the control (C2 and C4) *Chondrus crispus*, *Cladophora rupestris* and *Ulva lactuca*, figs 8 - 10, have shown an increase in covering degree. Since at least one of the controls shows identical increase to the oiled basins, the observed effect can be difficult to link to the diesel oil exposure. In contrast the fucoid germlings have shown a different development, fig. 6. HO-basin has given rise to fewer germlings than C2 and C4, and LO seems to lose several specimens of the original summer populations.

Among the five benthic animals given attention in figs 11 - 15 two are motile and three are sessile.

An expected result of the heavy reduction in all basins of *Asterias rubens* would be subsequence increase of the population of *Mytilus edulis*. This effect is not observed. On the contrary *Mytilus* has nearly disappeared in HO. The stability and new settlement observed in the three other basins indicate a negative effect of diesel oil on *Mytilus* only in HO.

There is no pronounced lethal effect of diesel oil on adult *Littorina littorea*. The reduction in C4 is probably normal due to the extreme high number at the start of the subproject. Still C4 shows the highest number of the four basins. The great variation in density in HO, especially the peak (fig. 14) in November 1982, reflects the start of the diesel oil dozing. At that time the snails avoided the oily water by staying in air. Recruitement was checked by separating small individuals from adults during the estimation. In contrast to adult snails the recruitement seems to be inhibited by diesel oil in both HO and LO basins. No effects of oil are detected for *Balanus balanoides*. But C4 has got a high settlement the last spring compared to the three other basins, even if all the basins show increases in settlements the last season.

At the start of this project in June 1982 no *Spirorbis* sp. was observed in any basin. Today only C4 can show an substantial population.

In conclusion the preliminary results indicate lethal effect for only one adult organism, *Mytilus edulis*. The more sensitive recruitement stages will be difficult to monitor using this method. But monitoring later stages h... shown possible lethal effects by diesel oil on fucoid germlings and sma... littorinids.

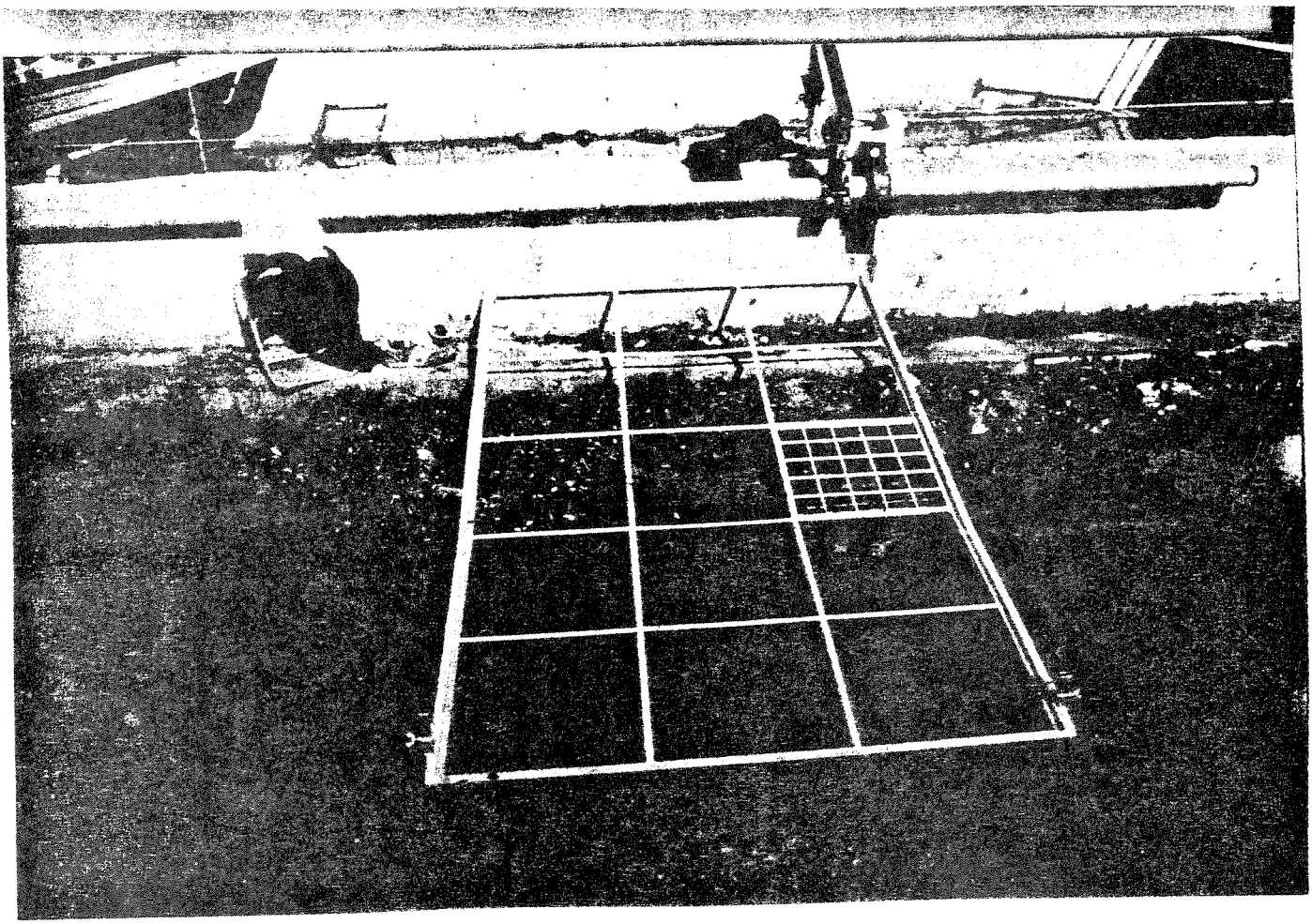


Fig. 1. A special made frame to estimate covering degree and numbers of organisms, with subsquares to make it more easy to estimate covering degree.

Table 1. Scheme for registration of organisms in the basins

DATE: _____ BASIN: _____

Square/No	1	2	3	4	5	6	7	8	9	Notes:
MOTILE ANIMALS/NO										
Acmaea sp.										
Asterias rubens										
Carcinus maenas										
Littorina littorea										
L. obtusata										
SESSILE ANIMALS/COVER										
Balanus balanoides										
B. improvisus										
Dynamena pumila										
Halichondria panicea										
Hydroider										
Membranipora/Electra spp.										
Mytilus edulis										
Pomatoceros triqueter										
Spirorbis sp.										
ALGAE COVER										
Ahnfeltia plicata										
Ascophyllum nodosum										
Ceramium rubrum										
C. strictum										
Chaetomorpha melagonium										
Chondrus crispus										
Chordaria flagelliformis										
Cladophora rupestris										
Cladophora spp.										
Cystoclonium purpureum										

cont. Table 1.

Square/No	1	2	3	4	5	6	7	8	9	Notes:
cont Algae cover										
Dictyosiphon foeniculaceus										
Dumontia incrassata										
Ectocarpus sp.										
Elachista fucicola										
Enteromorpha sp.										
Fucus distichus										
F. serratus										
F. spiralis										
F. vesiculosus										
Hildenbrandia rubra										
Laminaria digitata										
L. saccharina										
Petalonia fascia										
Phymatolithon lenormandii										
Pilayella littoralis										
Polysiphonia spp.										
Porphyra spp.										
Ralfsia spp.										
Rhodomela confervoides										
Scytosiphon lomentaria										
Spongonema tomentosum										
Ulothrix/Urospora										
Ulva lactuca										

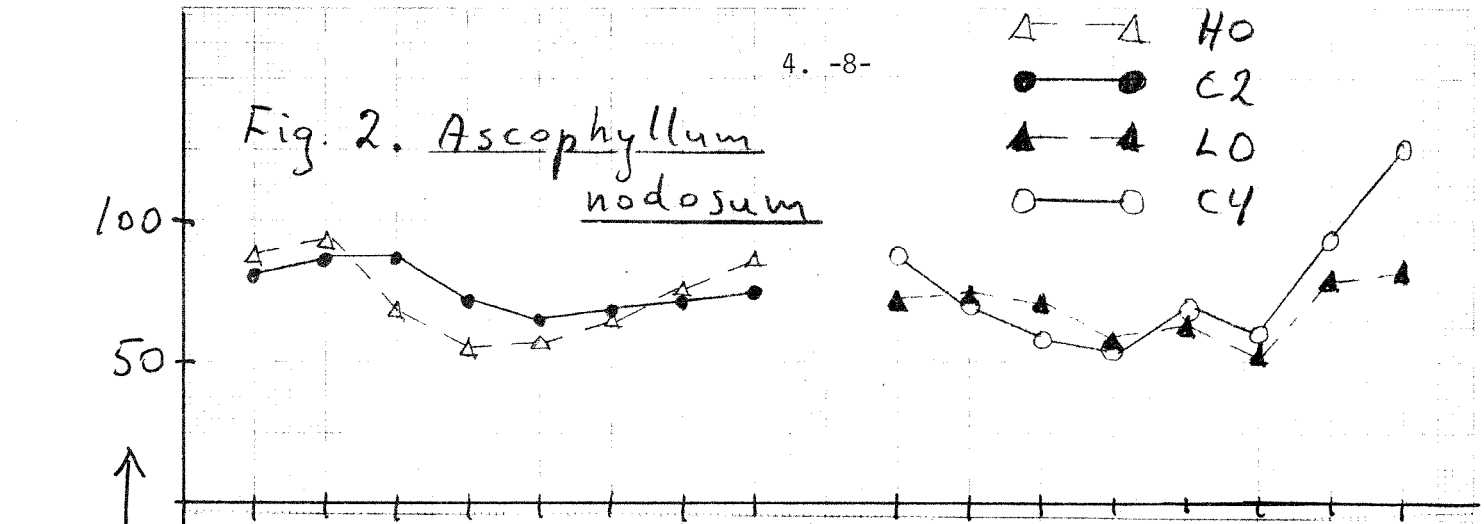


Fig. 2. Ascophyllum nodosum

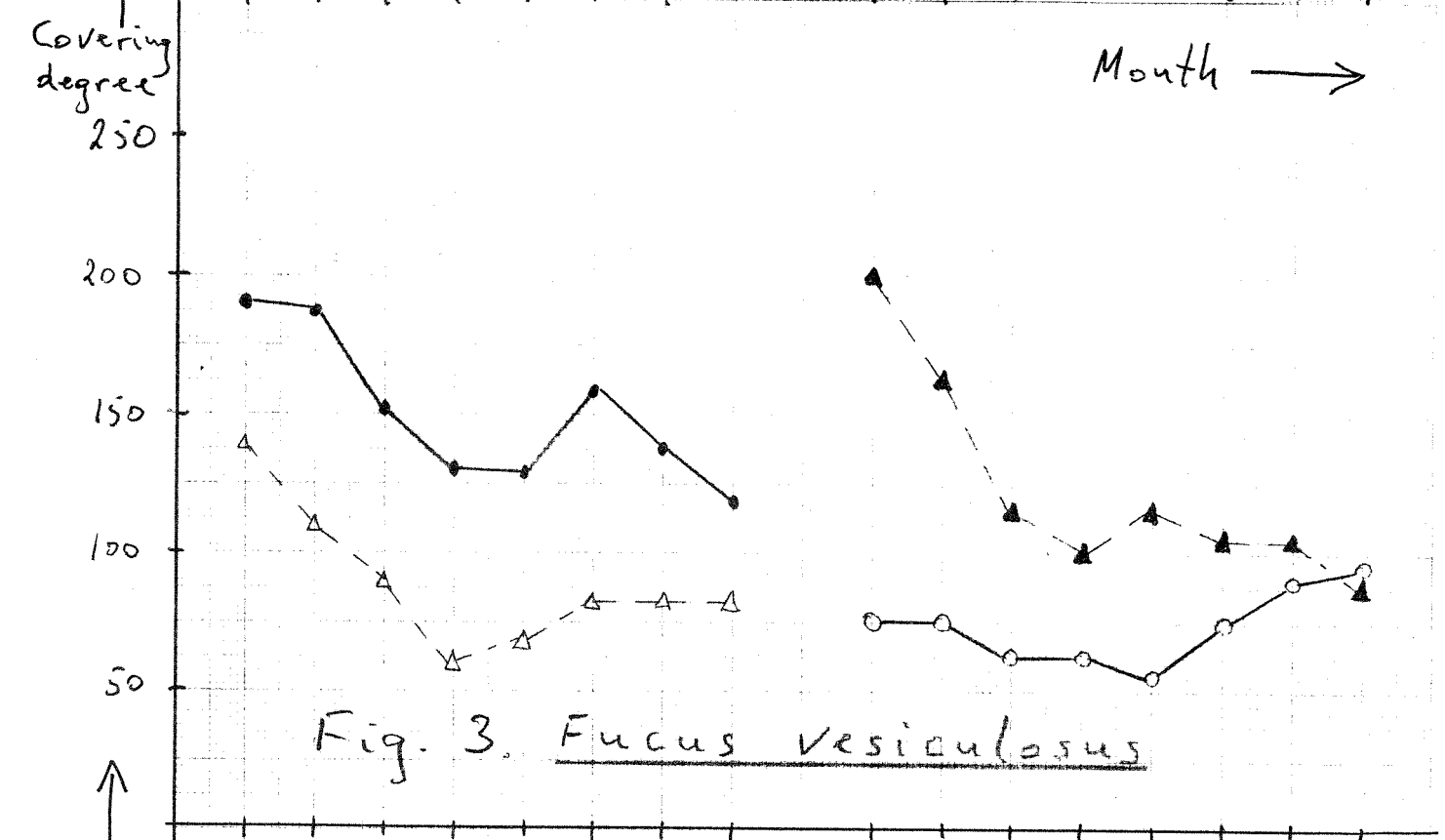


Fig. 3. Fucus vesiculosus

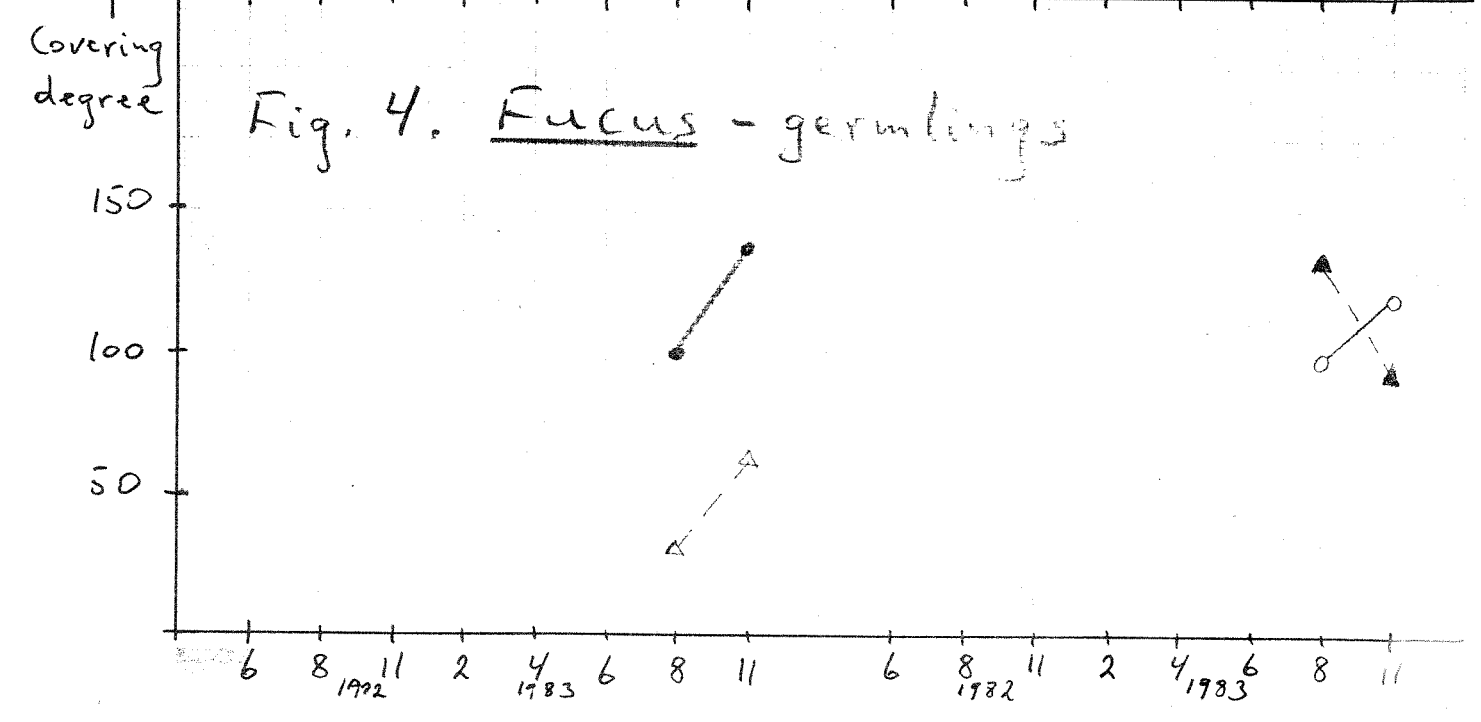


Fig. 4. Fucus - germlings

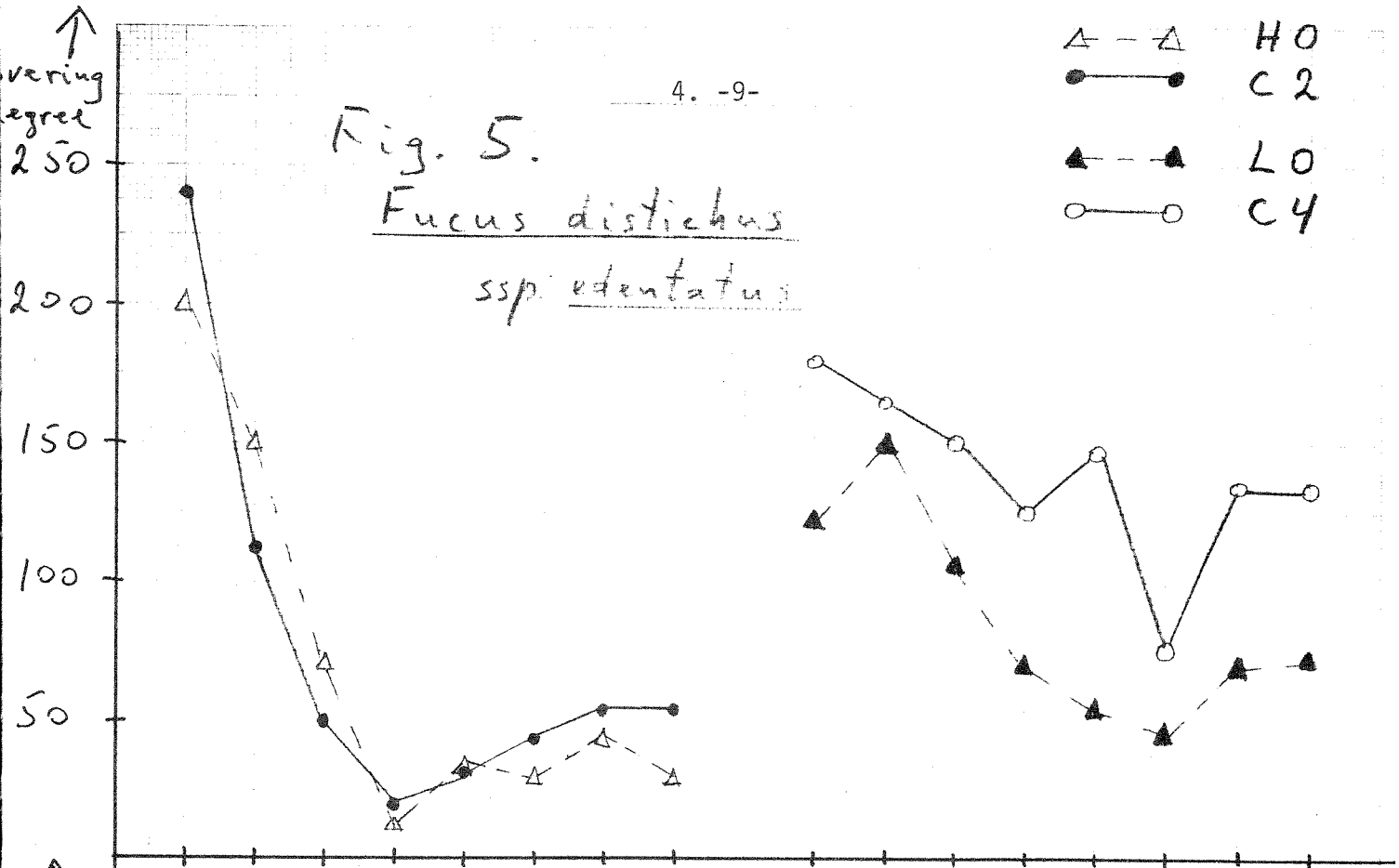


Fig. 5. Fucus distichus ssp. edentatus

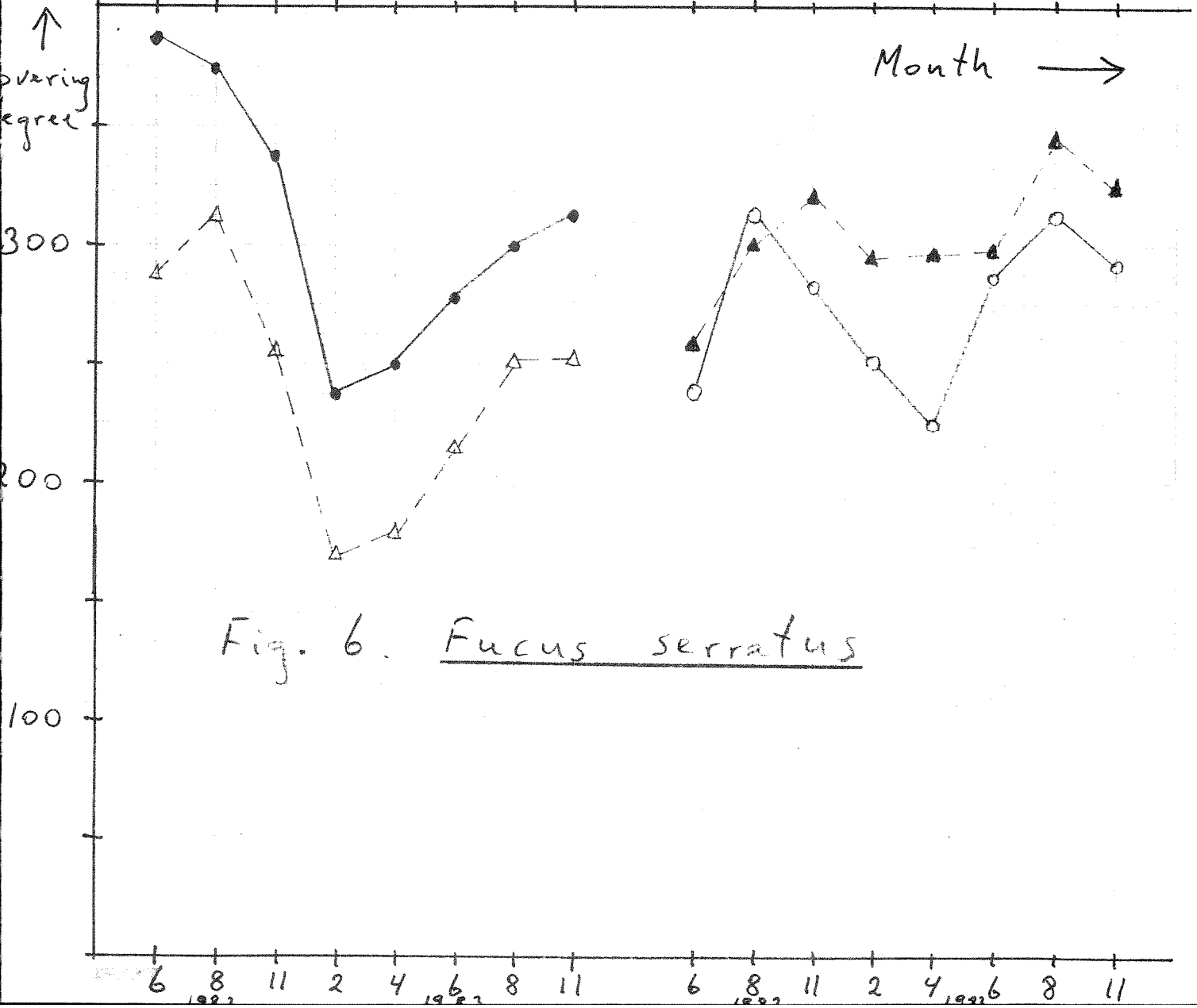
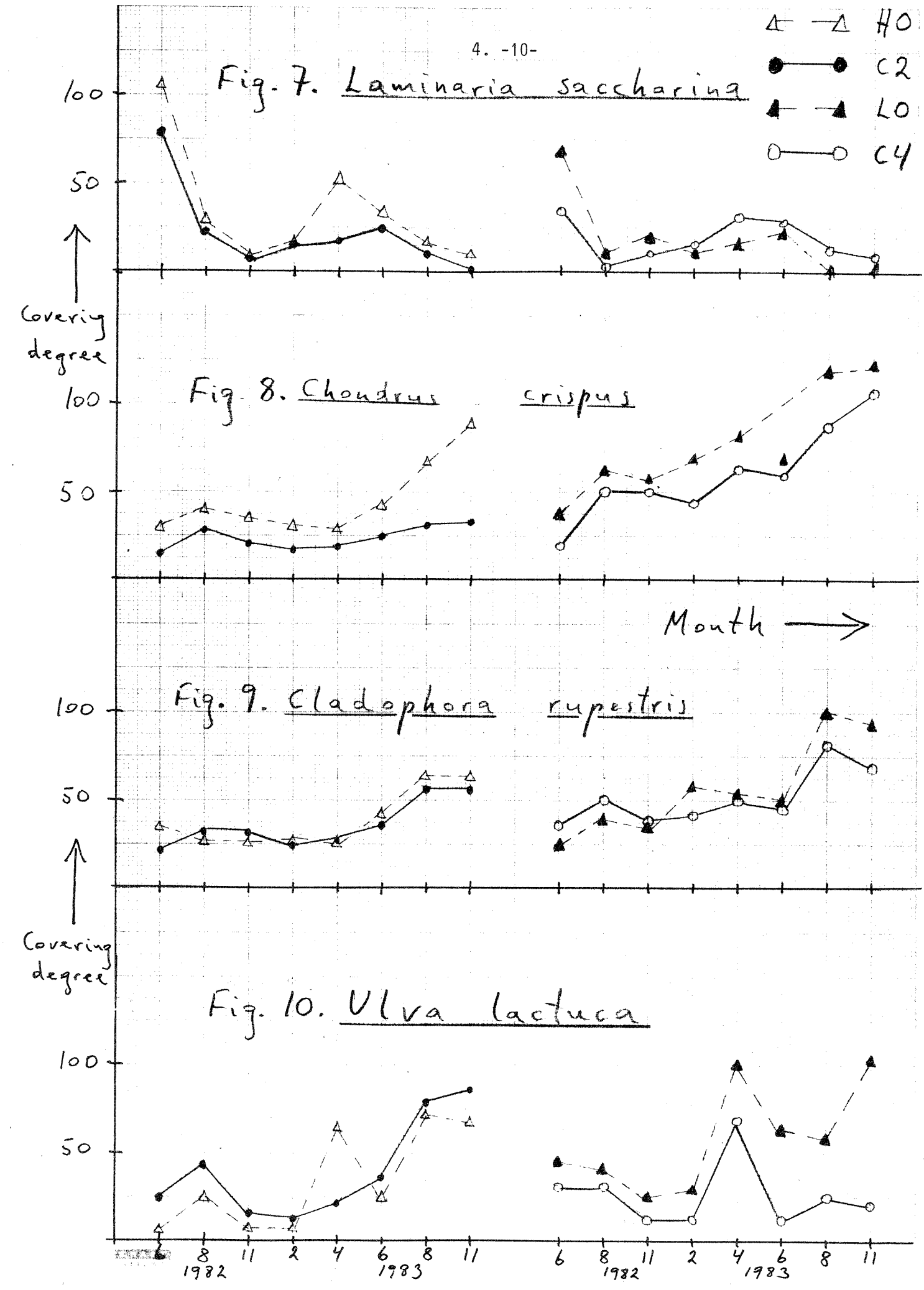


Fig. 6. Fucus serratus


HO
C2
LO
C4
Fig. 7. Laminaria saccharina
100
50
Covering degree
Fig. 8. Chondrus crispus
100
50
Month
Fig. 9. Cladophora rupestris
100
50
Covering degree
Fig. 10. Ulva lactuca
100
50
8 11 2 4 6 8 11
1982
1983
6 8 11 2 4 6 8 11
1982
1983

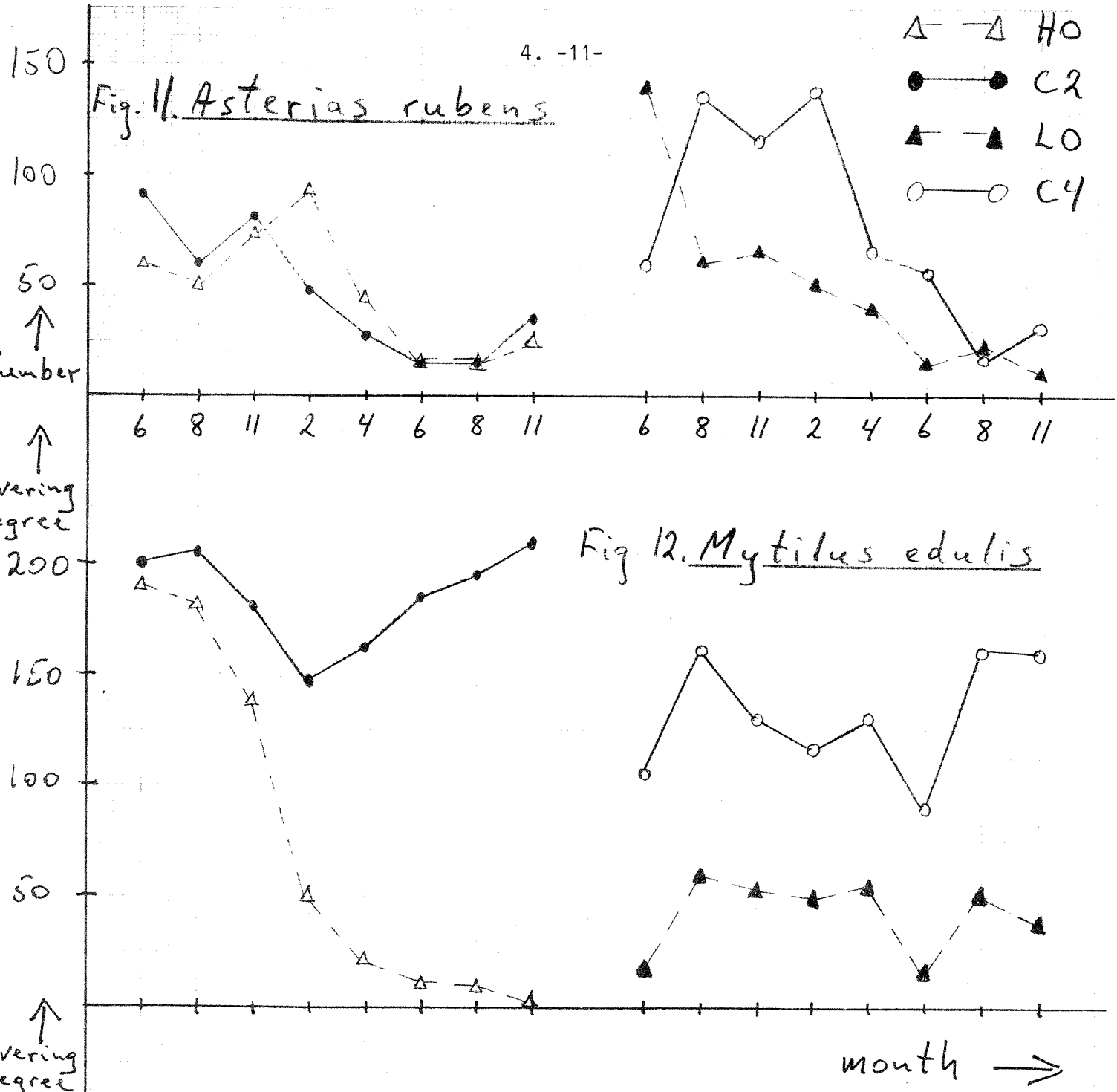


Fig. 11. Asterias rubens

Fig 12. Mytilus edulis

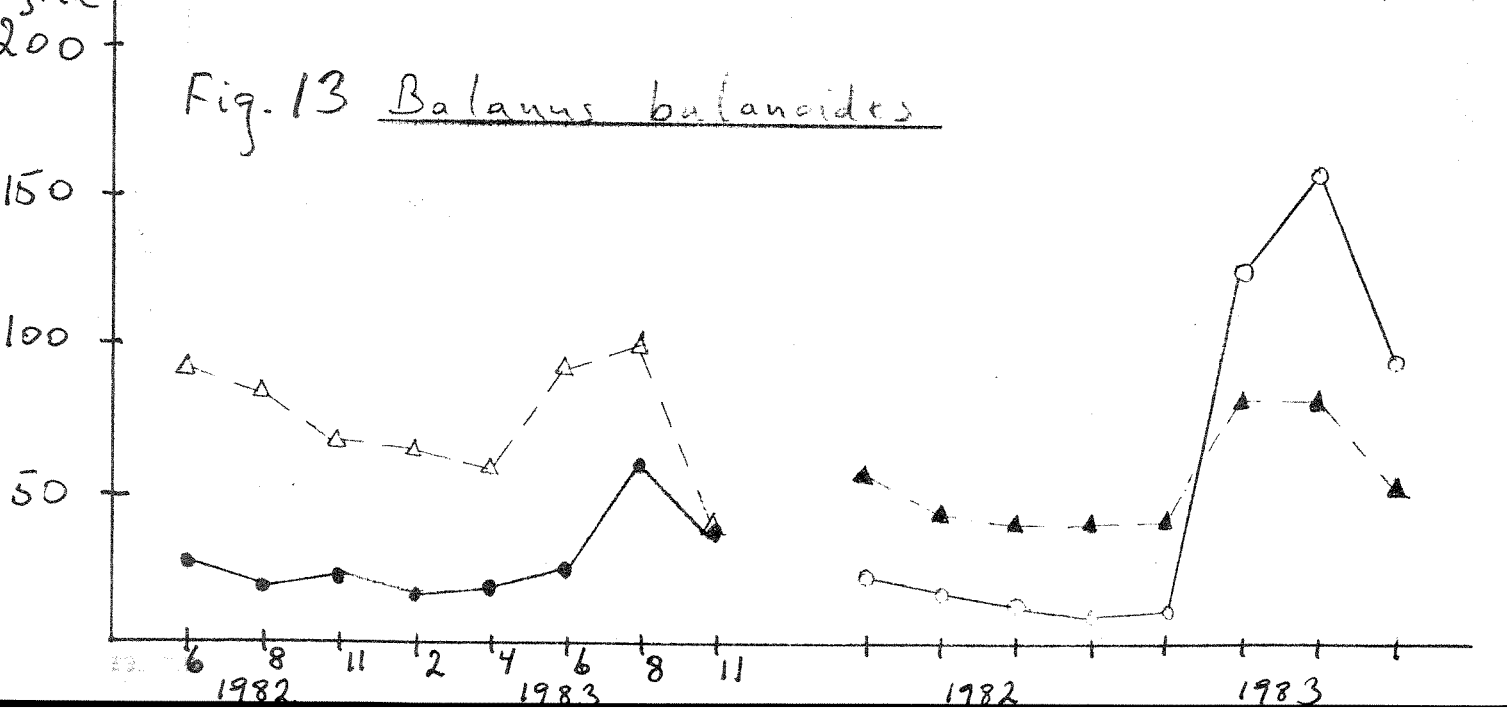


Fig. 13 Balanus balanoides

Fig. 14.
Littorina littorea

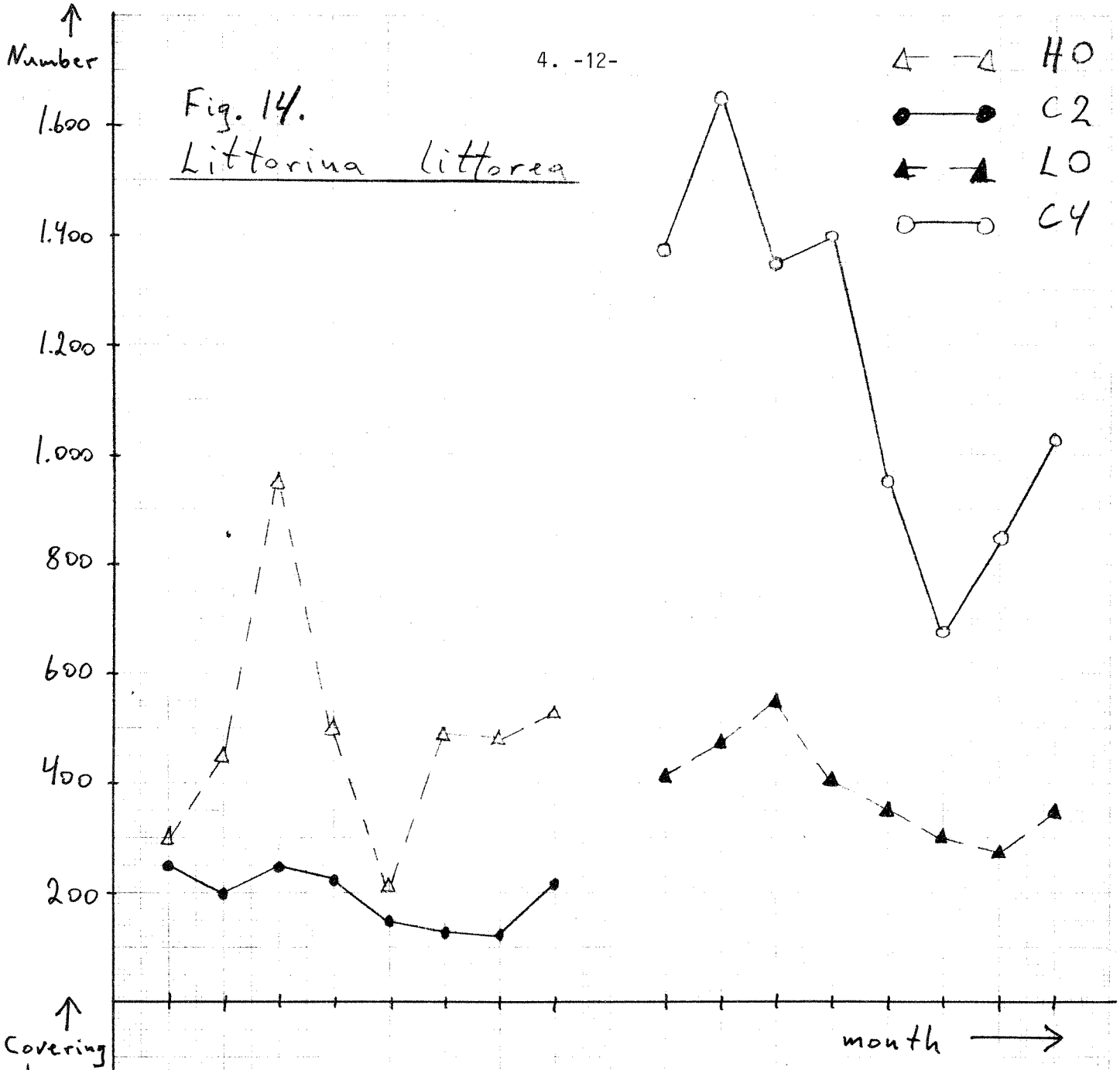
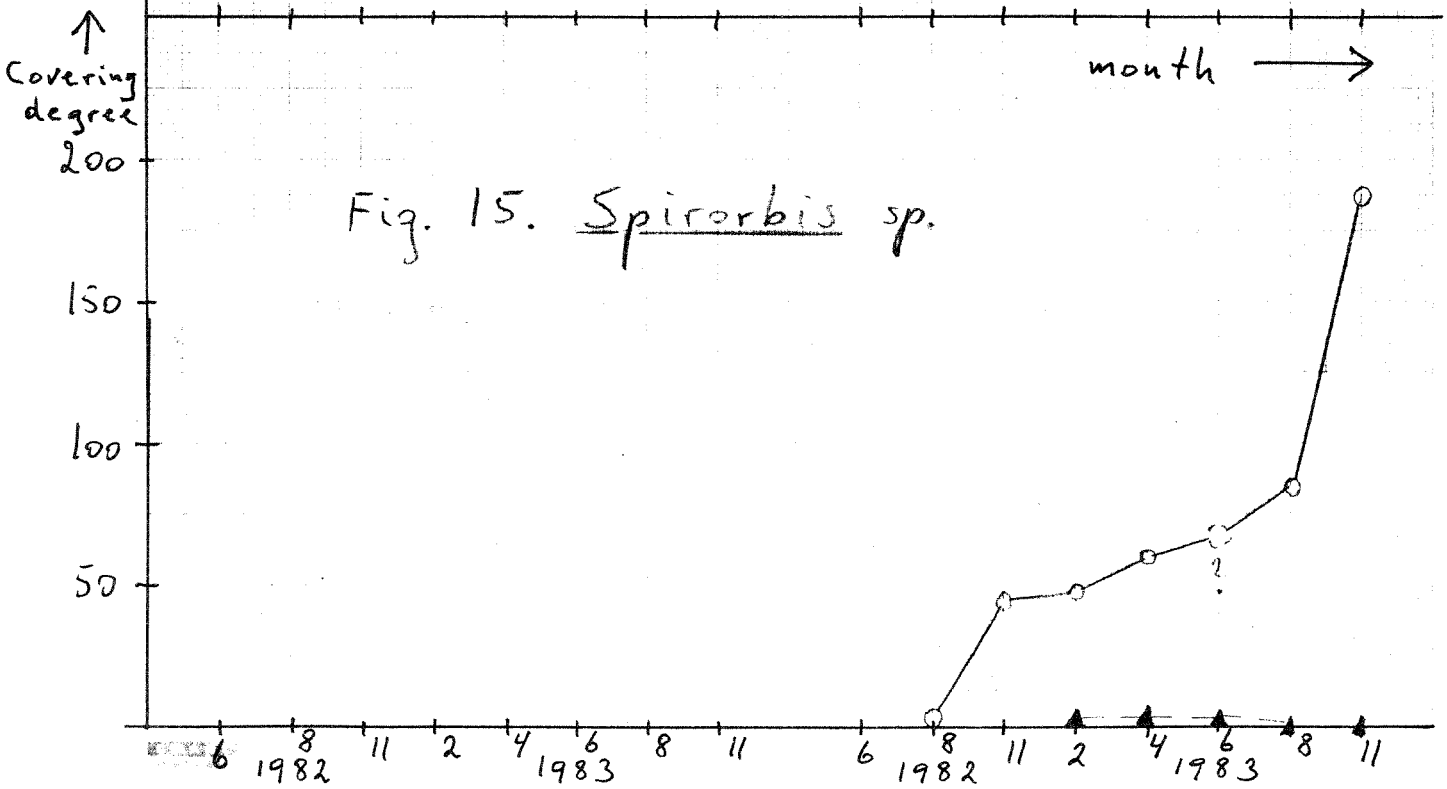


Fig. 15. Spirorbis sp.



MARINE RESEARCH STATION SOLBERGSTRAND

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5. RECOLONIZATION AND POPULATION STRUCTURE OF INTERTIDAL ORGANISMS

T. Bokn (NIVA).

Introduction

The aim of the subproject presented here is to see if low continuous exposure to diesel oil has any effects on colonization of intertidal organisms.

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. The surface used is uneven about ½ mm deep, in which spores and zygotes can better attach.

Originally 44 chips were located on steps in HO and C2 corresponding to the lower part of the intertidal in the basins. Tide is about 22cm, and exposure to air has been about 1½ hrs, to day about 3/4 hour. To avoid grazing by littorinids two racks with 44 chips are mounted in free water, one in each basin. Since 16 December 1982 the racks got its present design. The square chips are designed for one as well as several months experiments. Before located in the basins the chips are boiled to avoid any spores to survive. 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. Covering degree is estimated from every chip using a grid-system. The biomass is removed from half the chip and dry weight and ash free dry weight are determined by drying the dishes at 105^{o}C for 24 hours followed by incineration at 550^{o}C for 30 min. (APHA-AWWA-WPCF 1980), since June 1983 1 hour. The other half of the chips is harvested for identification of the organisms. The samples are conserved in 2-3% formalin. Identification has been carried out for the period October 1982 -

May 1983 only.

From September 1982 to October 1983 the average of diesel oil concentration in HO has been about 100 ppb. Biomass data have been obtained from October 1982 to October 1983. The samples are collected in the middle of each month (15-19).

Results and discussion

In Table 1 only the main species covering the chips have been paid attention. It was not possible to separate diatoms and tiny germlings when estimating the covering degree. The main species are therefore given identical per cent cover. Data from one month as well as several months growth is put together in the table.

Data of mean ash free dry weight (A.D.W.) from an area of 50 cm^2, half the chips, are presented in FIG. 1 . Table 2 shows the difference of the mean A.D.W. between the two racks, the two steps and between the rack and step in the oil and control basins respectively. Degree of significance is calculated by a paired t-test, indicated in the table.

Mean A.D.W. from the different areas of HO and C 2 are converted to g/m^2 in table 3. Number of parallel samples is also given.

A difference has been shown in the composition of the species as well as in the succession, table 1.

Species composition and succession on the racks

During the first month of oil exposure (16 September to 19 October) the chips on the rack in HO (B1 R) were overgrown by mainly diatoms, while the chips on the corresponding rack in C2 (B2 R) were dominated by *Enteromorpha* spp. Chips harvested in the middle of November and December showed very little growth due to low light energy (60^oN). In the middle of January recolonized diatom colonies were observed on

the B1 R and B2 R. The predominant species on B1 R was the cold water diatom *Melosira nummuloides* (Hendey 1964), while the main species on B2 R were *Navicula* spp.

Some reduction in covering degree was observed in February on both racks. On B1 R a mixture of *M. nummuloides* and *Navicula* spp. was found. Opposed to that pennate diatoms and the green algae *Ulothrix* spp/*Urospora* spp. dominated the cover of B2 R. In March the diatom flora seemed to change a lot. *M. nummuloides* was still dominating only on B1 R, but in mixture with pennate diatoms. *Ulothrix* spp/*Urospora* spp. were common on both racks, while another diatom *Fragilaria* sp. was the dominating diatom on B2 R. In addition to the former species observed during the first quarter of the year *Nitzschia closterium* and *Schizonema* colonies were found to be common on B1 R in April. In the May samples *M. nummuloides* was more seldom on B1 R. Instead *Gomphonema* sp. was dominating, as *N. closterium* was. At B2 R *Cocconeis* sp. was dominating together with *Fragilaria* sp.

In Table 4 the predominating species and their succession on the two racks are shown.

All predominating species on B1 R have been diatoms, and none has played any important role in the flora on B2 R with very few exceptions. Included in the predominating species of B2 R have been two annuals of Phaeo- and Chlorophyceae, which never showed the same appearance on B1 R (identification for the summer period has not yet been performed).

There is little evidence to state whether the predominating diatoms on B1 R are favoured by diesel oil or not. However, the winter diatom *Melosira nummuloides* was found to be the overall dominating organism in the supralittoral and littoral zone in SE Norway after an oil spill during late December 1982 (unpublished results).

Except for the month of October red algae have been absent. According to Huang and Boney (1983) diatom mucilage can inhibit growth of *Chondrus* and *Gigartina* opposed to the enchancement of six green and

brown algae. A possible inhibition by the diesel oil together with the known result from Huang and Boney's work can explain the absence of red algae on B1 R. Sousa (1979) found that *Ulva* inhibited the recruitment of perennial red algae. Thus the growth of *Enteromorpha* and *Ulothrix* might give identical effect on B2 R. The lack of multicellular species as predominants on B1 R can be an indirect or direct effect of diesel oil. During summer green algae have covered the long term chips in HO basin. Identification is not carried out.

An experiment performed by Steele (1977) with oil exposure of young stages of *Fucus edentatus* showed when exposure occured immediately, prior to and during release of gametes, that no germination or growth occured with any of the used oil types, even at extremely low concentrations (nominal 0,2 ppb and higher). However, in the present experiment germlings of *Fucus distichus* ssp. *edentatus* were found in equal number on B1 R and B2 R (mean: eight germlings each). Several cm long specimens have been observed growing on the long term chips in both basins during summer.

Species composition and succession on the steps

The chips on the steps in both basins (B1 S and B2 S) showed a notable similarity in covering degree. The only diatom more frequent on B2 S was *Fragilaria* sp. Spat of *Balanus balanoides* appeared earlier on B1 S than B2 S. Opposed to this fact Bonsdorff and Nelson (1981) found that newly settled spat of the same species in a Norwegian fjord could be negatively affected by oil pollution. Since May the barnacles on the long term chips in HO basin have been reduced in density compared to those in C2 basin.

The chips on B1 R were more heavily grown than corresponding chips on B1 S. Since summer no significant difference has been detected between rack and steps in C2. The green algae *Enteromorpha* spp. and *Ulothrix* spp. and the crustose algae *Ralfsia* sp. were the only algae found more frequent on the steps. The reason for the more scattered growth of algae on the steps is clear. The number of *Littorina littorea* on that step is calculated dependent of the season to 10-250/m^2. This snail controls

the abundance and type of algae according to Lubchenco (1978), but is not sufficient to keep an area free of diatoms (Castenholz 1961). On the racks the micrograzers as amphipods and isopods are not avoided, and these grazers control filamentous algal species (Brawley and Adey 1981). According to Shacklock and Doyle (1983) the crustaceans graze selectively on the algal cover. No work has been done to state if the oil has effected the number or the grazing intensity of the micro-grazers in HO.

Ash free dry weight (A.D.W.)

A significant higher weight was found in B2 R in October 1982 compared to B1 R likely due to a heavier growth of *Enteromorpha* spp. in B2 R. During the winter months there was no significant difference. From March to October 1983 significant difference was found, but inconclusive. The long term chips had a significant better growth on B1 R on two occasions. B1 S showed only a couple of times significant difference from B2 S in A.D.W. Except for the winter months significant difference between racks and steps in HO was found as expected (Fig. 1) From these data it seems that grazing by littorinids is extensive in the basin except in the winter months. The distinction of A.D.W. between grazed areas and none grazed areas in the two basins is different. The significance of the A.D.W. difference between B1 R and B1 S is very stable, opposed to corresponding data between B2 R and B2 S, which is less easy to interprete. During some of the months (every second) the first half year of 1983 B2 R had significant higher growth than B2 S. Since July none of the short term chips in C2 have been different in biomass. Too low water level and high temperature may have caused different condition in HO and C2 during summer and fall 1983. The present data of A.D.W. are inconclusive as to whether the diesel oil improves or inhibits the growth on the chips.

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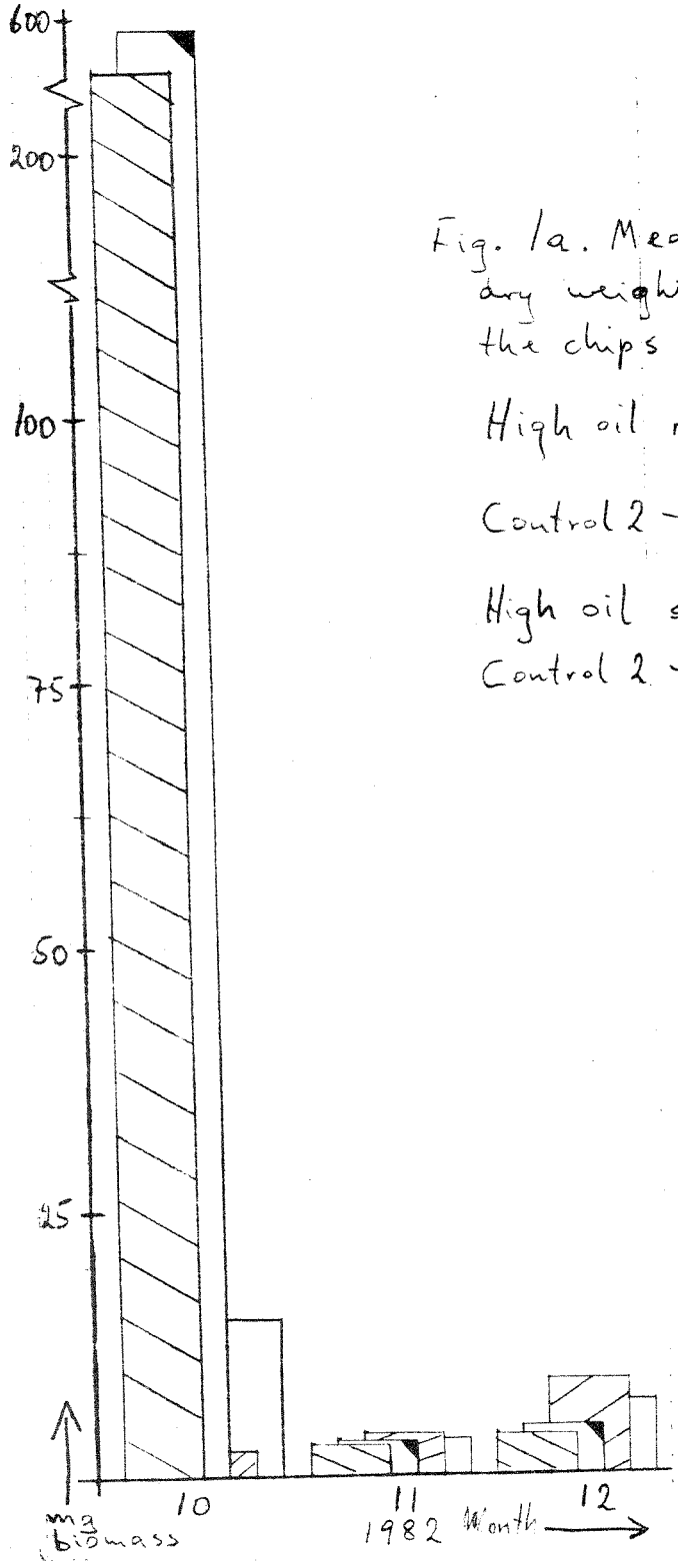

Fig. 1a. Mean ash free dry weight (A.D.W.) from the chips (50 cm² each) during 1982.

High oil rack (B1R)

Control 2 – " – (B2R)

High oil step (B1S)

Control 2 – " – (B2S)

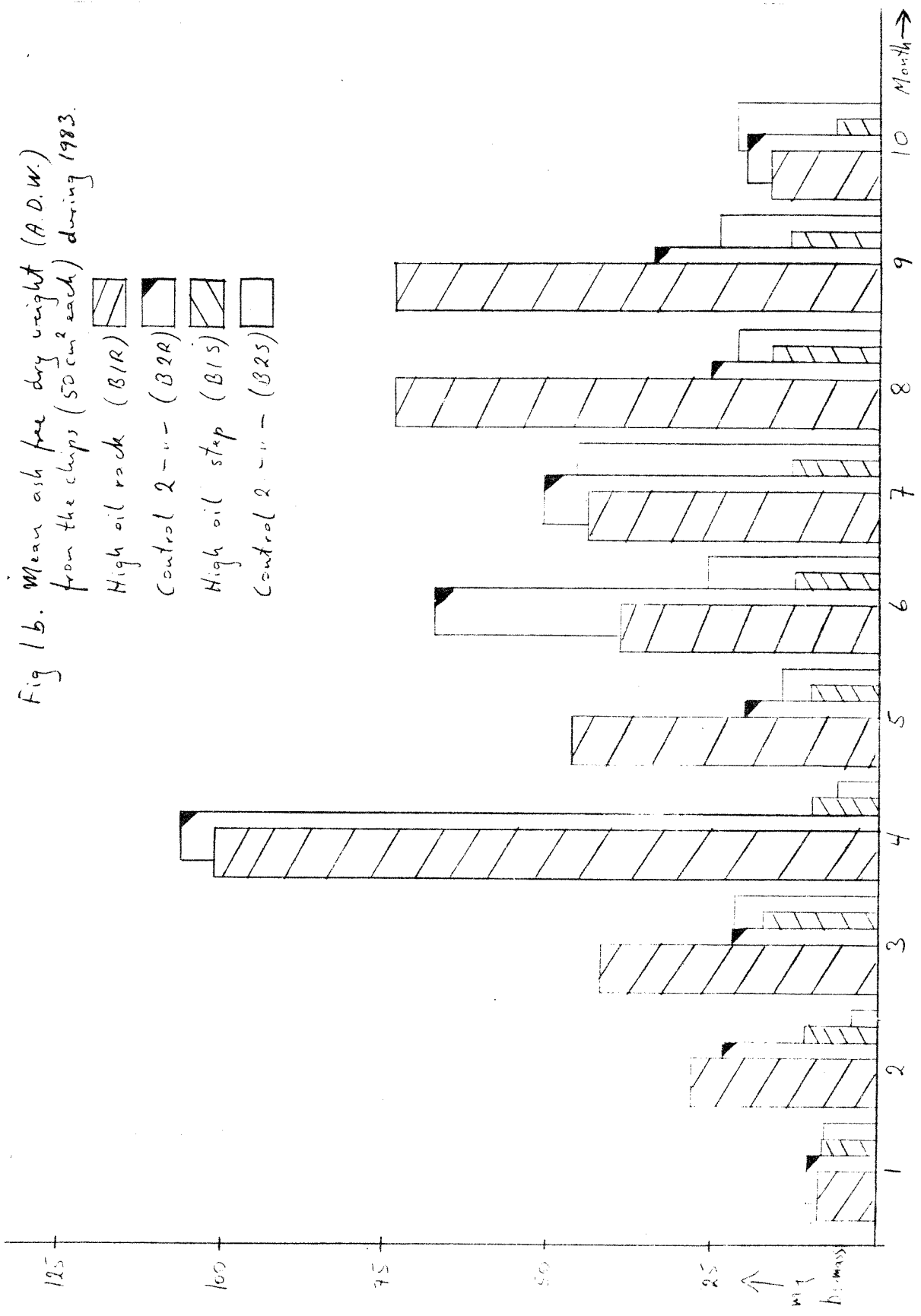

Fig 16. Mean ash free dry weight (A.D.W.) from the chips (50 cm² each) during 1983.

High oil rock (B1R)
Control 2 - " - (B2R)
High oil step (B1S)
Control 2 - " - (B2S)

TABLE 1. COVERING DEGREE (%) OF THE DOMINATING FOULING ORGANISM ON THE CHIPS

B1 R: chips in basin H0 on the rack
B2 R: chips in basin C2 on the rack
B1 S: chips in basin H0 on the step
B2 S: chips in basin C2 on the step

\- : absent or not dominating that month
* : Germlings
**: Southern chips 0, Northern chips 100

		Month: 10		11	12	1	2	3		4	5	
Duration of growth in months		1	4	1	1	1	1	1	3	1	1	5
Bacillariophyceae												
Cocconeis sp.	B1 R										-	
	B2 R										100	
	B1 S										-	
	B2 S										-	
Fragilaria sp.	B1 R							-	-	100	-	
	B2 R							90	100	100	100	
	B1 S							-	50	-	-	
	B2 S							90	50	90	-	
Gomphonema sp.	B1 R										80	
	B2 R										-	
	B1 S										-	
	B2 S										-	
Melosira nummuloides	B1 R					100	60	100	100	100		
	B2 R					-	-	-	-	-		
	B1 S					-	-	-	50	-		
	B2 S					-	-	-	100	-		
Navicula sp.	B1 R					-	60	100	100	100	-	100
	B2 R					90	30	-	-	100	100	-
	B1 S					-	100	-	50	-	-	-
	B2 S					-	-	90	50	-	-	-
Nitzschia closterium	B1 R									100	80	100
	B2 R									-	-	-
	B1 S									-	-	-
	B2 S									-	-	-
Unidentified pennate diatoms	B1 R	95	-				-	100	100	100	-	100
	B2 R	50	-				30	-	100	100	100	100
	B1 S	-	-				-	-	50	-	-	-
	B2 S	-	-				-	90	50	-	-	-
Schizonema colonies	B1 R	95	-						100	100		100
	B2 R	50	-						100	-		-
	B1 S	-	-						-	-		-
	B2 S	-	-						-	-		-
Florideophyceae												
Ceramium rubrum	B1 R	10	95									
	B2 R	15	25									
	B1 S	-	-									
	B2 S	-	-									
Phaeophyceae												
Ectocarpus siliculosus	B1 R											-
	B2 R											100
	B1 S											-
	B2 S											-
Ralfsia sp.	B1 R	-	-									
	B2 R	-	-									
	B1 S	-	100									
	B2 S	-	100									
Chlorophyceae												
Enteromorpha spp.	B1 R	20	10									
	B2 R	90	20									
	B1 S	100 *	-									
	B2 S	100 *	-									

TABLE 1. COVERING DEGREE (%) OF THE DOMINATING FOULING ORGANISM ON THE CHIPS

B1 R: chips in basin H0 on the rack
B2 R: chips in basin C2 on the rack
B1 S: chips in basin H0 on the step
B2 S: chips in basin C2 on the step

- : absent or not dominating that month
* : Germlings
**: Southern chips 0, Northern chips 100

		Month: 10		11	12	1	2		3	4	5	
Duration of growth in months		1	4	1	1	1	1	1	3	1	1	5
Ulothrix spp./*Urospora*	B1 R						-	100	-	-	-	
	B2 R						30	90	-	-	-	
	B1 S						-	-	75	100	60	
	B2 S						-	90	50	90	0-100	**
Ulva lactuca	B1 R	-	30									
	B2 R	-	40									
	B1 S	-	-									
	B2 S	-	-									
Sessile animals												
Balanus balanoides	B1 R									-	7	-
	B2 R									-	5	-
	B1 S									100	3	100
	B2 S									-	2	70
Balanus improvisus	B1 R	-	25									
	B2 R	-	10									
	B1 S	-	-									
	B2 S	-	-									

TABLE 2. DIFFERENCE OF THE MEAN ASH FREE DRY WEIGHT (A.D.W.), WITH CALCULATED SIGNIFICANCE ($_p$)

B1 R: Chips in basin H0 on the rack
B2 R: Chips in basin C2 on the rack
B1 S: Chips in basin H0 on the step
B2 S: Chips in basin C2 on the step

Month:	Growth duration (months)	B1 R - B2 R A.D.W. (mg/50 cm^2) mean	B1 S - B2 S A.D.W. (mg/50 cm^2) mean	B1 R - B1 S A.D.W. (mg/50 cm^2) mean	B2 R - B2 S A.D.W. (mg/50 cm^2) mean
10	1	- 137.9*	- 9.4	+ 425.2**	+ 550.8***
11	1	- 0.2	+ 0.2	- 0.8	- 0.4
12	1	- 0.8	+ 1.5	- 4.8	- 2.5
1	1	- 0.9	+ 0.3	+ 0.5	+ 1.7
2	1	+ 4.1	+ 6.7*	+ 17.6	+ 20.2**
3	1	+ 20.1*	- 4.5	+ 24.7*	+ 0.3
4	1	- 20.3	+ 3.5	+ 96.0*	+ 120.0***
5	1	+ 26.0**	- 4.1	+ 36.1**	+ 6.0
6	1	- 28.1 *	-13.5	+ 26.5***	+ 41.1*
7	1	- 6.9	-23.7*	+ 30.8***	+ 5.0
8	1	+ 47.9**	-5.1	- 57.0**	+ 4.0
9	1	+ 39.1*	-10.2	+ 60.0**	+ 10.7
10	1	- 3.5	-15.4	+ 10.2*	- 1.7
3	3	+165.0	+10.1	+ 443.7*	+ 288.8*
5	5	+128.1*	- 6.9	+ 220.7*	+ 85.7***
7	7	-31.5	-272.2*	+ 121.0**	- 119.7
9	9	+219.7*	-199.3	+ 259.6**	- 159.4

* = $p < 5$
** = $p < 1$
*** = $p < 0.1$

TABLE 3. MEAN ASH FREE DRY WEIGHT (A.D.W.) CONVERTED TO G/M^2

B1 R: Chips in basin H0 on the rack
B2 R: Chips in basin C2 on the rack
B1 S: Chips in basin H0 on the step
B2 S: Chips in basin C2 on the step

Month:	Growth duration months:	B1 R A.D.W.g/m^2 mean	No.	B2 R A.D.W.g/m^2 mean	No.	B1 S A.D.W.g/m^2 mean	No.	B2 S A.D.W.g/m^2 mean	No.
10	1	85.56	2	113.14	2	0.52	2	2.98	2
11	1	0.64	3	0.68	3	0.80	3	0.76	3
12	1	0.76	3	0.92	3	1.72	3	1.42	3
1	1	1.72	3	1.90	3	1.62	3	1.56	3
2	1	5.60	3	4.78	3	2.08	3	0.74	3
3	1	8.40	3	4.42	3	3.46	2	4.36	3
4	1	21.18	3	25.82	3	1.98	3	1.28	2
5	1	9.30	3	4.10	3	2.08	3	2.90	3
6	1	7.86	3	13.48	3	2.56	3	5.26	3
7	1	8.80	3	10.18	3	2.64	3	9.18	3
8	1	14.68	3	5.10	3	3.28	3	4.30	3
9	1	14.64	3	6.82	3	2.64	2	4.68	3
10	1	3.28	3	3.98	3	1.24	3	4.32	3
3	3	100.54	2	67.54	2	11.80	2	9.78	2
5	5	50.70	2	25.08	2	6.56	2	7.94	2
7	7	31.52	2	37.82	2	7.32	2	61.75	2
9	9	73.00	2	29.06	2	21.08	2	60.93	2

TABLE 4. PREDOMINATING SPECIES AND THEIR SUCCESSION ON THE RACKS

* only in May ** only in October

Succession ↓

B1 R (oil)	B2 R (control)
Melosira nummuloides	*Enteromorpha* spp.**
Schizonema colonies	*Fragilaria* sp.
Nitzschia closterium	*Ectocarpus siliculosus**
Gomphonema sp.*	*Cocconeis* sp.*

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6. INDIVIDUAL ASPECTS - GROWTH OF BENTHIC ALGAE

T. Bokn (NIVA)

Introduction

The aim of this subproject is to check if petroleum hydrocarbons dozed as diesel oil has effects on overall growth of macroalgae, measured as linear growth in selected species.

Materials and methods

During June 1982 and July 1983 25 tips of *Ascophyllum nodosum* - knobbed wrack - were tagged in HO, LO, C2, C4(23) and in HO, LO, C2, respectively. Immediately after tagging length growth from the youngest bladder to the end of the tip was measured. Parallel to these measurements corresponding tips were cut and measured in situ and then brought to the laboratory for drying and weighing. 33 tips each time were taken from three localities in the Oslofjord in the beginning of June and August 1982. In that way it will be possible to compare length growth with increase in weight. Tips were measured during June, July, September, October, December 1982 and February 1983. The mortality was large, and about 50 per cent of the specimens has been lost in different ways. The loss was most heavy in C4, and this basin is taken out of this subproject. The tagging system was changed during March. The same tips remaining after February were measured during March and April 1983. New tips developed from the 1983 bladder were tagged and measured during July, September and November.

At the start of March 1983 25 individuals of *Laminaria digitata* and *L. saccharina* were tagged in each of HO, LO and C2. (C4 had too few individuals). Growth was measured during March, April, June and July, see fig. 1.

The ratio between fertile and sterile tips of *Fucus vesiculosus* plants

was estimated in May 1983.

Results

Length growth of *Ascophyllum nodosum* tips is presented as diagrams in figs. 2 and 3. Fig. 2 illustrates the length growth during the first year of measurements. Standard deviation (S.D.) is only given for May 1983, which is representative for the high S.D. through the whole year. No significant difference in growth was stated. In fig. 3 corresponding data for July - November 1983 is presented. Significant difference was found between the C2 and the two oiled basins (HO and LO) in September and November, table 1.

Table 1. Significant difference in length growth of *Ascophyllum nodosum* tips (paired t-test)

	June 1982 - May 1983	July 1983	September 1983	November 1983
C2/HO	none	none	$p < 0.2$	$p < 1$
C2/LO	none	none	$p < 1$	$p < 1$
LO/HO	none	none	none	none

In figs. 4 and 5 histograms of *Laminaria digitata* and *L. saccharina* with S.D. are given. Between C2 and HO there was found a small significant difference in growth of *L. digitata* during March 1983, while a corresponding difference was found between C2 and LO for *L. saccharina* during April - June 1983.

The average proportion between fertile and sterile tips of *Fucus vesiculosus* individuals in the four basins is not found to be significant different. But in HO and LO several receptacles were deformed, and the maturity the receptacles of HO-plants was not so high as that of the plants in the other basins.

Discussion

The difference between measurements of *Ascophyllum* tips the first year of investigation and the corresponding data from the last half year is clear. During the first year no significant difference between the four basins was found (high S.D.). In September and November 1983 such a difference was stated. A possible explanation could be that all the tips in all the basins started their length growth without diesel oil, and their tissue had the opportunity to resist the diesel oil better than the next year tips, which started to grow in oil contaminated water.

Both species of laminarians have shown a typical growth curve during spring and early summer in all three basins. Only a slight significant difference between the control and diesel oil exposed basins has been stated. A considerable individual variability within each basin weakens the significance of this difference, figs. 4 and 5.

Results form May 1983 give no reason to state that diesel oil has had any effect on the development of the number of receptacles in *Fucus vesiculosus*. But the quality of the eggs and sperms is not stated. The deformed recepteacles and the low maturity in the oiled basins can be a sign of bad quality. An investigation of the quality of the receptacles of *F. vesiculosus* ought to be carried out in its season of fertility (May-June).

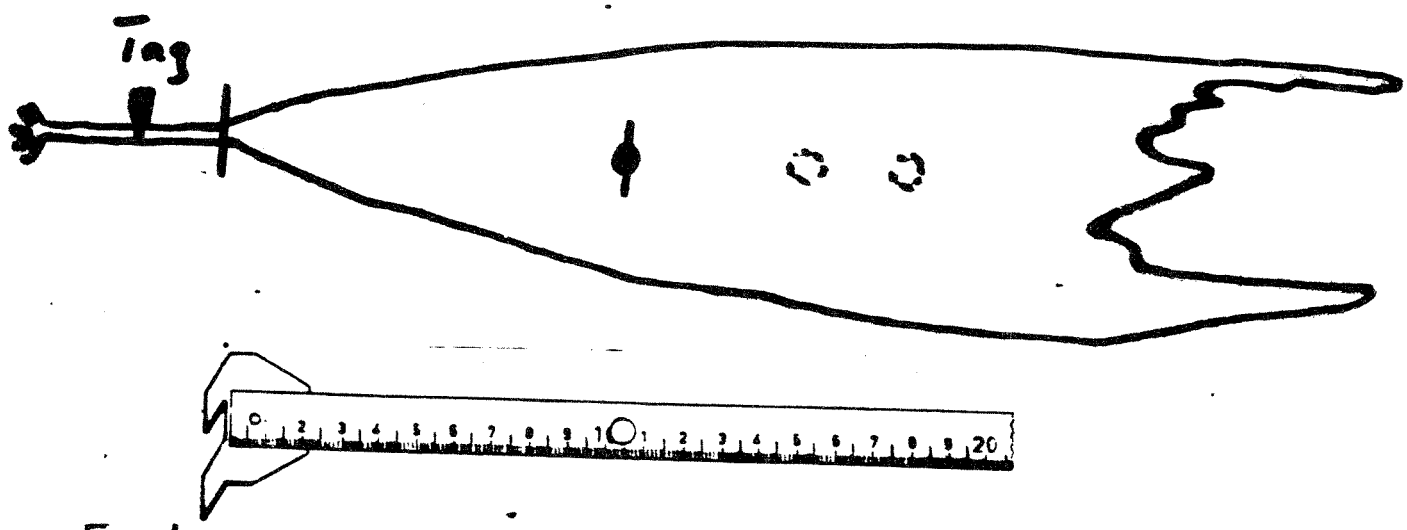
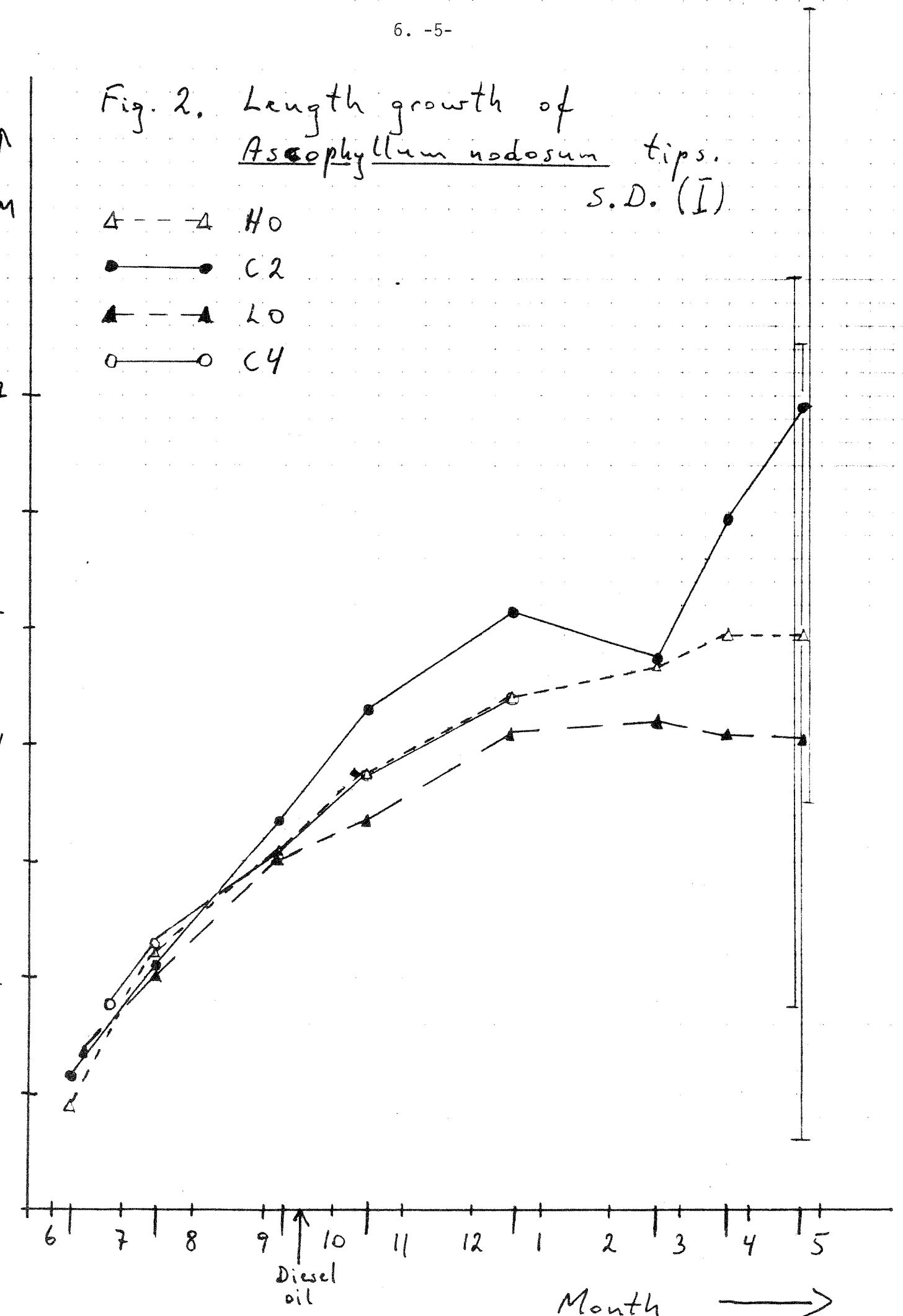


Fig. 1. Apparatus for growth measurements of the lowermost portion of the lamina. (Sundene 1964).

Fig. 2. Length growth of *Ascophyllum nodosum* tips. S.D. ($\bar{I}$)



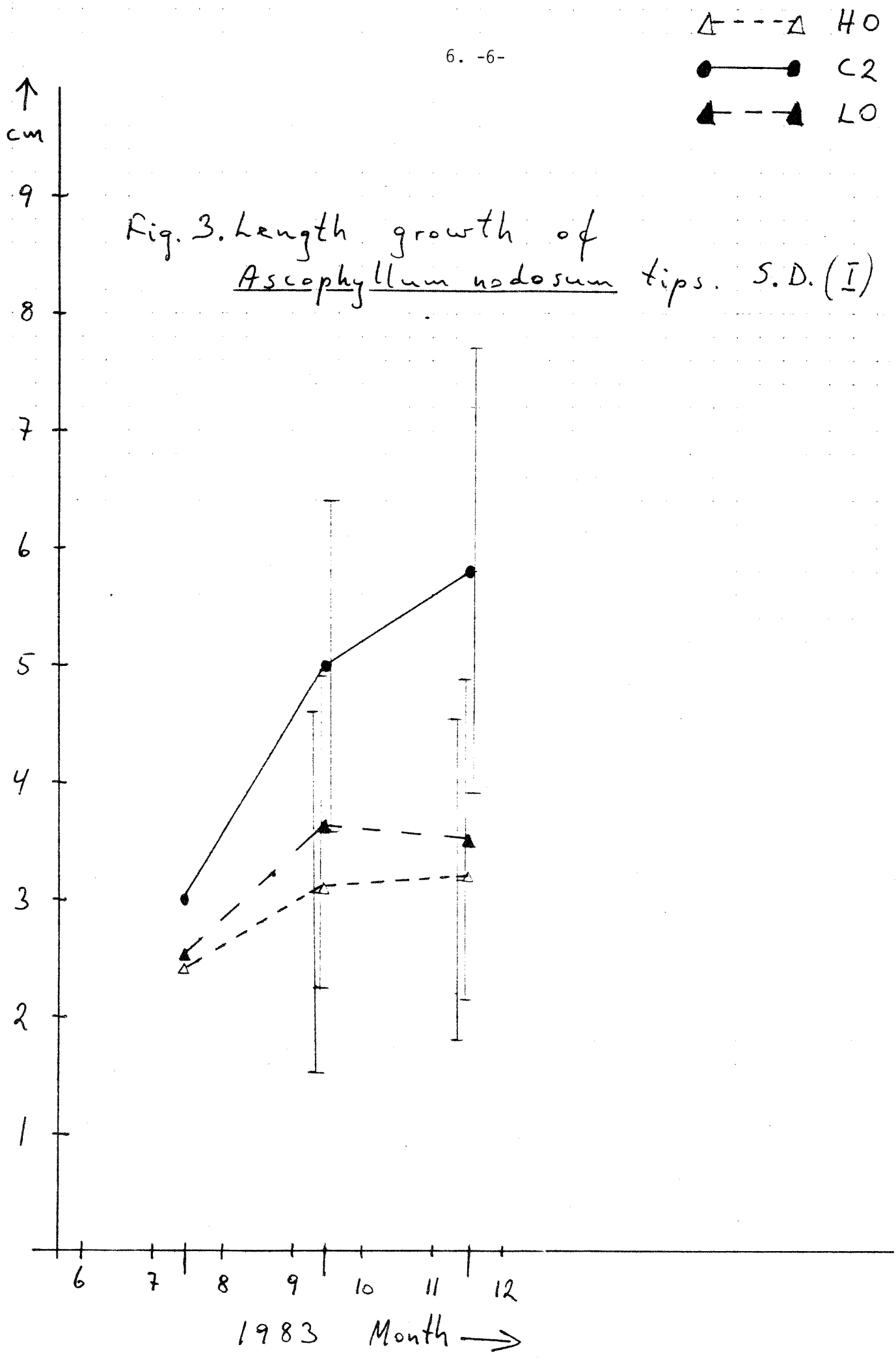
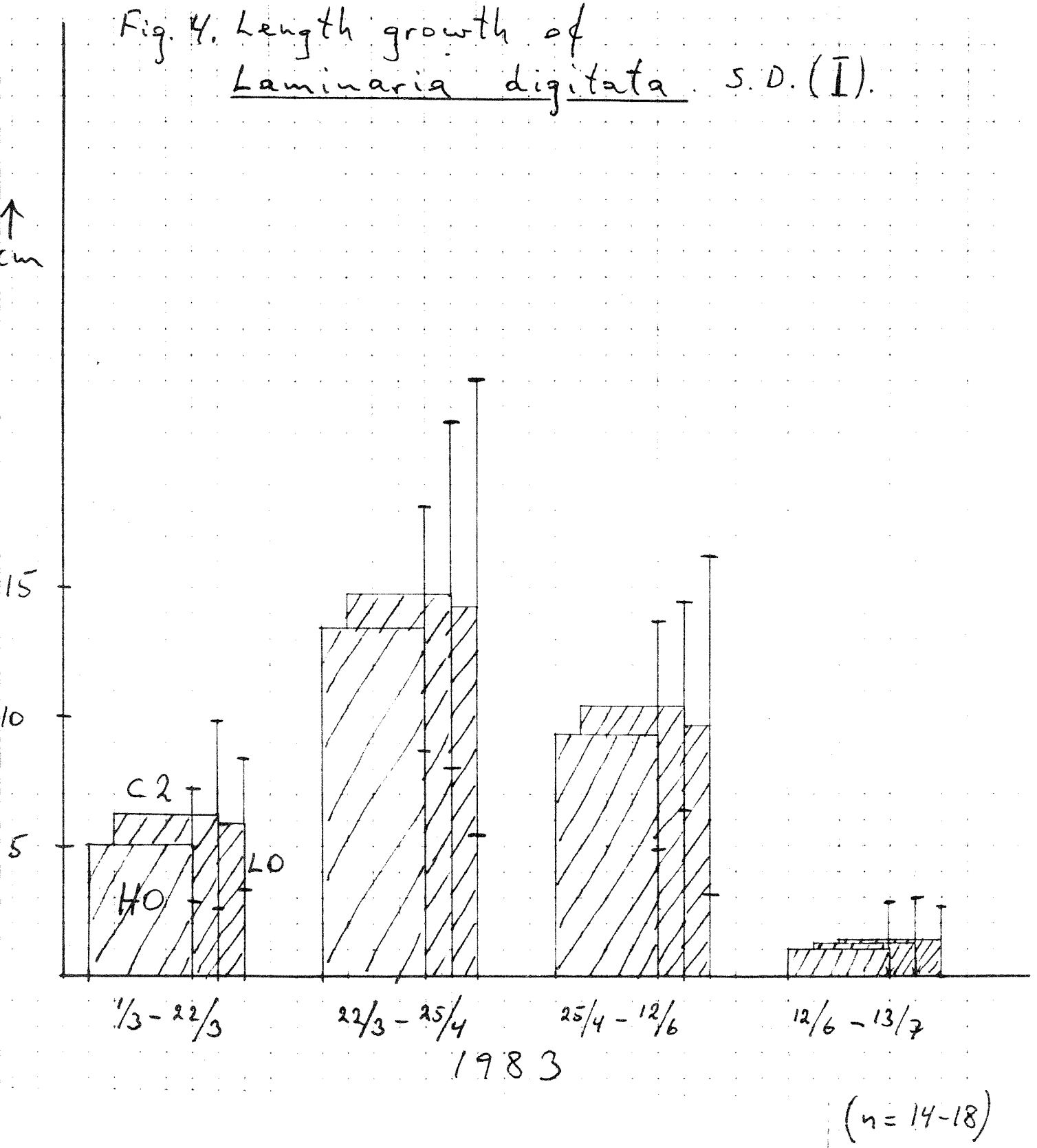


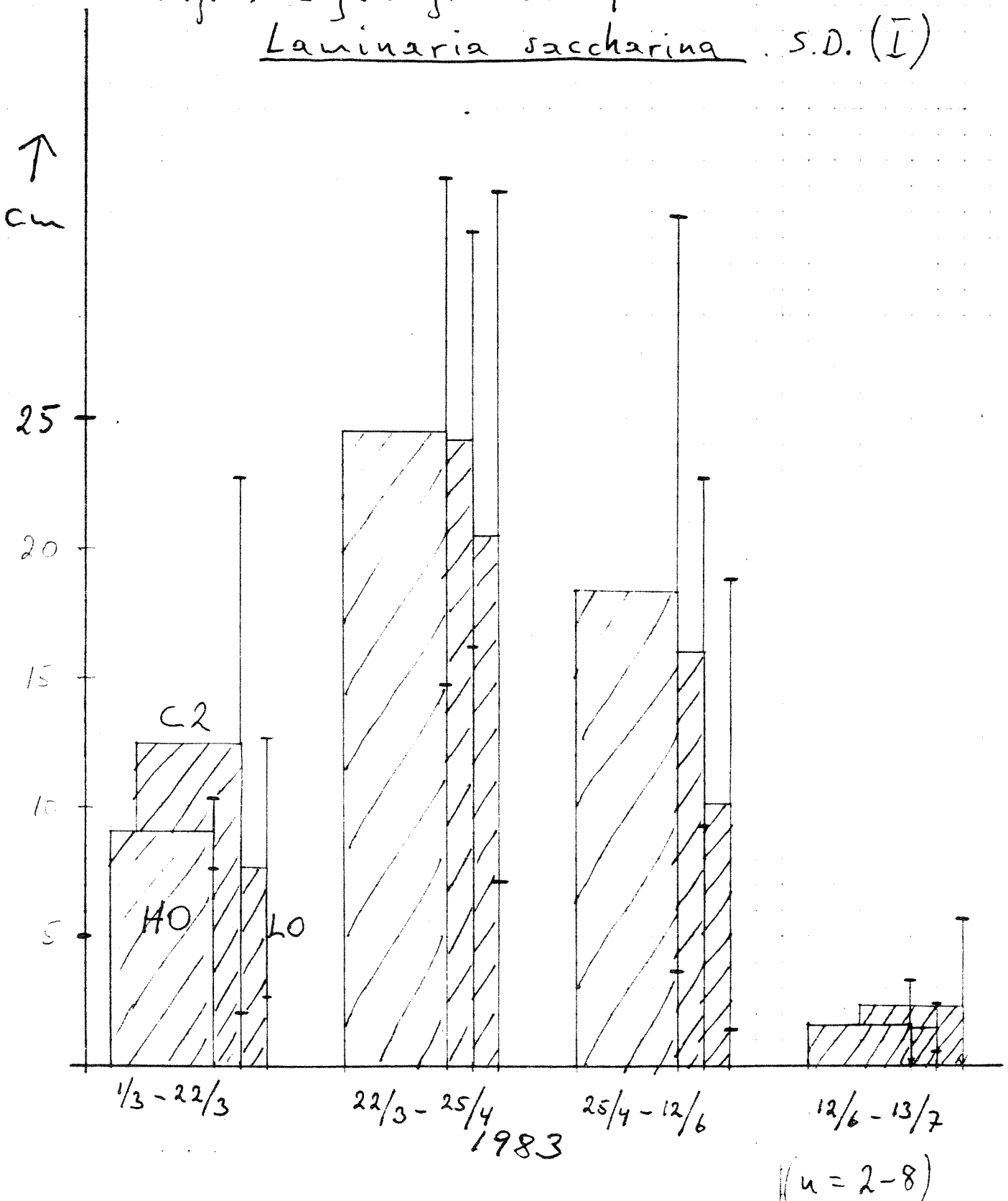
Fig. 3. Length growth of *Ascophyllum nodosum* tips. S.D. ($\bar{I}$)

Fig. 4. Length growth of *Laminaria digitata*. S.D. (Ī).



(n = 14-18)

Fig. 5. Length growth of
Laminaria saccharina . S.D. ($\bar{I}$)



(n = 2-8)

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7. COMMUNITY METABOLISM

A. Pedersen

Purpose of the work:

The project is a study concentrating on the variation and changes in metabolism i.e. production and respiration of natural as well as on contaminated communities. The aim is to determine if oil will effect the production and/or respiration of newly settled flora and fauna.

The work presented here is based on detecting differences between one contaminated basin and one control. Further testing will be done to determine wether the differences can be directly related to oil contamination.

Methods:

Sampling procedures have been performed according to the description in progress report no. 1, with modification described in progress report no. 2.

Sampling schedule for analysis of chlorophyll a (chl.a)/phaeopigments, C/N/P-ratios and production as a function of light and biomass have been performed according to Table 1.

Table 1. Sampling schedule.
ST = SHORT TERM EXPERIMENT. LT = LONG TERM EXPERIMENT.

Analyses / DATE	C/N		Metabolism		P		Chl a/phaeopigments	
	ST	LT	ST	LT	ST	LT	ST	LT
FEB	x				x		x	
MAR	x	x	x	x	x	x	x	x
APR	x		x		x		x	
MAY	x	x	x	x	x	x	x	x
JUN	x		x*		x		x	
JUL	x	x	x	x	x	x	x	x
AUG	x		x		x		x	
SEP	x	x	x	x	x	x	x	x
OKT	x		x		x		x	
NOV	x	x	x	x	x	x	x	x
DEC	x		x		x		x	

* Because of unforseen complications only the metabolism on the chip from the rack was measured.

The results from Carbon/Nitrogen/Phosphorous-analysis and light measurements are not yet available.

The chl.a measurements were analysed by spectrofluorometer and the absorption spectra were also identified for each chl.a. sample. This analysis was used to detect whether or not the chl.a. or other pigments whould give some unusual spectra or even be destroyed. Both results are impossible to determine by using traditional methods.

Due to late delivery of the respirometer (Progress Report no. 1), it was necessary to build a simple oxygen chamber for the metabolism-measurement. All measurements during the period March to December 83 have been performed using this O_2-jar in the laboratory. The jar has cooling facilities and different grey filters were put on the top of the O_2-jar giving different irradiances. The irradiance in $\mu E/m^2/sec.$ which the chips were exposed to (under 2 cm water column and the plexiglas top) were 279.3 - 145.6 - 104.6 - 73.5 - 48.1 - 24 - 11.7 and total darkness.

The oxygen increase or decrease was registrated on a x-y-recorder.

Calculations

All production measurements were based on changes in O_2-content in ppm (mg/dm^3) during one hour (h). The production values were corrected for the volume in the O_2-jar (x 1.91 litre) to give mg O_2/h. The O_2-change in one litre was then correlated to the area of the chip (100 cm^2). Change in one litre above the chip was then to be equivalent to O_2/100 cm^2/h. The next step was to correlate it to production per unit of biomass i.e. chl.a. Mg $O_2/m^2/h$ was divided by chl.a. content in mg/m^2 of the corresponding "production-chip" (chip used for production measurements) giving mg O_2/mg chl.a./h.

These values for each irradiance were used to construct a production v.s. light curve (P_{max}-curve).

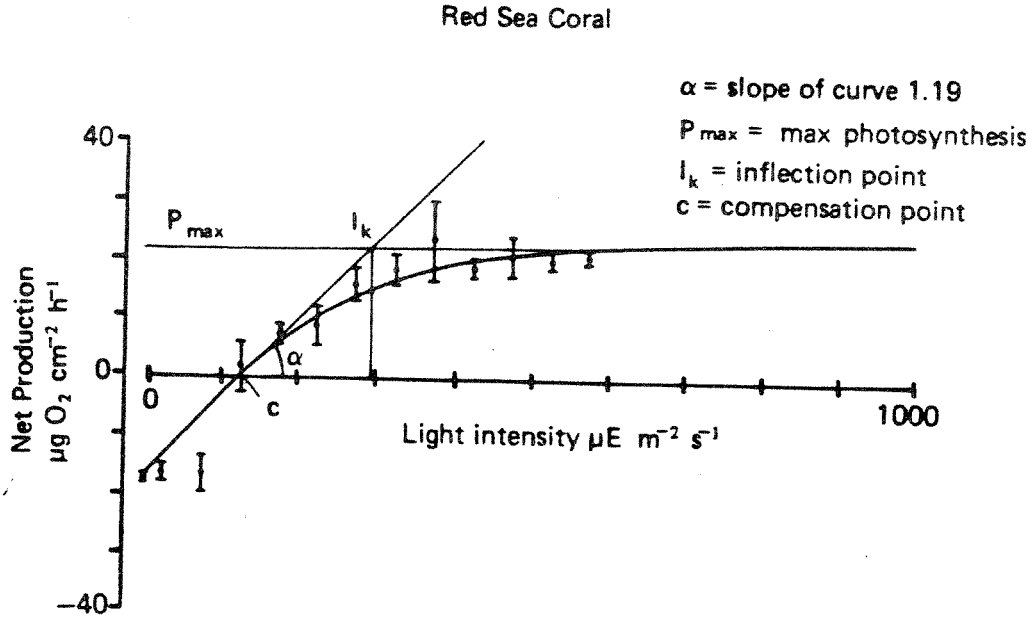

Fig. 1. Example on a production v.s. light-curve from Red Sea Coral.

The photochemical reaction at low light intensities are lineary correlated according to the equation

$$p^B = \alpha I + b$$

were p^B is the production per biomass (B) unit, α the slope, I the irradians and b the intercept or "resipiration" (Tables 2, 4, 6).

The production was also correlated to production per unit area as mg $O_2/m^2/h$ for each light intensity. The production in mg O_2/mg chl.a/h from one type of chip was multiplied with the mean chl.a. content (based on 3 or 2 replicates) for the corresponding type of chip. The production in mg O_2/mg chl.a./h will give the efficiency of the chl.a. unit. The production in mg $O_2/m^2/h$, however, will give the magnitude of production in the basins per m^2.

Unfortunately continuous light measurements have not yet been analysed and correlated to the production measurements. To obtain an idea of the energy input by the chips, however, one can assume that the irradiance probably will exceed 300 $\mu E/m^2/sec.$ (approximately up to p^B max) at least 6 hours a day as a mean for the year. These rough estimates for the production in g carbon (C)$/m^2$/year are as described in Tables 3, 5 and 7.

The equatation giving a theoretical conversion factor of 0.375 from gO_2 to gC is:

$$nCO_2 + 2\ nH_2O \text{ -------> } n(CH_2O) + nO_2 + nH_2O$$

$$nC : nO_2 = 12 : 32 = 0.375$$

The conversion from gO_2 to gC does not, however, strictly follow the cemical equation for the photo reaction. A conversion factor of 0.29 is more correct (Sepers 1981) and the conversion is performed according to the following equation:

$$gC/m^2/year = \frac{0.29 \times 6 \times 365 \times mg\ O_2/m^2/h}{1000}$$

All t-tests for significance are based on a one-tailed test. In further work nonparametric tests which may be applicable for this kind of data, will be utilized.

Results and discussion

1. Chlorophyll a (Chl.a.)

SHORT TERM EXPERIMENT (ST) (chips were exposed one month).

The first columns in Tables 2 and 3 show the chl.a. values for the chips at the steps from which the O_2-production measurements and the mean chl.a. values were taken. The mean chl.a. values are based on three replicates. There seems to be a higher chl.a. content in mg chl.a./m^2 in the control basin C2 than in the contaminated basin HO. The mean difference was significant ($P < 0.1$). Between the two basins there is an increase of 539 % in chl.a. content from HO to C2. There also seems to be a higher chl.a. content in the summer than in spring and autumn in both basins.

The chl.a. content showed a considerable variation during the experimental period. The smallest value was 0.19 mg chl.a./m^2 and the highest was 292 mg chl.a./m^2. The mean values in C2 and HO were 10.5 and 2.8 mg chl.a./m^2 respectively. Other authors describe values from 10 to 100 mg/m^2 as typical for a variety of surfaces. However, values up to 2000 mg/m^2 can occur under high nutrient supply and stable substrates. Our ST chips are just in the initial phase of a succession and therefore difficult to compare directly with these results.

The corresponding chips on the rack without grazing (Tables 4 and 5) do not show the same chl.a. content pattern as on the grazed chips. Here the opposite trends occur giving a higher chl.a. content in the contaminated basin than in the control. The mean difference was 30 % in favour of HO and significant at $p < 0.1$. The mean chl.a. values in C2 were 13.2 mg/m^2 and in HO 17.2 mg/m^2.

LONG TERM EXPERIMENT (LT) (chips have been exposed between 6 and 13 months).

Looking at the chl.a. content at the long term chips it seems that the mean values do not show any significant difference in chl.a. content between C2 and HO both at the rack and at the steps. The mean values for chl.a. at the steps were 150 mg/m^2 in C2 and 39.6 mg/m^2 in HO. The percentages difference is 280 % in favour of C2, but due to large variation

and only 4 samples thus far the difference is not significant with this parametric test.

Between the LT-chips at the rack there was a percentage increase of 57 % between C2 and HO. The values in C2 and HO were 12.43 and 19.46 mg chl.a./m^2 respectively.

The increase in chl.a. content at the steps in HO is mainly caused by a heavy growth of the bluegreen algae *Rivularia*. In C2 the chips were covered earlier with a continiuously increasing growth of *Ralfsia* cf. *verrucosa*.

At the rack the LT-chips show a small amount of chl.a. during the summer period in July. At this time the water level was too low especially in C2 and the wave generators were out of order for some time. The chip, therefore, may have been drying up and resulting in a poor algae community.

Compared to other publication the chl.a. values seem to be of the same magnitude, based on comparable substrates. The adsorbtion spectra have not yet been analysed, however, one can see that the amount of accessoric pigments are very small. As light is not a limiting factor there is no need for these pigments in the harvesting of light.

2. Production v.s. light (P max-curves)

No curves will be made for this report, but all the variables except for the slope of the curve, are presented in Tables 2, 4 and 6.

ST STEP (Table 2)

The I_k for the ST steps shows a significant increase (35 %) from C2 to HO ($p < 0.1$). This indicates that the production in mg O_2/mg chl.a./h in HO as mean of the period, shows a more slowly response to light than the community in C2. One should be aware of that the chl.a. spectra at the steps in HO in April contained mostly damaged pigments, giving a chl.a. concentration which was too low and thereby a "efficiency" which may be too high.

There is also a possibility that the net maximum production ($p \frac{NET}{MAX}$) sometimes has been underestimated because of too weak irradiance in the experiment. At the steps in the two basins the mean $p \frac{NET}{MAX}$ for the community at the ST chips are about equal (no significant difference). In C_2 the $p \frac{NET}{MAX}$ is 25 mg O_2/mg chl.a./h, whereas in HO the mean $p \frac{NET}{MAX}$ is 22 mg O_2/mg chl.a./h.

The intercept or "respiration" of the communities in Table 2 expressed as mg O_2/mg chl.a./h are different in the two basins ($p < 0.1$). The intercept in C2 is just half of that in HO, 7 and 14 mg O/mg chl.a./h.

The previous described factors give us a maximum gross community production ($p \frac{GROSS}{MAX}$) at about 32 mg O_2/mg chl.a./h in C2 and an increase of 16 % to 37 mg O_2/chl.a./h in HO. The difference was significant at $p < 0.1$.

As the "resipiration" in HO are much higher than in C2, the $p \frac{NET}{MAX}$ in C2 becomes higher, the $p \frac{GROSS}{MAX}$ which compensates for the loss of O_2 due to respiration by the whole community, concequently gives a somewhat higher production at the steps in the comtaminated HO than in C2.

The mean I_c = C (compensation point) is also higher in HO (38 mg O_2/mg chl.a./h) than in C2 (15 mg O_2/chl.a./h (see Fig. 1).

It is difficult to explain for the difference since "resipiration" in the two basins, may be caused by an increased biomass of bacteria living off the oil in basin HO. This in not yet to be checked.

ST - RACK (Table 4)

$p \frac{GROSS}{MAX}$, $p \frac{NET}{MAX}$ and the respiration are significantly higher in C2 than in HO ($p < 0.025$, $p < 0.025$, $p < 0.1$). The mean I_k values during March to October -83 show no significant differences, i.e. 94 $\mu E/m^2$/sec. in C2 and 96 $\mu E/m^2$/sec. in HO. It is sited in Parson and Takahasi that 4 littoral diatoms have a compensation irradiance I_c ranging from 3 to 31 $\mu E/m^2$/sec. and that they reached saturation (p MAX) in the range from 63 to 125 $\mu E/m^2$/sec. It is difficult to compare these values directly with the I_c values and saturation values obtained in this experiment because we are dealing with whol communities of bacteria, fauna and microflora.

The basic dark respiration by the algae obtained from many different species and growth conditions, however, is approximately 10 % of maximun gross photosyntesis (sited in Parson and Takahasi 1979). If one calculates with 20 % respiration from the algae the mean I_c at ST-step in HO 11 $\mu E/m^2/sec$. and at the C2-ST-step will be 7 $\mu E/m^2/sec$. At the rack the corresponding values are 8 and 9 $\mu E/m^2/sec$.

ST STEP & RACK

Concerning the long term experiment it seems that the HO basin has a higher $p\frac{GROSS}{MAX}$, a higher $p\frac{NET}{MAX}$ - a higher respiration and higher I_k values than basin C2. This corresponds to both "grazed" and "nongrazed" communities.

At the step the percentage differences between C2 and HO for $p\frac{NET}{MAX}$, Tespiration, I_k and $p\frac{GROSS}{MAX}$, are 18 %, 79 %, 13 % and 35 % respectively. At the rack the corresponding percentage differences were 24 %, 183 %, 6 % and 89 %.

3. Production v.s. biomass

ST STEP

The production described has been correlated to mg O_2/mg chl.a./h. v.s. light. The production per m^2 is of interest when calculating the energy budget of the basins. Maximum net production, respiration and maximum gross production for the short term experiment of the step are listed in Table 3.

Net Max-, Gross Max production and respiration are significantly higher in C2 than in HO. The net max production in C2 during March to October -83 was 216 mg $O_2/m^2/h$ and 50 mg $O_2/m^2/h$ in HO. The corresponding values for the respiration were 66 and 30 mg $O_2/m^2/h$ and for max gross production are 281 and 80 mg $O_2/m^2/h$.

As mentioned before light measurements are not available. The estimated gross production in the two basins with the assumptions previously described are presented in Table 3. The production 51 mg $C/m^2/year$ in HO and 179 in C2.

ST RACK

The same trends as described for the ST-steps are found in the data from the rack i.e. max gross- and net production and respiration are 38 to 39 % higher in the controll basin than in the contaminated one. The estimates for the gross production during the year in the two basins are 100 mg C/m^2/year in HO and 138 mg C/m^2/year in C2. These values in contrast to those at the steps, were found to be nonsignificant.

ST STEP

The four samplings of the LT chips showed no significant differences between the two basins. Respiration was the same in the two basins. A somewhat higher (30 %) maximum net production and consequently also a higher max gross production occurred at the steps in C2 than in HO (as the respiration is about the same).

ST RACK

The max gross production at the LT chips at the rack was 62 % higher in HO than in C2, although not significant. The respiration, however, was significantly higher in HO ($p < 0.05$). The mean max gross production was 742 mg $O_2/m^2/h$ in HO and 459 mg $O_2/m^2/h$ in C2.

The estimates for gross production during the year are 337 mg C/m^2/year in C2 and 545 mg C/m^2/year in HO.

4. Respiration

The last table compares the respiration between the HO and the C2 during the period March to October 1983 expressed as percent of gross production. Normally the respiration of a mono culture of algae is about 10 % of p-max. The respiration of the communities in this experiment at the ST chips is 24 % at the steps in C2 and 38 % in HO. At the reck the respiration of the ST chips is the same in C2 and HO, 20 %. For the ST chips the respiration gives the same pattern as for the ST chips, giving a higher respira-

tion at the steps in HO (30 %) and less in C2 (24 %). The respiration at the racks does not differ from C2 til HO, giving a respiration at both of 19 %. The respiration at the racks is less than at the steps both for LT- and ST chips.

5. General remarks

Production for epibenthic algae has been estimated by other authors to be about 200 g C/m^2/year. For intertidal sandflats estimates from 0 to 325 g C/m^2/year have been published.

The preliminary production values for the "fouling" communities at the LT chips are somewhat high, at the rack in C2 and HO 337 to 545 mg C/m^2/year respectively. For the steps the production values seems to fall in the same range as described in others publications.

It is too early to detect any significant differences between the LT chips in the controll and the oil contaminated basin, i.e. both for the rack and the steps. Some trends are noted in the ST experiment. Additional analyse will be performed.

TABLE 2. Maximum production (Net and Gross) and "respirations" (Intercept) responses in mg O_2/mg chla/h for the chips at the steps in the short term experiment (S.T.) during Mar. to Oct. 1983. I_k-values for the P_{max}-curve are also listed. C2 = Control basin, HO = Contaminated basin.

DATE	Chla on the production-chip mg/m^2		Community P^{Net}_{Max} mg O_2/mg chla/h		Intercept "Respiration" mg O_2/mg chla/h		I_k (explan. see text) µE/m^2/sec.		Community P^{Gross}_{Max} ("resp"+ P^{Net}_{Max}) mg O_2/chla/h.	
	C2	HO	C2	HO	C2	HO	C2	HO	C2	HO
MAR	1.2	2.3	29.48	24.91	- 11.05	- 8.30	96	115	40.53	33.21
APR	1.4	0.5	38.98	27.85	- 15.87	-47.75	81	65	54.85	75.60
MAY	7.0	3.0	4.36	7.64	- 2.73	- 9.55	50	80	7.09	17.19
JUN	-	-	-	-	-	-	-	-	-	-
JUL	21.4	3.2	7.32	2.39	- 2.14	-12.53	62	168	9.46	14.92
AUG	7.6	5.9	14.52	13.21	- 5.51	- 4.51	77	94	20.03	17.72
SEP	32.6	8.3	5.04	11.51	- 1.52	- 4.37	66	89	6.56	15.88
OKT	2.5	1.3	74.11	67.58	- 9.17	-16.16	62	60	83.28	83.74
Mean values per month	10.52	2.78	24.83	22.16	6.85	14.3	71	96	31.68	36.89
Magnitude of difference	278%		12%		109%		35%		16%	
t-test	$p < 0.1$		n.s.		$p < 0.1$		$p < 0.1$		$p < 0.1$	

TABLE 3. Maximum production (Net and Gross) in mg $O_2/m^2/h$ and respiration of the community for the short term chips at the steps during Mar. to Oct. 1983. The yearly production in g C/m^2/year is preliminary, based on a saturation of light 6 hours a day. C2 = Control basin, HO = Contaminated basin.

DATE:	MEAN Chla mg/m^2		MAX Net. Production mg $O_2/m^2/h$		MAX Community Respiration mg $O_2/m^2/h$		MAX Gross. Production mg $O_2/m^2/h$		PRELIMINARY ESTIMATES for Gross Production g C/m^2/year	
	C2	HO	C2	HO	C2	HO	C2	HO	C2	HO
MAR	4.40	1.60	129.71	40.60	48.62	13.53	178.33	54.13	113	34
APR	2.60	0.30	101.35	8.36	41.26	14.33	142.61	22.68	91	14
MAY	12.90	3.70	56.24	28.27	35.21	35.34	91.46	63.60	58	40
JUN	-	-	-	-	-	-	-	-	-	-
JUL	111.70	5.00	817.64	11.95	239.04	62.65	1056.68	74.60	671	47
AUG	6.10	4.40	88.57	58.12	33.61	19.84	122.18	77.97	78	50
SEP	30.20	10.40	152.21	119.70	45.90	45.45	198.11	165.15	126	105
OKT	2.20	1.20	163.04	81.10	20.17	19.39	183.22	100.49	116	64
Mean values per month	24.3	3.8	215.53	49.72	66.25	30.07	281.30	79.80	179	51
Magnitude of diff.	539%		333%		120%		253%		251%	
t-test	n.s		$p < 0.1$		$p < 0.1$		$p < 0.1$		$p < 0.1$	

TABLE 4. The maximum production (Net and Gross) and "respiration" (Intercept) respons in mg O_2/mg chla/h for the chips at the rack in the short term experiment (S.T.) during Mar. to Oct. 1983. I_K-values for the P_{max}-curve are also listed.
C2 = Control basin HO = Contaminated basin.

DATE	Chla on the production-chip mg/m^2		Community P^{Net}_{Max} mg O_2/mg chla/h		Intercept "Respiration" mg O_2/mg chla/h		I_k (explan. see text) µE/m^2/sec.		Community P^{Gross}_{Max} ("resp"+ P^{Net}_{Max}) mg O_2/chla/h.	
	C2	HO	C2	HO	C2	HO	C2	HO	C2	HO
MAR	15.7	9.3	10.22	5.37	- 2.83	- 1.65	97	76	13.05	7.02
APR	38.0	76.9	7.84	4.78	- 1.56	- 0.78	100	82	9.40	5.56
MAY	11.2	28.0	4.26	2.05	- 2.38	- 0.96	50	93	6.64	3.01
JUN	15.3	16.7	11.35	0.92	- 4.54	- 2.06	84	92	15.89	2.98
JUL	1.8	8.4	30.77	2.73	-25.47	- 6.14	97	85	56.24	8.86
AUG	2.5	4.7	17.93	21.94	-15.59	- 5.28	116	104	33.52	27.22
SEP	3.5	14.3	25.10	14.29	- 7.64	- 3.47	97	112	32.74	17.76
OKT	4.4	2.6	92.90	69.79	- 9.12	-13.22	108	120	102.02	83.01
Mean values per month	11.6	20.1	25.05	15.23	- 8.64	- 4.20	94	96	33.68	19.43
Magnitude of difference	73%		64%		106%		2%		73%	
t-test	p < 0.1		p < 0.025		p < 0.1		n.s.		p < 0.025	

TABLE 5. Maximum production (Net and Gross) in mg $O_2/m^2/h$ and respiration of the community for the short term chips at the rack during Mar. to Oct. 1983. The yearly production is preliminary, based on a saturation of light 6 hours a day.
C2 = Control basin, HO = Contaminated basin.

DATE:	MEAN Chla mg/m^2		MAX Net. Production mg $O_2/m^2/h$		MAX Community Respiration mg $O_2/m^2/h$		MAX Gross. Production mg $O_2/m^2/h$		PRELIMINARY ESTIMATES for Gross Production g C/m^2/year	
	C2	HO	C2	HO	C2	HO	C2	HO	C2	HO
MAR	11.8	9.3	120.6	49.9	- 33.4	- 15.3	154.0	65.3	98	41
APR	58.5	69.6	458.6	332.7	- 91.3	- 54.3	549.9	387.0	349	245
MAY	7.5	20.2	32.0	41.4	- 17.9	- 19.4	49.8	58.4	32	37
JUN	15.3	9.0	137.7	8.3	- 69.5	- 18.5	243.1	26.8	154	17
JUL	1.2	4.0	36.9	10.9	- 30.6	- 24.6	67.5	35.4	43	22
AUG	2.5	4.4	44.8	96.5	- 39.0	- 23.2	83.8	119.8	53	76
SEP	4.4	18.6	110.4	265.8	- 33.6	- 64.5	144.1	330.3	92	210
OKT	4.4	2.8	408.8	195.4	- 40.7	- 37.0	448.9	232.4	285	148
Mean values per month	13.2	17.2	173.23	125.11	- 44.5	- 32.1	217.64	156.92	138	100
Magnitude of diff.	30%		38%		39%		39%		38%	
t-test	p < 0.1		n.s		n.s		n.s		n.s	

TABLE 6. The maximum production (Net and Gross) and "respiration" (Intercept) respons in mg O_2/mg chla/h for the chips both at steps and racks in the long term experiment in the control (C2) and contaminated basin (HO). I_k-values for the P_{max} -curve are also listed.

	Mean Chla on the production-chip mg/m²		Community P^{Net}_{Max} mg O_2/mg chla/h		Intercept "Respiration" mg O_2/mg chla/h		I_k (explan. se text) µE/m²/sec.		Community P^{Gross}_{Max} ("resp + P^{Net}_{Max}) mg O_2/chla/h	
	C2	HO	C2	HO	C2	HO	C2	HO	C2	HO
STEP:										
MAR	10.5	9.00	22.46	3.42	- 7.85	- 1.02	93	131	30.31	4.44
MAY	10.3	5.9	4.78	4.89	- 3.49	- 6.11	73	48	8.27	11.00
JUL	357.3	4.6	0.70	22.84	- 0.25	-12.46	63	81	0.95	35.30
SEP	219.2	57.9	1.27	3.20	- 0.22	- 1.35	83	91	1.49	4.55
Mean values per month:	150.5	39.6	7.30	8.59	- 2.95	- 5.24	78	88	10.26	13.82
Magnitude of difference:	280%		18%		78.6%		13%		35%	
t-test:	n.s		n.s		n.s		n.s		n.s	
RACK:										
MAR	232.5	266.8	3.74	4.21	- 0.62	- 0.79	113	107	4.36	5.00
MAY	186.6	199.6	1.54	0.61	- 0.44	- 0.67	84	82	1.98	1.28
JUL	4.6	1.5	13.29	19.10	-13.70	-42.02	92	82	26.99	61.12
SEP	73.3	310.4	3.73	3.68	- 0.76	- 0.46	94	137	4.49	4.15
Mean values per month:	12.43	19.46	5.58	6.90	- 3.88	-10.99	96	102	9.46	17.89
Magnitude of difference:	57%		24%		183%		6%		89%	
t-test:	n.s.		n.s.		n.s.		n.s.		n.s.	

TABLE 7. Maximum production (Net and Gross) in mg $O_2/m^2/h$ and respiration of the community for the long term chips at both the steps and the rack during Mar. to Oct. 1983. The yearly production in g C/m^2 are preliminary, based on a saturation of light 6 hours a day. C2 = Control basin, HO = Contaminated basin.

	MAX Net Production mg $O_2/m^2/h$		MAX Community Respiration mg $O_2/m^2/h$		MAX Gross Production mg $O_2/m^2/h$		Preliminary (se text) estimates for Gross Production in g C/m^2/year	
	C2	HO	C2	HO	C2	HO	C2	HO
STEP:								
MAR	235.83	307.8	- 82.43	- 91.8	318.26	399.6	202	254
MAY	49.23	28.85	- 35.95	- 36.05	85.18	64.9	54	41
JUL	250.11	105.06	- 89.33	- 57.32	339.46	162.38	216	103
SEP	278.38	185.28	- 48.22	- 78.17	326.61	263.45	207	167
Mean values per month:	203.39	156.75	64.14	65.83	267.38	222.58	196	141
Magnitude of difference:	20%		3%		20%		39%	
t-test:	n.s		n.s		n.s		n.s	
RACK:								
MAR	869.55	1123.23	- 144.15	- 210.77	1013.7	1334.0	644	847
MAY	287.36	121.76	- 82.20	- 133.73	369.47	255.49	235	62
JUL	63.94	28.65	- 63.02	- 63.03	124.15	91.68	79	58
SEP	273.41	1145.38	- 55.71	- 142.78	329.12	1288.16	209	818
Mean values per month:	373.57	604.76	- 86.25	- 137.58	459.11	742.33	337	544
Magnitude of difference:	62%		60%		62%		62%	
t-test:	n.s		$p < 0.05$		n.s		n.s	

TABLE 8. The mean respiration expressed as percent of mean Gross production during Mar. to Oct. 1983.

SHORT TERM				LONG TERM			
STEP		RACK		STEP		RACK	
C2	HO	C2	HO	C2	HO	C2	HO
24%	38%	20%	20%	24%	30%	19%	19%

MARINE RESEARCH STATION SOLBERGSTRAND

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8. POPULATION GENETICS OF MYTILUS EDULIS AND LITTORINA LITTOREA

Svein E. Fevolden and Susan P. Garner

Aims and purposes

To investigate at the population level potential effects from oil pollution for electrophoretic variants at selected gene-loci of Mytilus edulis and Littorina littorea. Electrophoretic analyses of these animals will provide data on the amount of genetic differences between oil-exposed and non oil-exposed populations. The electrophoretic data will be evaluated for the presence of any diagnostic differences that might be used to classify individuals according to whether they come from polluted or unpolluted water.

Work progress

Mytilus edulis: Approximately 100 animals sampled in fall 1982 from each of the four basins have been run on starch gel electrophoresis for a series of 17 enzymes. Also about 100 animals from the fjord immediately adjacent to Solbergstrand have been electrophoretically analyzed for the same enzymes. Out of 39 scorable loci 12 (31%) were polymorphic, but only five loci (phosphoglucoseisomerase, isocitratedehydrogenase, leucine-aminopeptidase-2 and -3, and phosphoglucomutase) were polymorphic to any significant extent. Average heterozygosity in these animals is about 5.5%. These 500 specimens provide the necessary background data that will be used to detect any change in genetic structure of A) animals that have now been exposed to oil for several months, and B) new recuits that have successfully settled in the oil-exposed basins.

About 125 animals that survived the longest in the basin with high oil concentration (HO) were sampled in June 1983, and have now been analyzed for genetic variation at the same loci. Preliminary results indicated a disappearance of rare alleles and a heterozygous overdominance in these survivors. More animals have now been analyzed since these first results were obtained, but not until this additional data has been processed can we say if this difference is statistically significant.

New recruits from the basin with low oil concentration (LO) and from the control basins were sampled in late October 1983 and will be electrophoretically analyzed this December. Also 40 oil-exposed Mytilus edulis that have been classified by Dr. J. Widdows as fast or slow feeders (with a high or low filtration rate respectively) will be electrophoretically analyzed in December.

Littorina littorea: At this time, out of 400 basin animals sampled before oil-exposure, about 300 have been run for a series of 24 enzymes, resulting in a frequency of polymorphic loci of about 28%. Also about 100 animals from the fjord outside Solbergstrand have been analyzed. In this species only one locus, 6-phosphogluconate-dehydrogenase shows a distinct polymorphism (48.4% average heterozygosity), while the other polymorphic loci show a heterozygous frequency of less than 2.5%. Because of this moderate polymorphism, Littorina littorea is less suitable than Mytilus edulis to be used for detecting changes in allelic frequencies. A very high number of animals would have to be run to obtain statistically significant differences between controls and oil-exposed animals.

Over the last few months new recruits from this season's brood of Littorina have settled in all four basins, although in very low numbers in the high oil basin (HO). These new recruits will be sampled in early December and their allelic frequencies will be analyzed for selected polymorphic loci. Also in early December numbered animals from the high oil basin will be sampled. Since detailed knowledge of growth for each specimen is available, potential allelic differences between fast and slow growing oil-exposed animals can be studied.

Data processing

All data obtained will now be put on files to be processed by means of a computer program, BIOSYS-1 (developed by Swofford and Selander), that has now been installed at the University Computer Center. This program performs most types of electrophoretic data analyses commonly employed in biochemical population genetics. These include measurements of allele frequencies, deviation of genotype frequencies from Hardy-Weinberg expectations, F-statistics, heterogeneity Chi-square analyses, calculation of a variety of similarity and distance coefficients between samples, and construction of phenograms using cluster analyses.

Besides comparing data from oil-exposed and non oil-exposed basins, the data processing will also be very useful in testing "basin-effects", that is differences between the basins and differences between the basins and the fjord that could not be due to oil exposure.

Conclusion

The population genetics program for analyses of Mytilus edulis and Littorina littorea is running fairly well according to schedule. Only the data processing has been delayed due to problems in compiling BIOSYS-1 on the University computer. These

problems are now being solved.

The amount of processed data to be expected from the Mytilus and Littorina work, together with similar data from two newly commenced student projects on Balanus balanoides and Balanus improvisus, should provide sufficient evidence to determine whether the chosen levels of oil exposure might cause genetic changes of the kind that was outlined in the project objectives.

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9. *Balanus improvisus.*

By Odd A Frydenberg, The University of Oslo

Material and Methods

The project started in September 1982 with 1.423 *B. improvisus* on 15 plexiglass plates. Each plate is 20 x 20 cm. 4 of these were positioned in the HO basin, 4 plates in the LO basin, 4 plates in the C4 basin and 3 plates at a fjord station. In November 1982 the 3 plates at the fjord station were detached, and all animals were eaten by predators.
On November 2nd the plates from C4 were transferred to the fjord station. Between July 1983 and September 1983 these plates also disappeard.

Periods of in situ measurements have been:

13/9 - 16/9 1982
30/11- 2/12 1982
19/1 - 21/1 1983
17/3 - 19/3 1983
10/5 - 12/5 1983
5/7 - 6/7 1983
13/9 - 14/8 1983

RESULTS

Mortality

From the start the mortality at the fjord station appears to be larger than in the C4 basin. Some of this was caused by the plates with barnacles and the "stockings" with mussels rubbing against one another. After the January measurements the barnacle plates were put into an aluminium frame to reduce this problem. Since the fjord station also disappeared between July and September 1983, no measurements were obtained from this period.

The station is therefore not included in figs 1 and 2.

The results from HO, LO and C4 basins

From figs. 1 and 2 it seems that the mortality was about equal in all populations from September 1982 to March 1983. From March 1983 to September 1983 the mortalities differed. Cumulative mortality (fig. 1): There was a statistical significant difference between the HO basin and the C4 basin from September 1982 to September 1983. No statistical significant difference was found between the LO basin and C4 basin during the same period.

Growth

Statistical tests show that it was the smallest barnacles which died in the HO basin. Hence, on figs. 3 and 4 it is the largest barnacles which have grown somewhat from September 1982, while almost all small barnacles from September 1982 have died. There was no statistical difference between the LO basin, C4 basin and the fjord station with respect to the barnacles which died during the year. On all these 3 stations the barnacles have grown much more than in the HO basin.

The histogram also show that the barnacles from Frogner have grown more than those from Holmestrand. It is possible to explain this by the mean value of the barnacles from Frogner being 2-3 mm smaller than those from Holmestrand in September 1982.

Odd Frydenberg

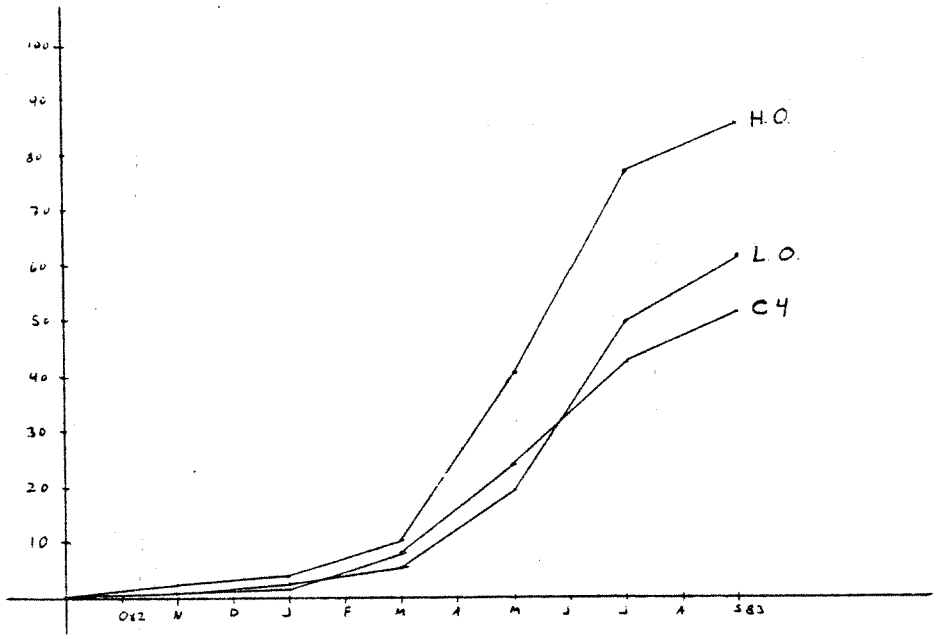

Fig. 1

Cumulative mortality from Sep 82 - Sep 83

HO ,LO, C4 basins

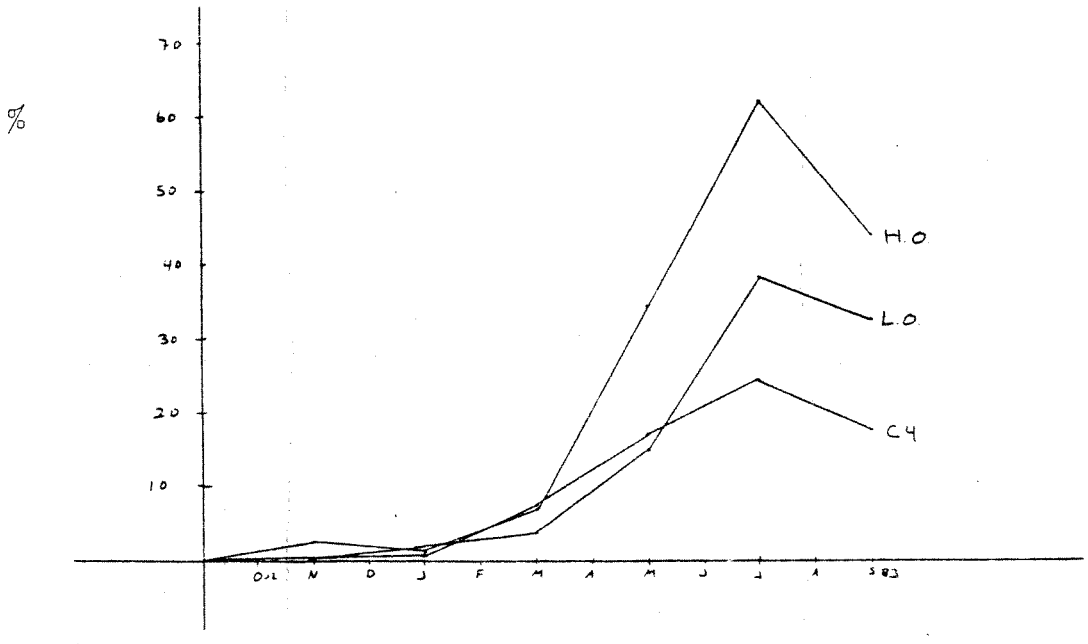

Fig. 2

Periodic mortality Sep 82- Sep 83

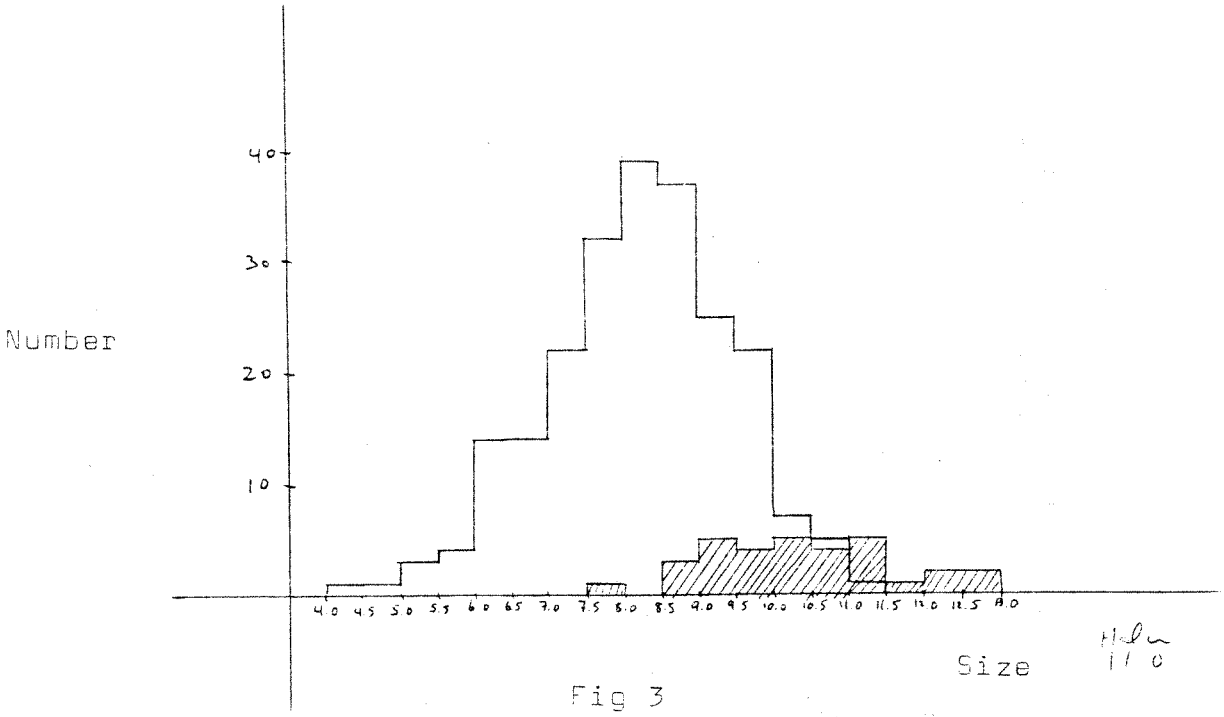

Fig 3

Histogram Sep. 82 and Sep. 83

HO basin Holmestrand pop.

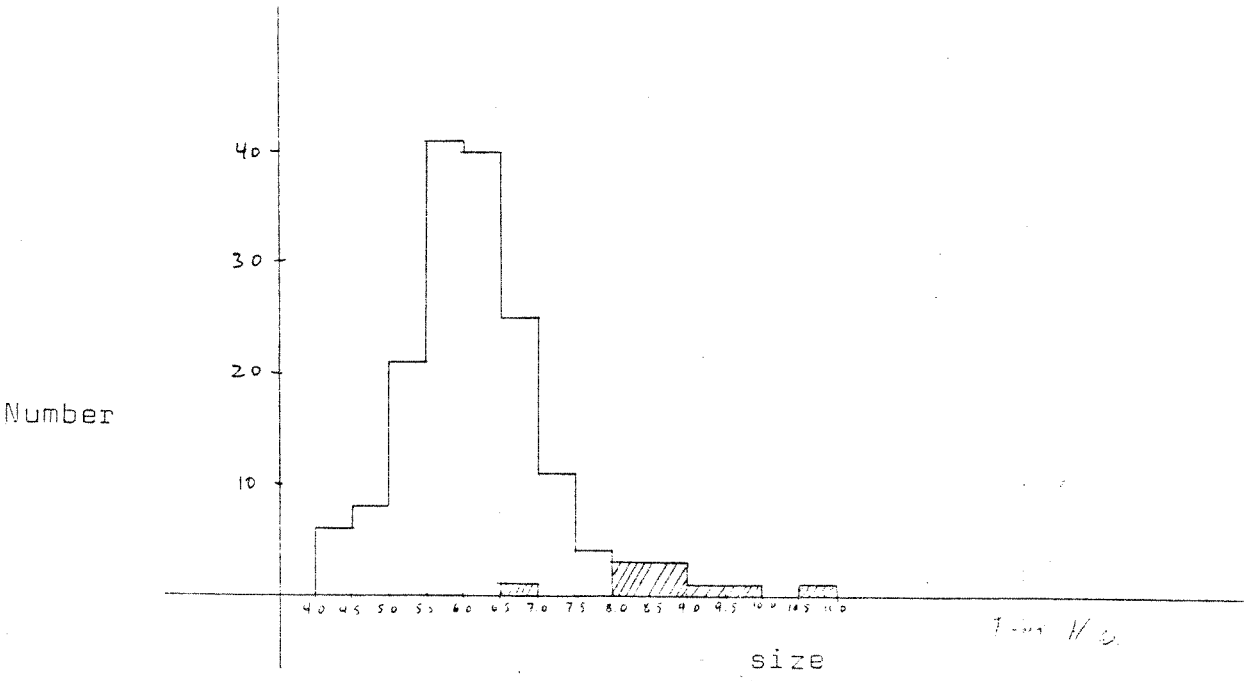

Fig 4

Histogram Sep 82 and Sep 83

HO basin Frogner pop.

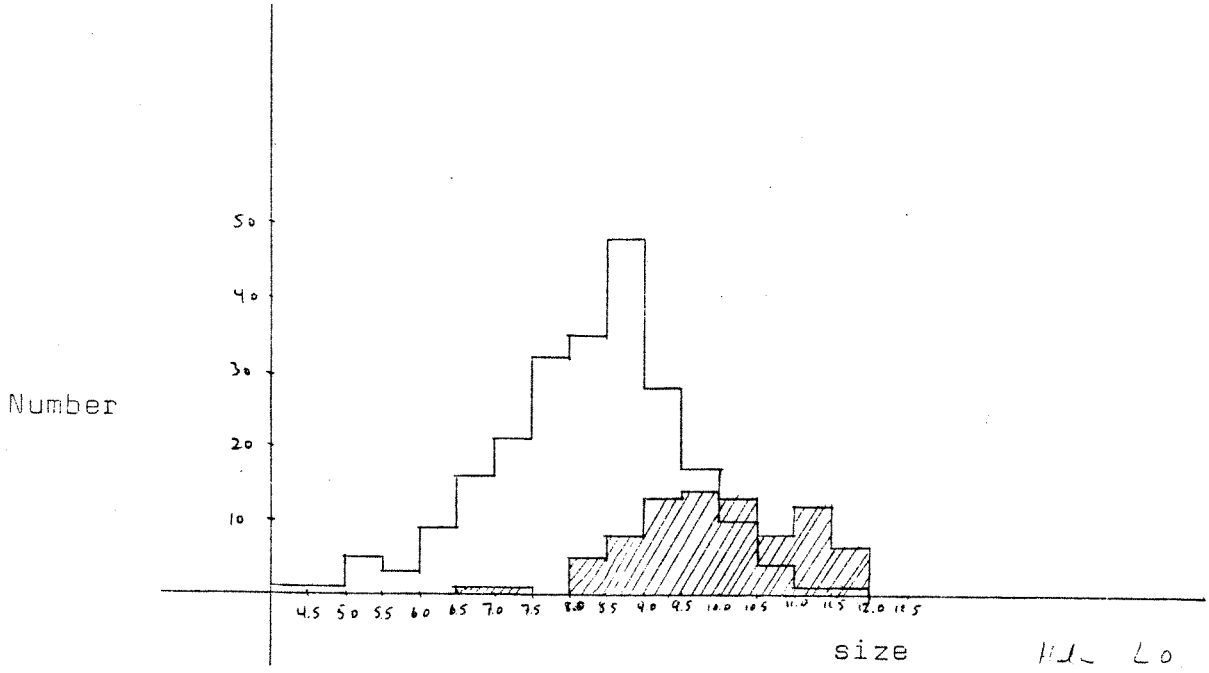

Fig 5

Histogram Sep 82and Sep 83

LO basin Holmestrand pop.

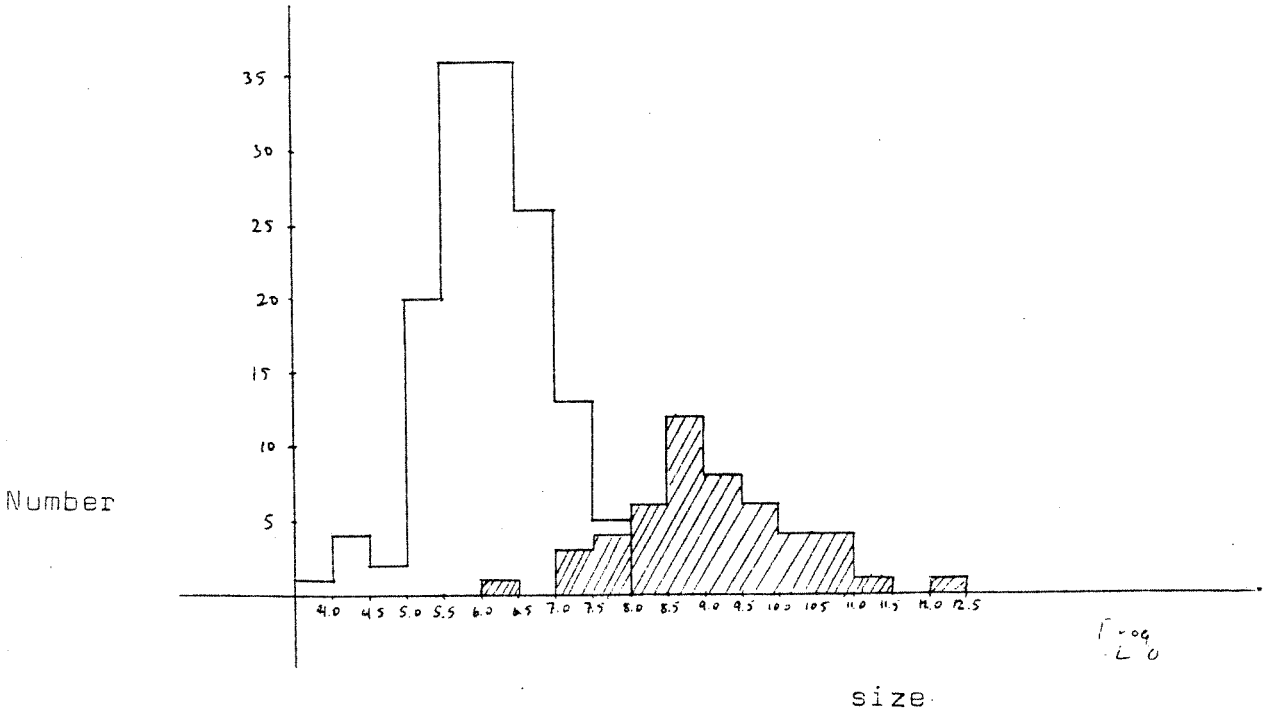

Fig 6

Histogram Sep 82 and Sep 83

LO basin Frogner pop.

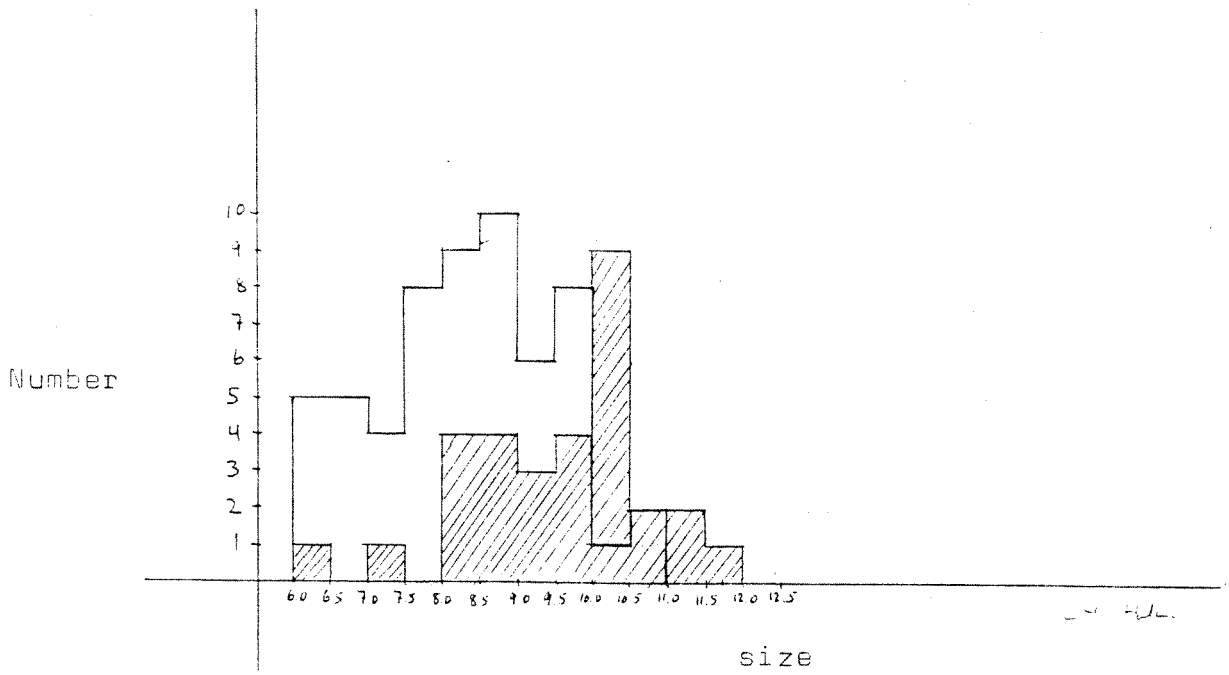

Fig 7

Histogram Sep 82 and Sep 83

C4 basin Holmestrand pop.

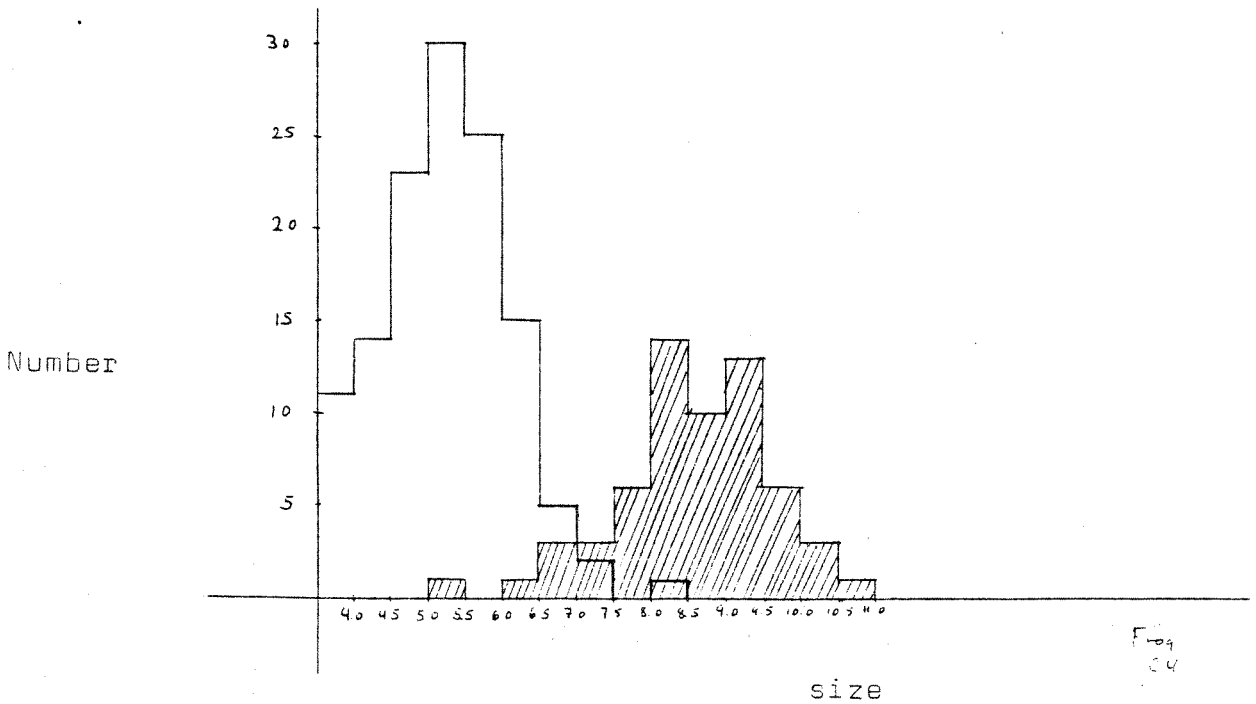

Fig 8

Histogram Sep 82 and Sep 83

C4 basin frogner pop.

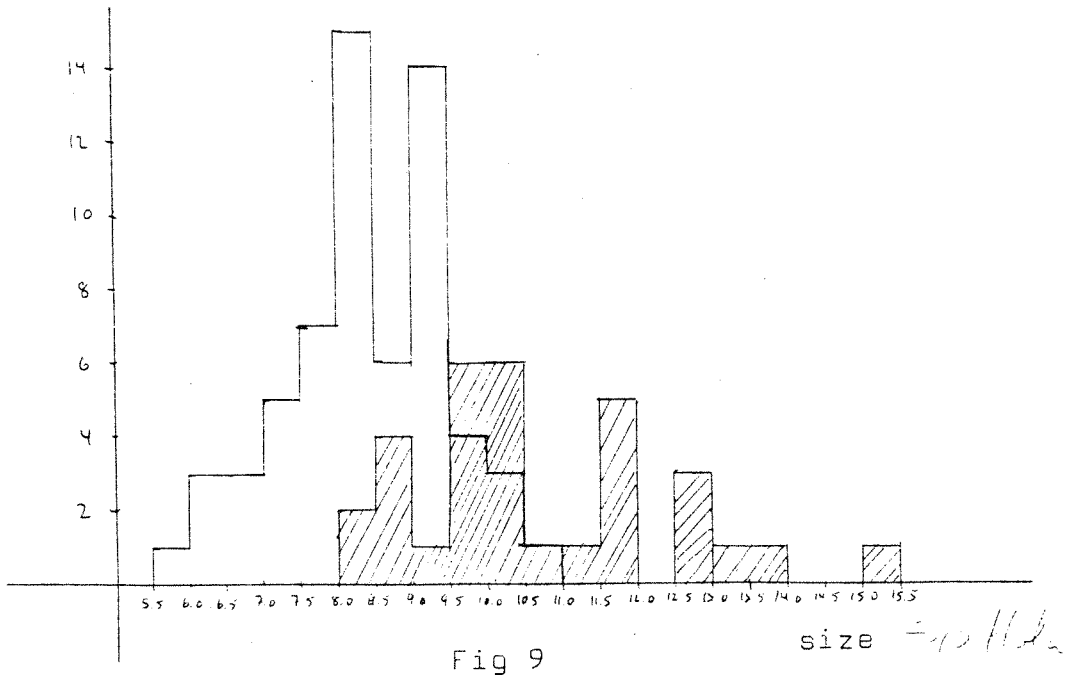

Fig 9

Histogram Sep 82 And July 83

Fjord station Holmestrand pop.

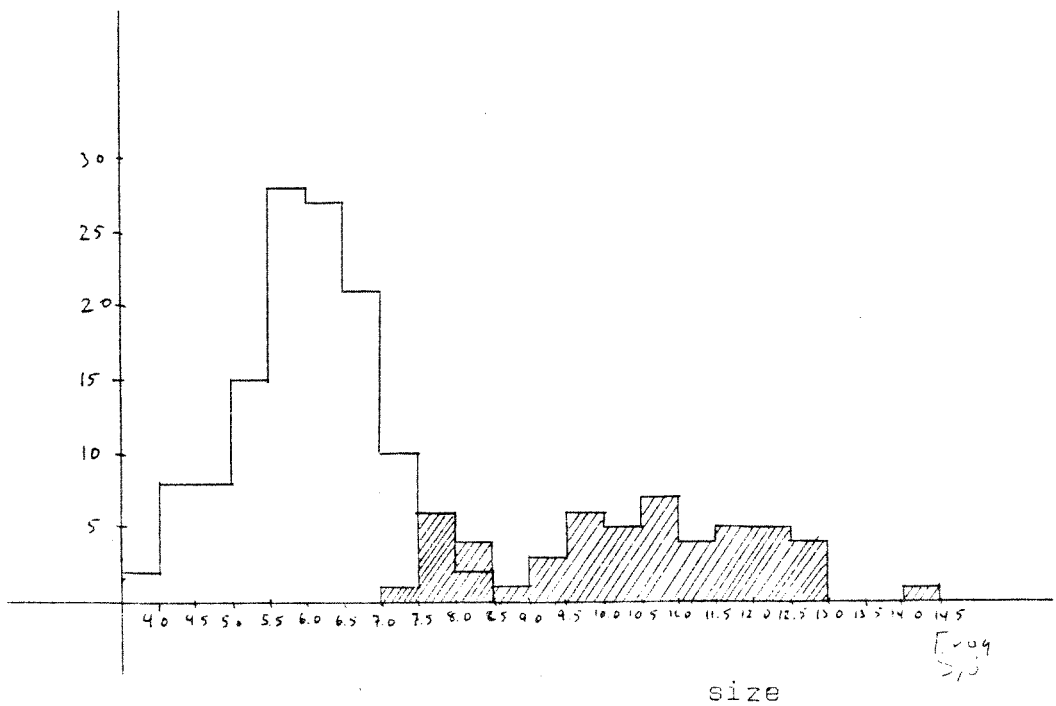

Fig 10

Histogram Sep 82 and July 83

Fjord station Frogner pop.

MARINE RESEARCH STATION SOLBERGSTRAND

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10. *Mytilus edulis*, POPULATION STRUCTURE AND DYNAMICS

Participants: Pål Thome
Mats Walday

GROWTH AND MORTALITY.

MATERIALS AND METHODS.

In July 1982 about 10 000 Mytilus edulis were sampled from the basins and the fjord.They were sorted by size;10-15mm,15-20mm.....35-40mm,transferred to bags of polypropylene fibre,and replaced in basin 1 (HIGH OIL),basin 4 (REFERENCE)and out in the fjord (CONTROL).

Because of the extremely high mortality in interval 10-15mm in basin 1 at the begining of the experiment, an extra population of 10-15mm (230 individuals) were put out in basin 1.This population were kept separatly and not mixed with the rest of the original 10-15mm population.(Fig.1,curve B1A.)

In January 1983,600 mussels (300 from the 15-20mm interval and 300 from the 20-25mm interval) were transferred from basin 4 to basin 3(LOW OIL).

Every second month (approx.) all the mussels were taken out of their bags and the byssus-net carefully torn apart. 200 random individuals (or less, depending on how many available) were measured from each interval.

Max.length and
Mean volume were measured.

The lengths of dead mussels still in the bags were also measured and the total number of living animals in each interval counted to estimate mortality.

After this treatment the mussels were gently put into new bags and replaced at their respective stations.

Samples for further analysis were taken in Dec.82,March 83 and May 83.

Max.length,
Volume,
Wet weight and
Freeze-dry weight were measured.

Some of these mussels will be sent to SI for hydrocarbon-analysis,and some will be put through CHN-analysis at Uio.

The rest of the living mussels in basin 1(H%H OIL)(from our "bag-populations"),a total of 11 animals, were sampled in the end of November 83.Together with approx.70 other mussels sampled from the other stations at the same time, they will be sent to SI for hydrocarbon-analysis.

ABBREVIATIONS

B0=fjord station ▲
B1=basin 1 ○
B3=basin 3 □
B4=basin 4 +

P1=Sampling 1 (Sept.82)
P2=Sampling 2 (Nov.82)
P3=Sampling 3 (Jan.83)
P4=Sampling 4 (March 83)
P5=Sampling 5 (May 83)
P6=Sampling 6 (June 83)
P7=Sampling 7 (July 83)
P8=Sampling 8 (Sept.83)

I1=Interval 10-15mm
I2=Interval 15_20mm
I3=Interval 20-25mm
I4=Interval 25-30mm
I5=Interval 30-35mm
I6=Interval 35-40mm

RESULTS.

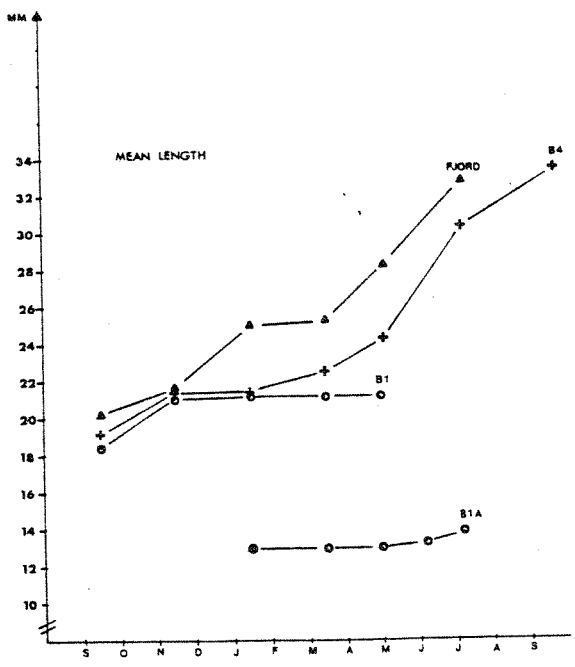

Fig.1 Musselgrowth in mm throughout one year(Sept.82-Sept.83) for interval 10-15mm.

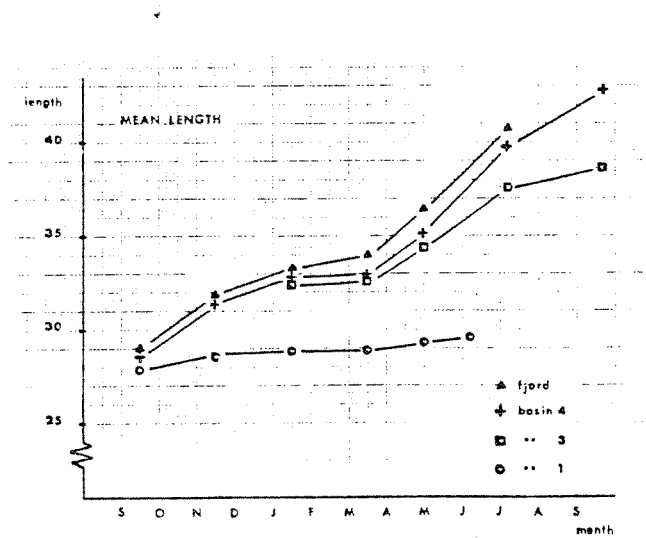

Fig.2 Musselgrowth in mm throughout one year for interval 20-25mm.

From Fig.1 and Fig.2 notice the great difference in growth between basin 1(HIGH OIL) and the others.

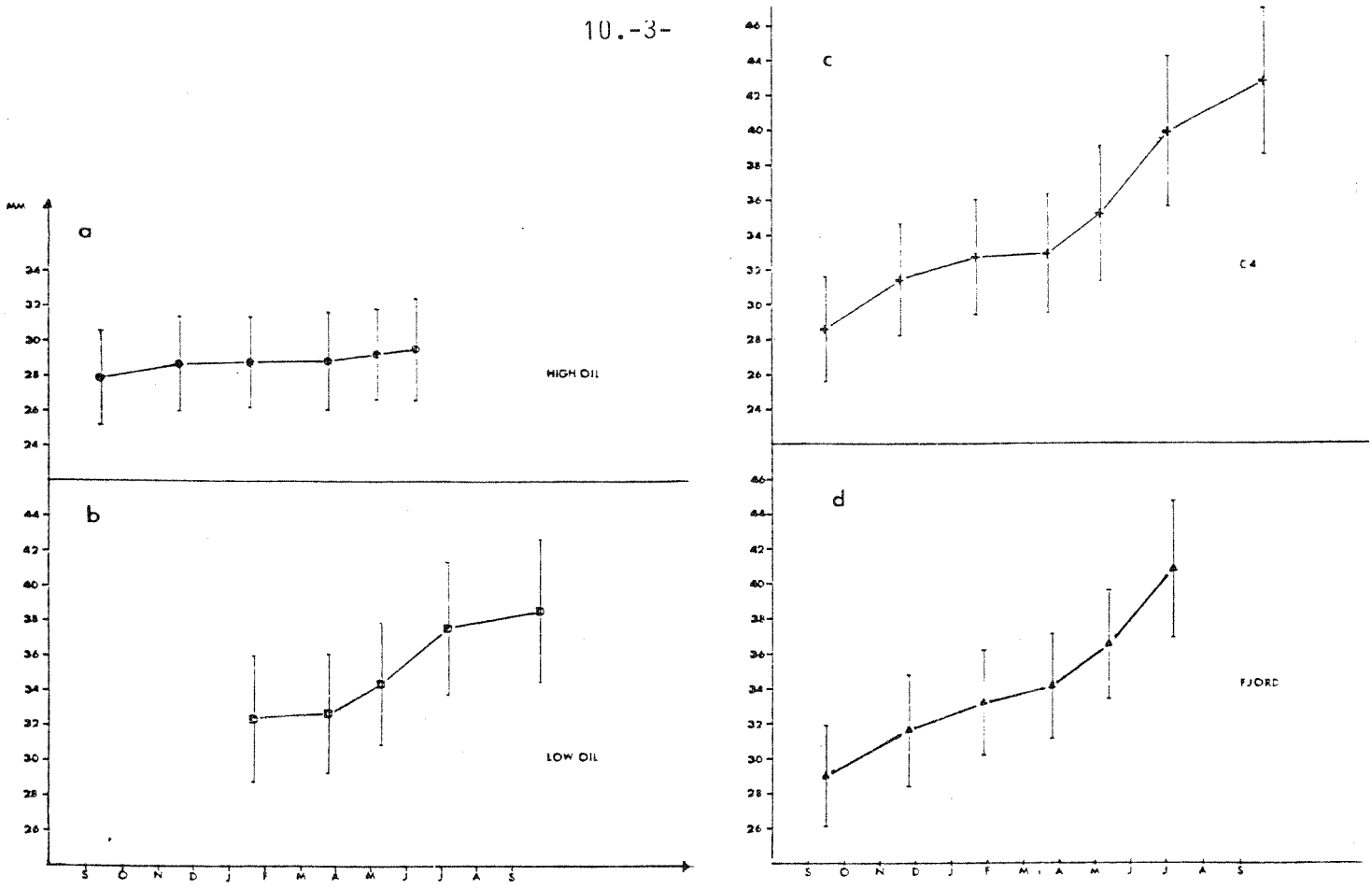

Fig.3 a,b,c and d shows the same as Fig.2 but in addition the S.D is plotted.

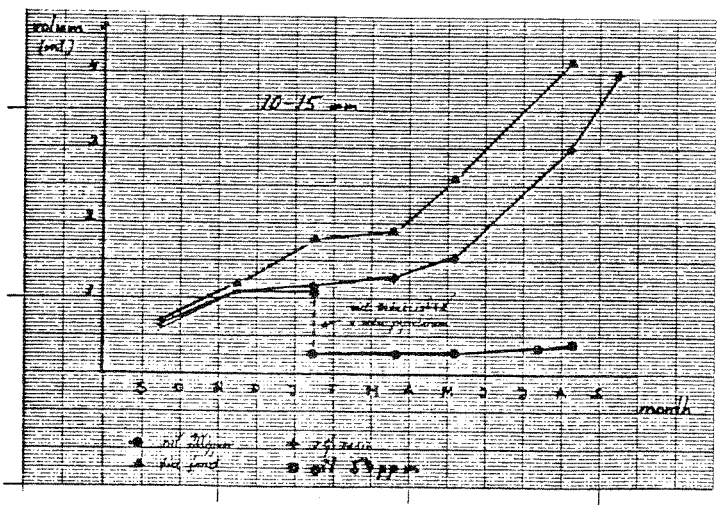

Fig.4 Mean growth in ml (water displacement) throughout one year for interval 10-15mm.

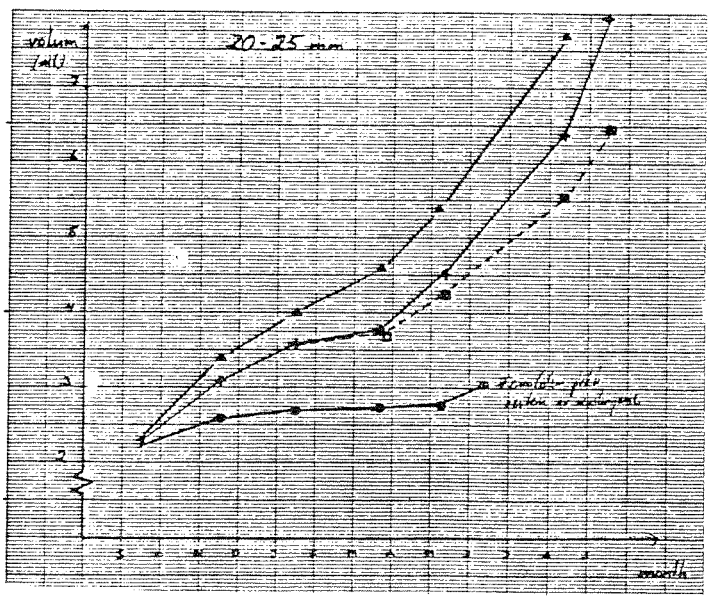

Fig.5 Mean growth in ml throughout one year for interval 20-25mm.

From Fig.4 and Fig.5 notice the difference between basin 1 and the other stations.

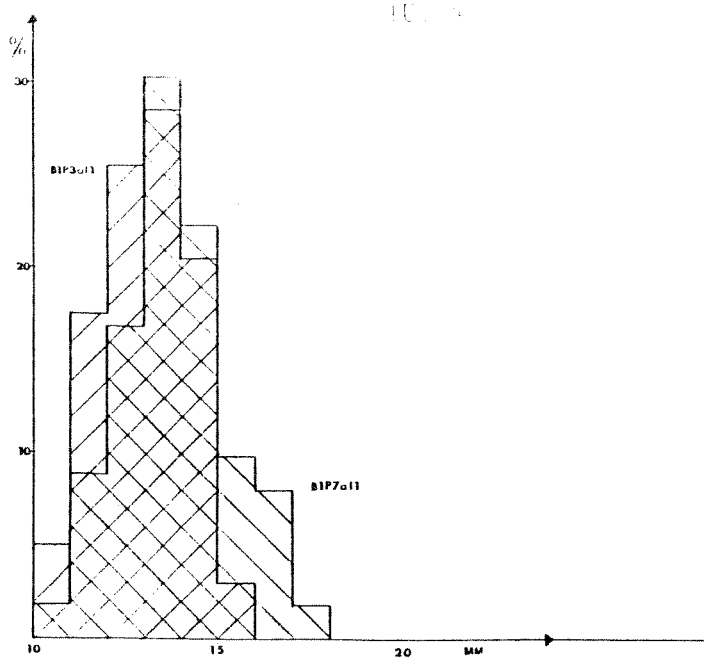

Fig.6 Size-frequency at two sampling periods(Jan.83 and July 83) in basin 1.Interval 10-15mm. (extra population)

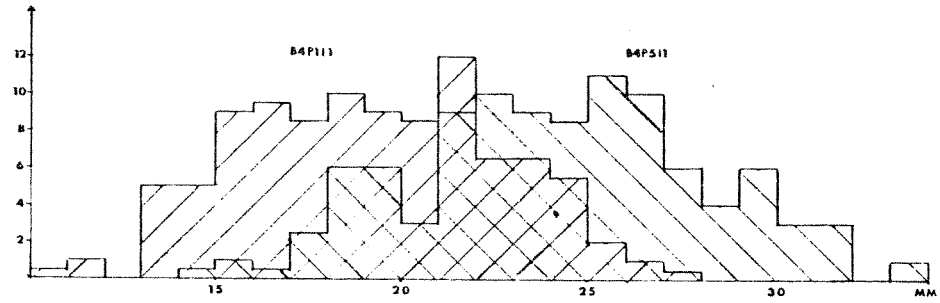

Fig.7 Size-frequency in basin 4.Sampling periods in Sept.82 and May 83.Interval 10-15mm.

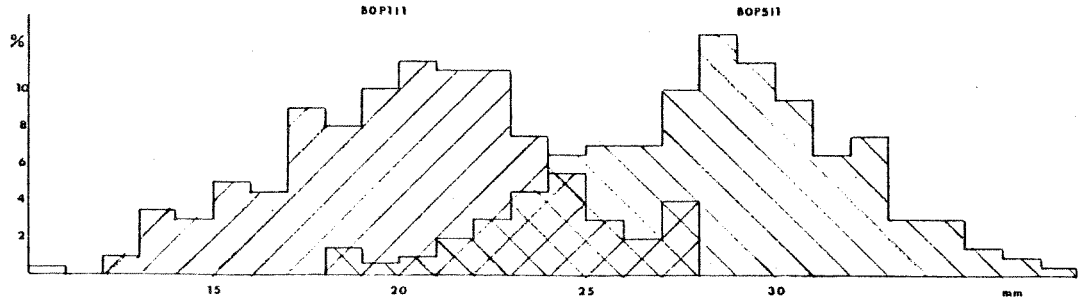

Fig.8 Size-frequency in the fjord station .Sampling periods Sept.82 and May 83.Interval 10-15mm.

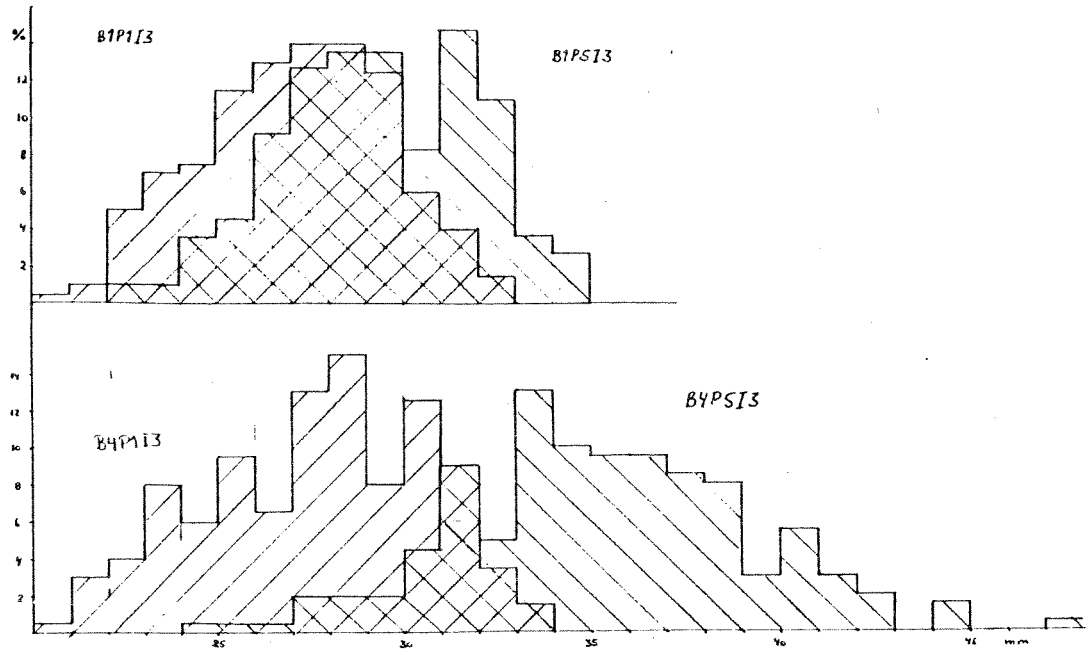

Fig.9 Size-frequency in basin 1(B1) and basin 4(B4) in Sept.82(P1) and May 83(P5). Interval 20-25mm.

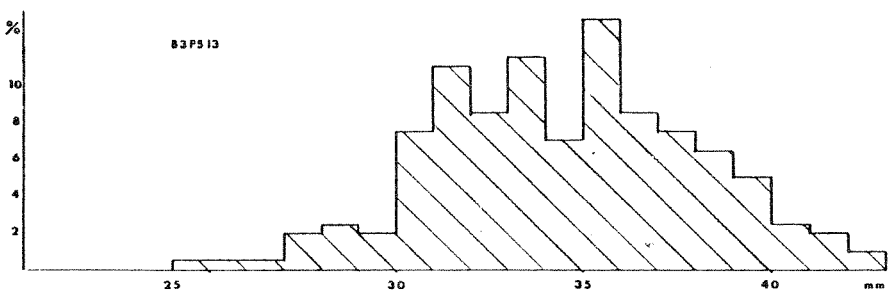

Fig.10 Size-frequency in basin 3(LOW OIL), May 83. Interval 20-25mm.

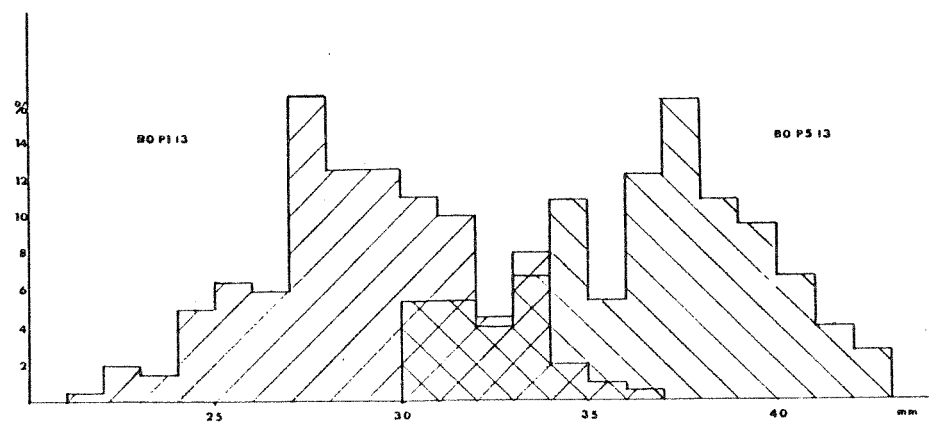

Fig.11 Size-frequency in the fjord. Sept.82 and May 83. Interval 20-25mm.

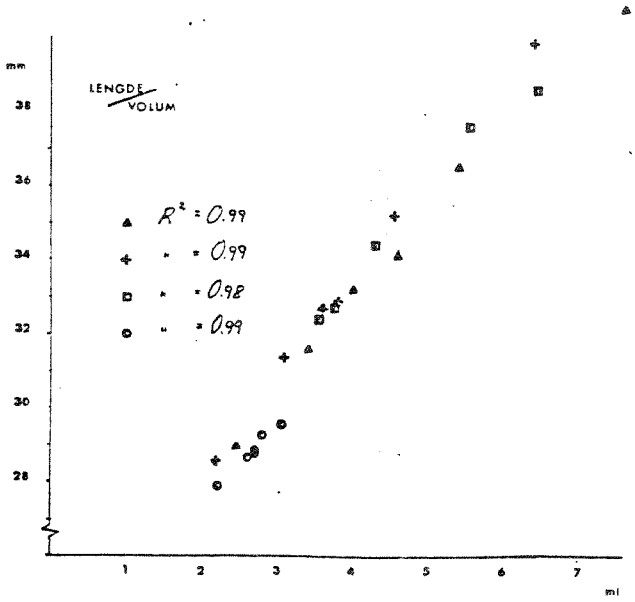

Fig.12 Length/volume for the four stations. Interval 20-25mm.

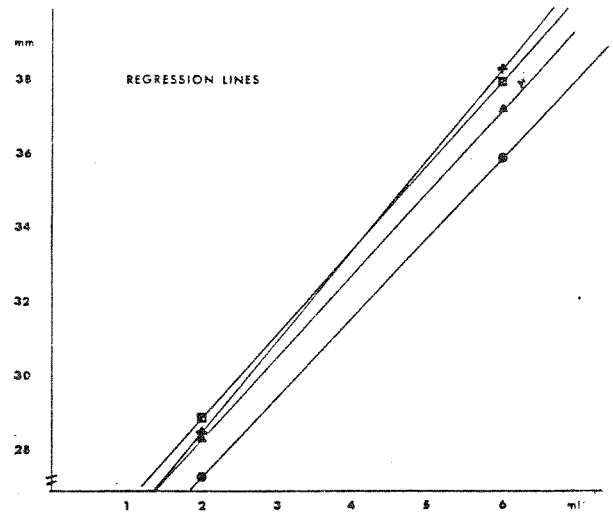

Fig.13 Length/volume regression lines estimated statistically from the data in Fig.12.

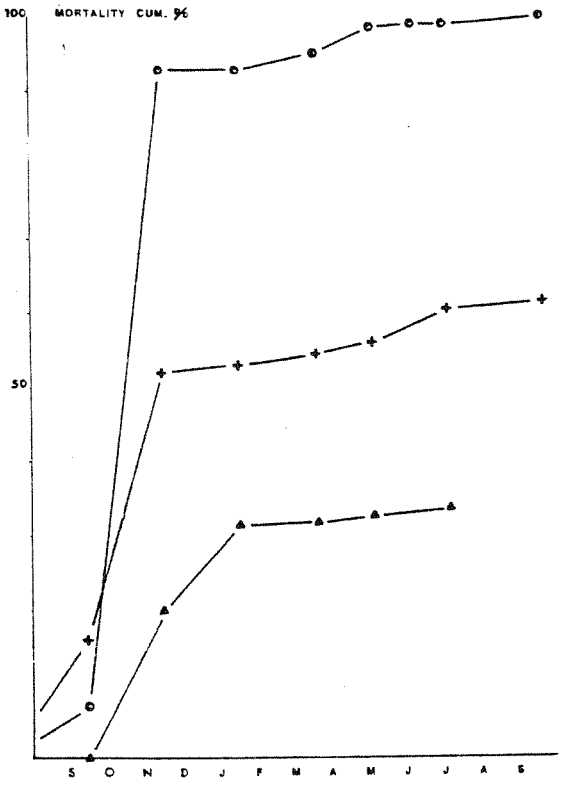

Fig.14 Mortality, cumulative %, for interval 10-15mm.

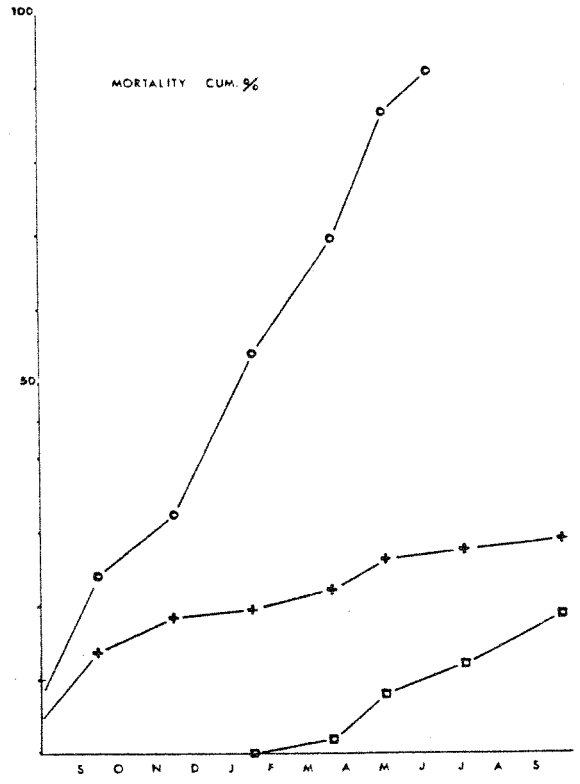

Fig.15 Mortality, cumulative %, for interval 20-25mm.

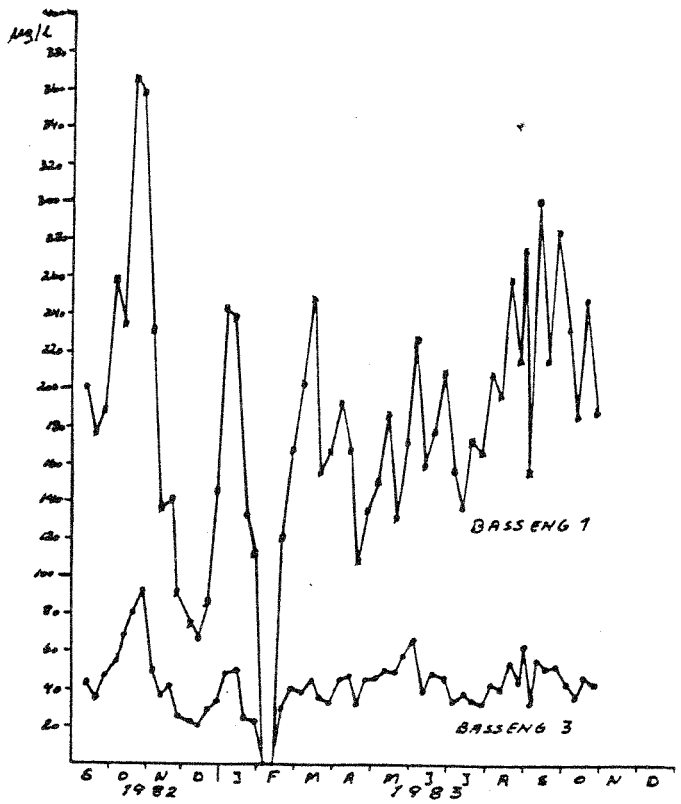

Fig.16 Theoretical mean (measured every week) of the total hydrocarbon concentration in basin 1 and basin 3. (See Torgeir Bakke)

RECRUITMENT

MATERIALS AND METHODS.

The collectors we used for settling-experiments were made of green polypropylene threads woven into 5 cm wide bands.The same type of collector-bands are also used by commercial mussel farmers.

Collectors,cut to a lenght of exactly 50 cm ,were placed in the basins and in the fjord.The arrangement of the collectors in the basins were exactly alike, and the collectors in the fjord as much as possible like those in the basins with respect to water-level and space.

The field work was carried out from 9/6--21/7 1983. Collectors staying out for one week were preserved in 96% ethanol and at the same time replaced with a new one.

In addition, one collector at each station were exposed to settling the whole period (6 weeks) and then preserved (not counted yet).

Counting was done on each side of the collector and in the preservation fluid.

RESULTS

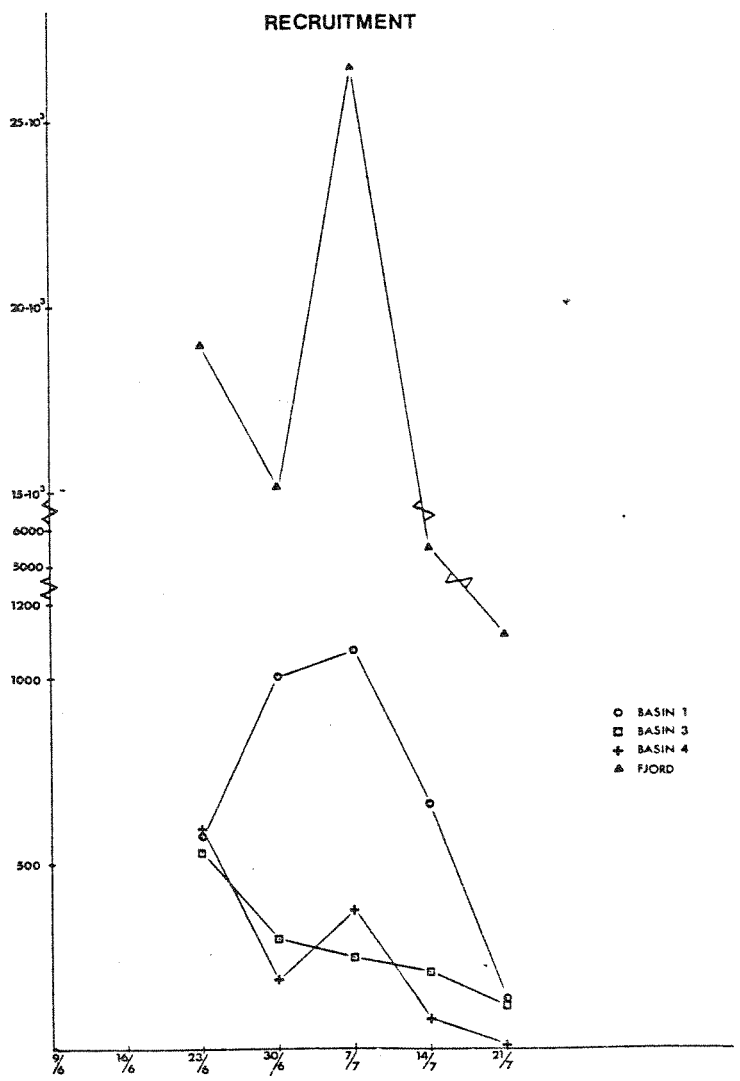

Fig.17 Total number of Mytilus larvaes on the collectors.

DISCUSSION

It seems to be a clear difference in recruitment between the fjord and the basins.

Maybe damage on the larvaes made by the waterpump and the high settling in the water pipes limits the amount of Mytilus ready for settling in the income water,or maybe the larvaes prefer to leave without settling in the basins.

The difference among the basins is not so clear, but high oil pollution seems to have no negative effect on settling in the basins. Maybe the transport distance of water from the fjord to the different basins can explain some of the relatively small difference in settling among the basins.(No statistic tests for significant differences between the stations have been executed so far.)

PÅL THOME MATS WALDAY

MARINE RESEARCH STATION SOLBERGSTRAND

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11. Population dynamics of *Littorina littorea* in an oil contaminated environment at Solbergstrand Experimental Station.

Participants: Kjell Moe & Einar Lystad, both students at the University of Oslo.

Aim and Purpose

Register effects of low oil contaminated water on populations of the common periwinkle, Littorina littorea. Both individual and population responce will be estimated and evaluated. In addition the relationship with an open population at the pier will be a part of our work. The five populations examined will be designated as follows B 1 - high oil basin (200ppb), C 2 - control basin, B 3 - low oil basin (50ppb), C 4 - control basin and C P - open control population at the pier.

Description of the work.

Sampling, marking and recapture have taken place every second month from July 82 until Sept. 83, exception made for the pier Sept. 83. This due to disturbance by people.

Every month periwinkles have been deep-frozen for CN-analysis and others put on Bakers formol solution for tissue examination. The latter especially for gamete production inquiries. Length is measured with a digital calipher along the columella axis.

Results

Size distribution and numbers of individuals marked for each of the five populations by July 82 are given in fig. 1.

B 1 - 200ppb

VALUE-COMB.	FREQ.	PERCENT	ONE X REPRESENTS 9 SUBJECTS.
07(,)	1	0.1%	X
08(,)	3	0.2%	X
09(,)	10	0.5%	XX
10(,)	21	1.1%	XXX
11(,)	32	1.6%	XXXX
12(,)	52	2.6%	XXXXXX
13(,)	83	4.2%	XXXXXXXXXX
14(,)	93	4.7%	XXXXXXXXXX
15(,)	131	6.6%	XXXXXXXXXXXXXX
16(,)	223	11.3%	XXXXXXXXXXXXXXXXXXXXXXXX
17(,)	292	14.8%	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
18(,)	260	13.2%	XXXXXXXXXXXXXXXXXXXXXXXXXXXXX
19(,)	274	13.9%	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
20(,)	163	8.3%	XXXXXXXXXXXXXXXXXX
21(,)	120	6.1%	XXXXXXXXXXXX
22(,)	96	4.9%	XXXXXXXXXX
23(,)	71	3.6%	XXXXXXX
24(,)	28	1.4%	XXX
25(,)	5	0.3%	X
26(,)	7	0.4%	X
27(,)	1	0.1%	X
29(,)	1	0.1%	X

total of 1970 individuals marked

C 2 - control 2

VALUE-COMB.	FREQ.	PERCENT	ONE X REPRESENTS 3 SUBJECTS.
06(,)	1	0.1%	X
08(,)	2	0.3%	X
10(,)	3	0.4%	X
11(,)	11	1.6%	XXXX
12(,)	17	2.4%	XXXXXX
13(,)	28	4.0%	XXXXXXXXX
14(,)	29	4.1%	XXXXXXXXX
15(,)	45	6.4%	XXXXXXXXXXXXX
16(,)	49	7.0%	XXXXXXXXXXXXXXX
17(,)	79	11.3%	XXXXXXXXXXXXXXXXXXXXXXXX
18(,)	81	11.5%	XXXXXXXXXXXXXXXXXXXXXXXXX
19(,)	84	12.0%	XXXXXXXXXXXXXXXXXXXXXXXXX
20(,)	72	10.3%	XXXXXXXXXXXXXXXXXXXXXX
21(,)	68	9.7%	XXXXXXXXXXXXXXXXXXXX
22(,)	51	7.3%	XXXXXXXXXXXXXX
23(,)	38	5.4%	XXXXXXXXXXX
24(,)	25	3.6%	XXXXXXXX
25(,)	11	1.6%	XXX
26(,)	5	0.7%	XX
27(,)	2	0.3%	X
28(,)	1	0.1%	X

total of 702 individuals marked

B 3 - 50ppb

VALUE-COMB.	FREQ.	PERCENT	ONE X REPRESENTS 2 SUBJECTS.
10(,)	1	0.4%	X
11(,)	3	1.2%	XX
12(,)	6	2.4%	XXX
13(,)	13	5.3%	XXXXXX
14(,)	14	5.7%	XXXXXXX
15(,)	20	8.1%	XXXXXXXXX
16(,)	21	8.5%	XXXXXXXXXX
17(,)	41	16.7%	XXXXXXXXXXXXXXXXXXXX
18(,)	35	14.2%	XXXXXXXXXXXXXXXXX
19(,)	27	11.0%	XXXXXXXXXXXXX
20(,)	27	11.0%	XXXXXXXXXXXXX
21(,)	22	8.9%	XXXXXXXXXX
22(,)	8	3.3%	XXXX
23(,)	3	1.2%	XX
24(,)	2	0.8%	X
25(,)	2	0.8%	X
27(,)	1	0.4%	X

total of 246 individuals marked

C 4 - control 4

VALUE-COMB.	FREQ.	PERCENT	ONE X REPRESENTS 3 SUBJECTS
05(,)	1	0.3%	X
06(,)	1	0.3%	X
08(,)	2	0.5%	X
10(,)	2	0.5%	X
11(,)	6	1.6%	XX
12(,)	15	4.1%	XXXXX
13(,)	19	5.1%	XXXXXX
14(,)	53	14.4%	XXXXXXXXXXXXXXXXX
15(,)	73	19.8%	XXXXXXXXXXXXXXXXXXXXXXX
16(,)	80	21.7%	XXXXXXXXXXXXXXXXXXXXXXXXX
17(,)	55	14.9%	XXXXXXXXXXXXXXXXX
18(,)	35	9.5%	XXXXXXXXXX
19(,)	12	3.3%	XXXX
20(,)	7	1.9%	XXX
21(,)	3	0.8%	X
23(,)	3	0.8%	X
24(,)	1	0.3%	X
25(,)	1	0.3%	X

total of 369 individuals marked

C P - control pier

VALUE-COMB.	FREQ.	PERCENT	ONE X REPRESENTS 3 SUBJECTS.
05(,)	2	0.2%	X
06(,)	1	0.1%	X
07(,)	4	0.4%	X
08(,)	6	0.5%	XX
09(,)	7	0.6%	XX
10(,)	6	0.5%	XX
11(,)	11	1.0%	XXX
12(,)	12	1.1%	XXX
13(,)	11	1.0%	XXX
14(,)	23	2.1%	XXXXX
15(,)	45	4.0%	XXXXXXXXX
16(,)	80	7.1%	XXXXXXXXXXXXXXXX
17(,)	158	14.1%	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
18(,)	174	15.5%	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
19(,)	158	14.1%	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
20(,)	117	10.4%	XXXXXXXXXXXXXXXXXXXXXXXX
21(,)	108	9.6%	XXXXXXXXXXXXXXXXXXXXX
22(,)	85	7.6%	XXXXXXXXXXXXXXXXX
23(,)	58	5.2%	XXXXXXXXXXX
24(,)	26	2.3%	XXXXXX
25(,)	17	1.5%	XXXX
26(,)	4	0.4%	X
27(,)	5	0.4%	X
28(,)	2	0.2%	X

total of 1127 individuals marked

Fig. 1. Histograms showing size-frequency distribution for all 5 populations that were marked during July 82. Note each X is not equal !!!

C4 differs from the others by having a lower mean and a cluster at sizes from 14-17mm. B1, C2, B3 and CP have greater resemblance.

Growth patterns for each population and each cohort are different but there are resemblances to be outlined in fig. 2.

B 1

VALUE INTERVAL	FREQ.	PROS.	ONE X REPRESENTS 3 SUBJECTS	FREQ.	PROS.	ONE X REPRESENTS 3 SUBJECTS
			07-82			**07-83**
9. - 10.>	1.0	0.2%				
10. - 11.>	1.0	0.2%				
11. - 12.>	4.0	0.9%	X			
12. - 13.>	8.0	1.7%	XXX			
13. - 14.>	21.0	4.3%	XXXXXXX			
14. - 15.>	24.0	5.0%	XXXXXXXX			
15. - 16.>	25.0	5.2%	XXXXXXXX	2.0	0.4%	X
16. - 17.>	58.0	12.0%	XXXXXXXXXXXXXXXXXX	1.0	0.2%	
17. - 18.>	60.0	12.4%	XXXXXXXXXXXXXXXXXXX	21.0	4.3%	XXXXXXX
18. - 19.>	60.0	12.4%	XXXXXXXXXXXXXXXXXXX	39.0	8.1%	XXXXXXXXXXXXX
19. - 20.>	93.0	19.2%	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	89.0	18.4%	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
20. - 21.>	40.0	8.3%	XXXXXXXXXXXX	104.0	21.5%	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
21. - 22.>	33.0	6.8%	XXXXXXXXXX	103.0	21.7%	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
22. - 23.>	30.0	6.2%	XXXXXXXXX	67.0	13.8%	XXXXXXXXXXXXXXXXXXX
23. - 24.>	20.0	4.1%	XXXXXX	41.0	8.5%	XXXXXXXXXXXX
24. - 25.>	5.0	1.0%	XX	10.0	2.1%	XXX
25. - 26.A	1.0	0.2%		5.0	1.0%	XX
NO.OF S:	487.		MEAN: 18.312			MEAN: 20.920

C 2

VALUE INTERVAL	FREQ.	PROS.	ONE X REPRESENTS 1 SUBJECTS	FREQ.	PROS.	ONE X REPRESENTS 2 SUBJECTS
11. - 12.>	3.0	1.4%	XXX			
12. - 13.>	2.0	0.9%	XX			
13. - 14.>	8.0	3.6%	XXXXXXXX			
14. - 15.>	8.0	3.6%	XXXXXXXX			
15. - 16.>	11.0	5.0%	XXXXXXXXXXX			
16. - 17.>	17.0	7.7%	XXXXXXXXXXXXXXXXX	3.0	1.4%	XX
17. - 18.>	21.0	9.5%	XXXXXXXXXXXXXXXXXXXX	3.0	1.4%	XX
18. - 19.>	29.0	13.1%	XXXXXXXXXXXXXXXXXXXXXXXXXXX	11.0	5.0%	XXXXXX
19. - 20.>	25.0	11.3%	XXXXXXXXXXXXXXXXXXXXXXXX	20.0	9.0%	XXXXXXXXXX
20. - 21.>	23.0	10.4%	XXXXXXXXXXXXXXXXXXXXXX	29.0	13.1%	XXXXXXXXXXXXXX
21. - 22.>	26.0	11.8%	XXXXXXXXXXXXXXXXXXXXXXXXX	42.0	19.0%	XXXXXXXXXXXXXXXXXXXX
22. - 23.>	18.0	8.1%	XXXXXXXXXXXXXXXXX	50.0	22.6%	XXXXXXXXXXXXXXXXXXXXXXXX
23. - 24.>	17.0	7.7%	XXXXXXXXXXXXXXXX	44.0	19.9%	XXXXXXXXXXXXXXXXXXXX
24. - 25.>	9.0	4.1%	XXXXXXXXX	15.0	6.8%	XXXXXXX
25. - 26.>	3.0	1.4%	XXX	2.0	0.9%	X
26. - 27.>	1.0	0.5%	X	2.0	0.9%	X
27. - 28.A	0.0	0.0%		0.0	0.0%	
NO.OF S:	222.		MEAN: 19.419			MEAN: 20.892

B 3

VALUE INTERVAL	FREQ.	PROS.	ONE X REPRESENTS 1 SUBJECTS	FREQ.	PROS.	ONE X REPRESENTS 1 SUBJECTS
12. - 13.>	1.0	1.2%	X			
13. - 14.>	5.0	6.2%	XXXXX			
14. - 15.>	5.0	6.2%	XXXXX			
15. - 16.>	6.0	7.4%	XXXXXX	1.0	1.2%	X
16. - 17.>	10.0	12.3%	XXXXXXXXXX	4.0	4.9%	XXXX
17. - 18.>	11.0	13.6%	XXXXXXXXXXX	8.0	9.9%	XXXXXXXX
18. - 19.>	10.0	12.3%	XXXXXXXXXX	10.0	12.3%	XXXXXXXXXX
19. - 20.>	8.0	9.9%	XXXXXXXX	11.0	13.6%	XXXXXXXXXXX
20. - 21.>	12.0	14.8%	XXXXXXXXXXXX	18.0	22.2%	XXXXXXXXXXXXXXXXXX
21. - 22.>	8.0	9.9%	XXXXXXXX	18.0	22.2%	XXXXXXXXXXXXXXXXXX
22. - 23.>	2.0	2.5%	XX	6.0	7.4%	XXXXXX
23. - 24.>	1.0	1.2%	X	5.0	6.2%	XXXXX
24. - 25.>	1.0	1.2%	X	0.0	0.0%	
25. - 26.>	1.0	1.2%	X	0.0	0.0%	
26. - 27.>	0.0	0.0%		0.0	0.0%	
27. - 28.>	0.0	0.0%		0.0	0.0%	
28. - 29.>	0.0	0.0%		0.0	0.0%	
29. - 30.A	0.0	0.0%		0.0	0.0%	
NO.OF S:	81.000		MEAN: 16.384			MEAN: 21.257

C 4

VALUE INTERVAL	07-82 FREQ.	07-82 PROS.	07-82 ONE X REPRESENTS 1 SUBJECTS	07-83 FREQ.	07-83 PROS.	07-83 ONE X REPRESENTS 1 SUBJECTS
11. - 12.>	1.0	0.8%	X			
12. - 13.>	1.0	0.8%	X			
13. - 14.>	4.0	3.4%	XXXX	1.0	0.8%	X
14. - 15.>	10.0	8.4%	XXXXXXXXXX	0.0	0.0%	
15. - 16.>	25.0	21.0%	XXXXXXXXXXXXXXXXXXXXXXXXX	0.0	0.0%	
16. - 17.>	31.0	26.1%	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	5.0	4.2%	XXXXX
17. - 18.>	21.0	17.6%	XXXXXXXXXXXXXXXXXXXX	29.0	24.4%	XXXXXXXXXXXXXXXXXXXXXXXXXXXX
18. - 19.>	14.0	11.8%	XXXXXXXXXXXXX	33.0	27.7%	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
19. - 20.>	7.0	5.9%	XXXXXXX	32.0	27.7%	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
20. - 21.>	4.0	3.4%	XXXX	16.0	13.4%	XXXXXXXXXXXXXXXX
21. - 22.>	0.0	0.0%		1.0	0.8%	X
22. - 23.A	1.0	0.8%	X	1.0	0.8%	X
NO.OF S:	120.000	MEAN:	16.797	MEAN:		18.765

CP

VALUE INTERVAL	FREQ.	PROS.	ONE X REPRESENTS 1 SUBJECTS	FREQ.	PROS.	ONE X REPRESENTS 1 SUBJECTS
7. - 8.>	2.0	1.2%	XX			
8. - 9.>	1.0	0.6%	X			
9. - 10.>	0.0	0.0%				
10. - 11.>	0.0	0.0%				
11. - 12.>	1.0	0.6%	X	2.0	1.2%	XX
12. - 13.>	2.0	1.2%	XX	0.0	0.0%	
13. - 14.>	2.0	1.2%	XX	1.0	0.6%	X
14. - 15.>	5.0	3.1%	XXXXX	0.0	0.0%	
15. - 16.>	4.0	2.5%	XXXX	0.0	0.0%	
16. - 17.>	7.0	4.3%	XXXXXXX	6.0	3.7%	XXXXXX
17. - 18.>	14.0	8.7%	XXXXXXXXXXXXX	6.0	3.7%	XXXXXX
18. - 19.>	27.0	16.8%	XXXXXXXXXXXXXXXXXXXXXXXXXX	15.0	9.3%	XXXXXXXXXXXXXX
19. - 20.>	23.0	14.3%	XXXXXXXXXXXXXXXXXXXXXX	19.0	11.7%	XXXXXXXXXXXXXXXXXX
20. - 21.>	22.0	13.7%	XXXXXXXXXXXXXXXXXXXXX	30.0	18.5%	XXXXXXXXXXXXXXXXXXXXXXXXXXXXX
21. - 22.>	17.0	10.6%	XXXXXXXXXXXXXXXX	37.0	22.8%	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
22. - 23.>	14.0	8.7%	XXXXXXXXXXXXX	22.0	13.6%	XXXXXXXXXXXXXXXXXXXX
23. - 24.>	10.0	6.2%	XXXXXXXXX	11.0	6.8%	XXXXXXXXXX
24. - 25.>	7.0	4.3%	XXXXXXX	8.0	4.9%	XXXXXXXX
25. - 26.>	2.0	1.2%	XX	4.0	2.5%	XXXX
26. - 27.A	1.0	0.6%	X	1.0	0.6%	X
NO.OF S:	163.	MEAN:	16.638	MEAN:		20.931

Fig. 2. Twelve months of growth based on size distribution for individuals of all size classes marked July 82 and recaptured July 83. Mark the overall mean increase. Size classes given in intervals of 1 mm.

The histograms of sizegroup 15-18mm for all five populations by July 82 and after one year (fig. 3), shows the different growthpatterns registered. Following ranking of growth is outlined (tab. 1.) C2, B3, B1, C4, CP. C2 shows the highest mean increase of 4.95 mm/individual. (It does not seem to be good conditions for growth in C4 and CP compared to C2, B3 and B1).

B 1

VALUE INTERVAL		07-82 FREQ.	07-82 PROS.	07-82 ONE X REPRESENTS 1 SUBJECTS	07-83 FREQ.	07-83 PROS.	07-83 ONE X REPRESENTS 1
15.000 -	15.526>	18.0	9.1%	XXXXXXXXXXXX	0.0	0.0%	
15.526 -	16.053>	19.0	13.3%	XXXXXXXXXXXXXXXXX	1.0	0.7%	X
16.053 -	16.579>	23.0	16.1%	XXXXXXXXXXXXXXXXXXXXX	0.0	0.0%	
16.579 -	17.105>	34.0	23.8%	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX	0.0	0.0%	
17.105 -	17.632>	31.0	21.7%	XXXXXXXXXXXXXXXXXXXXXXXXXXXXX	3.0	2.1%	XXX
17.632 -	18.158>	23.0	16.1%	XXXXXXXXXXXXXXXXXXXXX	5.0	3.5%	XXXXX
18.158 -	18.684>	0.0	0.0%		11.0	7.7%	XXXXXXXXXX
18.684 -	19.211>	0.0	0.0%		18.0	12.6%	XXXXXXXXXXXXXXXXX
19.211 -	19.737>	0.0	0.0%		24.0	16.8%	XXXXXXXXXXXXXXXXXXXXXXX
19.737 -	20.263>	0.0	0.0%		32.0	22.4%	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
20.263 -	20.789>	0.0	0.0%		28.0	19.6%	XXXXXXXXXXXXXXXXXXXXXXXXXX
20.789 -	21.316>	0.0	0.0%		9.0	6.3%	XXXXXXXXX
21.316 -	21.842>	0.0	0.0%		10.0	7.0%	XXXXXXXXXX
21.842 -	22.368>	0.0	0.0%		2.0	1.4%	XX
22.368 -	22.895>	0.0	0.0%		0.0	0.0%	

C 2

VALUE INTERVAL		07-82 FREQ.	07-82 PROS.	07-82 ONE X REPRESENTS 1	07-83 FREQ.	07-83 PROS.	07-83 ONE X REPRESENTS 1
15.000 -	15.526>	4.0	8.2%	XXXX	0.0	0.0%	
15.526 -	16.053>	8.0	16.3%	XXXXXXXX	0.0	0.0%	
16.053 -	16.579>	7.0	14.3%	XXXXXXX	0.0	0.0%	
16.579 -	17.105>	10.0	20.4%	XXXXXXXXXX	0.0	0.0%	
17.105 -	17.632>	13.0	26.5%	XXXXXXXXXXXX	0.0	0.0%	
17.632 -	18.158>	7.0	14.3%	XXXXXXX	0.0	0.0%	
18.158 -	18.684>	0.0	0.0%		1.0	2.0%	X
18.684 -	19.211>	0.0	0.0%		1.0	2.0%	X
19.211 -	19.737>	0.0	0.0%		4.0	8.2%	XXXX
19.737 -	20.263>	0.0	0.0%		1.0	2.0%	X
20.263 -	20.789>	0.0	0.0%		3.0	6.1%	XXX
20.789 -	21.316>	0.0	0.0%		7.0	14.3%	XXXXXXX
21.316 -	21.842>	0.0	0.0%		7.0	14.3%	XXXXXXX
21.842 -	22.368>	0.0	0.0%		7.0	14.3%	XXXXXXX
22.368 -	22.895>	0.0	0.0%		10.0	20.4%	XXXXXXXXXX
22.895 -	23.421>	0.0	0.0%		5.0	10.2%	XXXXX
23.421 -	23.947>	0.0	0.0%		2.0	4.1%	XX
23.947 -	24.474>	0.0	0.0%		1.0	2.0%	X

B 3

VALUE INTERVAL		07-82 FREQ.	07-82 PROS.	07-82 ONE X REPRESENTS 1	07-83 FREQ.	07-83 PROS.	07-83 ONE X REPRESENTS 1
15.000 -	15.526>	5.0	18.5%	XXXXX	0.0	0.0%	
15.526 -	16.053>	2.0	7.4%	XX	0.0	0.0%	
16.053 -	16.579>	6.0	22.2%	XXXXXX	0.0	0.0%	
16.579 -	17.105>	4.0	14.8%	XXXX	1.0	3.7%	X
17.105 -	17.632>	7.0	25.9%	XXXXXXX	0.0	0.0%	
17.632 -	18.158>	3.0	11.1%	XXX	1.0	3.7%	X
18.158 -	18.684>	0.0	0.0%		1.0	3.7%	X
18.684 -	19.211>	0.0	0.0%		1.0	3.7%	X
19.211 -	19.737>	0.0	0.0%		6.0	22.2%	XXXXXX
19.737 -	20.263>	0.0	0.0%		3.0	11.1%	XXX
20.263 -	20.789>	0.0	0.0%		6.0	22.2%	XXXXXX
20.789 -	21.316>	0.0	0.0%		6.0	22.2%	XXXXXX
21.316 -	21.842>	0.0	0.0%		0.0	0.0%	
21.842 -	22.368>	0.0	0.0%		2.0	7.4%	XX

C 4

VALUE INTERVAL		07-82 FREQ.	07-82 PROS.	07-82 ONE X REPRESENTS 1	07-83 FREQ.	07-83 PROS.	07-83 ONE X REPRESENTS 1
15.000 -	15.526>	12.0	15.6%	XXXXXXXXXXX	0.0	0.0%	
15.526 -	16.053>	16.0	20.8%	XXXXXXXXXXXXXXX	0.0	0.0%	
16.053 -	16.579>	17.0	22.1%	XXXXXXXXXXXXXXXX	0.0	0.0%	
16.579 -	17.105>	13.0	16.9%	XXXXXXXXXXXX	2.0	2.6%	XX
17.105 -	17.632>	9.0	11.7%	XXXXXXXX	7.0	9.1%	XXXXXXX
17.632 -	18.158>	10.0	13.0%	XXXXXXXXX	18.0	23.4%	XXXXXXXXXXXXXXXXX
18.158 -	18.684>	0.0	0.0%		15.0	19.5%	XXXXXXXXXXXXXX
18.684 -	19.211>	0.0	0.0%		18.0	23.4%	XXXXXXXXXXXXXXXXX
19.211 -	19.737>	0.0	0.0%		11.0	14.3%	XXXXXXXXXX
19.737 -	20.263>	0.0	0.0%		5.0	6.5%	XXXXX
20.263 -	20.789>	0.0	0.0%		1.0	1.3%	X

C P

VALUE INTERVAL		07-82 FREQ.	07-82 PROS.	07-82 ONE X REPRESENTS 1	07-83 FREQ.	07-83 PROS.	07-83 ONE X REPRESENTS 1
15.000 -	15.526>	2.0	8.0%	XX	0.0	0.0%	
15.526 -	16.053>	4.0	16.0%	XXXX	0.0	0.0%	
16.053 -	16.579>	1.0	4.0%	X	0.0	0.0%	
16.579 -	17.105>	4.0	16.0%	XXXX	1.0	4.0%	X
17.105 -	17.632>	8.0	32.0%	XXXXXXXX	0.0	0.0%	
17.632 -	18.158>	6.0	24.0%	XXXXXX	1.0	4.0%	X
18.158 -	18.684>	0.0	0.0%		8.0	32.0%	XXXXXXXX
18.684 -	19.211>	0.0	0.0%		8.0	32.0%	XXXXXXXX
19.211 -	19.737>	0.0	0.0%		3.0	12.0%	XXX
19.737 -	20.263>	0.0	0.0%		3.0	12.0%	XXX
20.263 -	20.789>	0.0	0.0%		1.0	4.0%	X

Fig. 3. Histograms of sizegroup 15-18mm for all populations by 07.82 and after one year. (Tab. 1 points out clearly the difference in growth for this group).

Tab. 1. Size group 15-18mm by July 82 and the same individuals after 12 months. Note the mean growth for each group and the differencies.

Population \ Date		July 82	July 83		Mean growth
B 1	Mean	16.74	19.78	=	3.04 mm
	st.dev	0.77	1.04		
C 2	Mean	16.74	21.69	=	4.95 mm
	st.dev	0.80	1.29		
B 3	Mean	16.58	20.10	=	3.52 mm
	st.dev	0.85	1.17		
C 4	Mean	16.45	18.55	=	2.10 mm
	st.dev	0.83	0.80		
C P	Mean	16.96	18.92	=	1.97 mm
	st.dev	0.85	0.78		

To stress the fact that the populations show differentiations in growth pattern and size distribution (fig. 1, 2, 3 and tab. 1), 15 individuals were chosen randomly within three groups (15-18mm, 18-21mm and 21-24mm) and their growth plotted (fig. 4.).

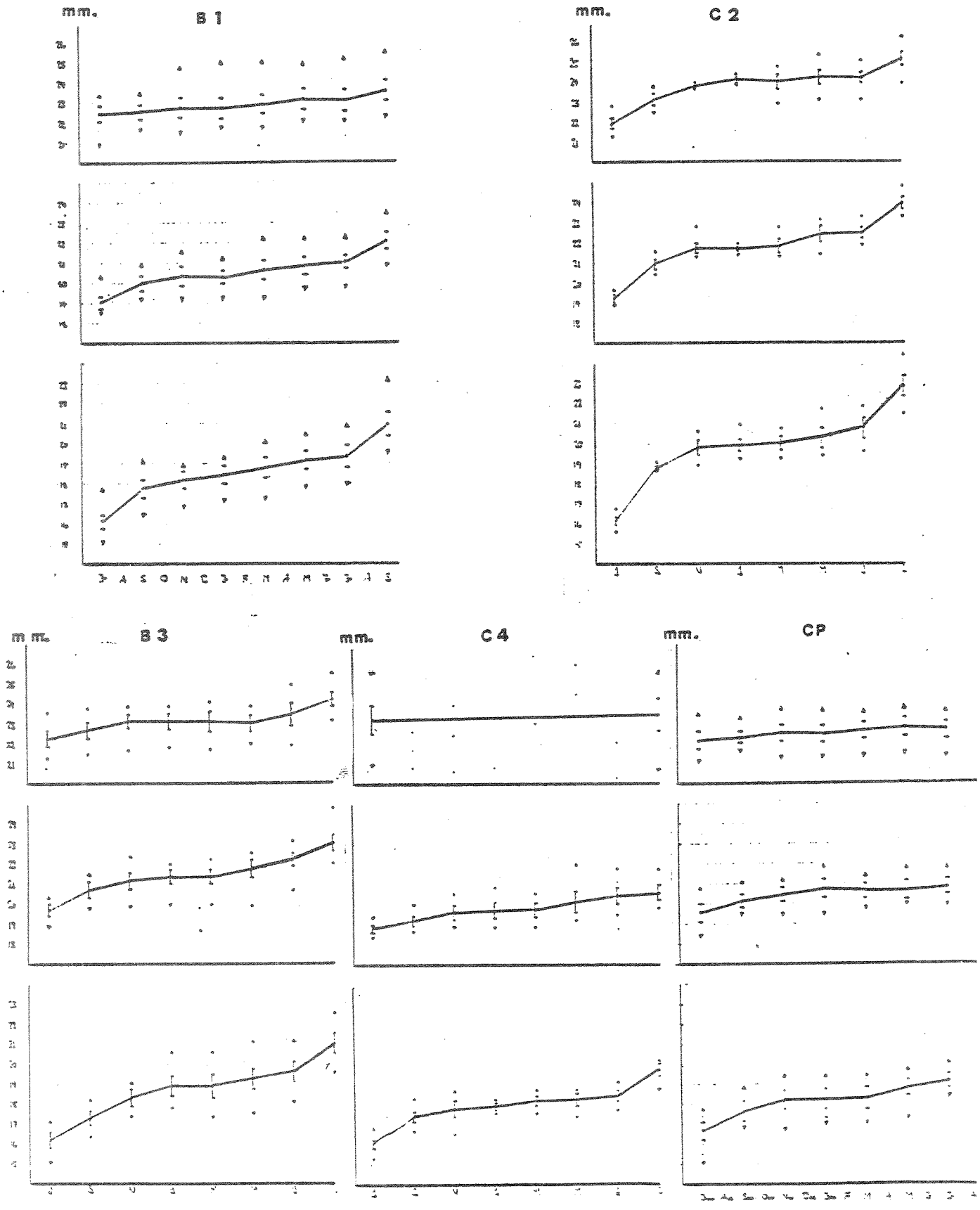

Fig. 4. Growth curves of 15 (randomly chosen) L.l. within three size intervals (15-17mm, 18-21mm and 21-24mm) from each basin and Cp.

A seasonability can be traced and the year split into periods of growth (shell growth) and quiescence. Three different size intervals based on recapture every second month outlines July-Sept/Oct as a heavy growth period. From November until March there seems to be little if no growth at all, which also is supported by field observations during this period. Activity and growth starts from March and onward, but the fast growing period does not seem to start before midsummer. These data also show a slower growth for both contaminated and stressed populations in B1 and B3 compared to C2, C4 and CP both have rather slow growth, but no conclusions are brought forward at this stage.

The interval 15-17mm stress the fact that L.l. of C2 (Fig. **5**.) are of best physological condition using growth as a method. Growth curves for B1, B3, C4 and Cp outlines some differentiations. Tests on significance are in preparation, but at this stage not yet available.

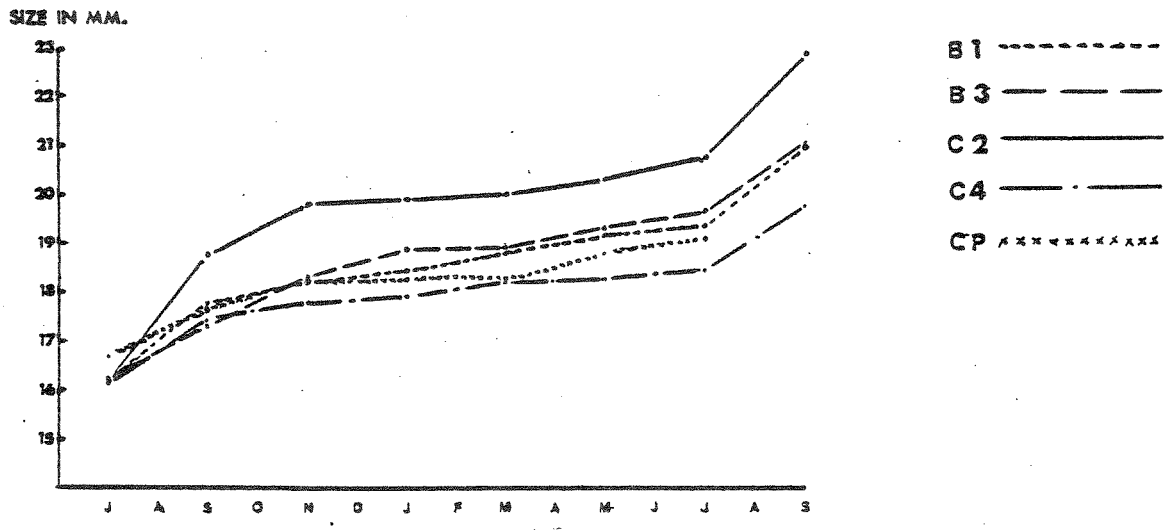

Fig. **5**. Growth curves of the interval 15-17mm for all basins and Cp. Note the level of C2 growth.

Another measurment for growth is accomplished by transects. Here all captured winkles both marked and unmarked are measured and give rise to fig. **6**. This gives an idea about recruitement which is not covered by the standard

capture-recapture procedure. The occurence of small winkles in C2 and C4 is striking in the March sample compared to the absence of such in B1 and B3 at 20/9-83.

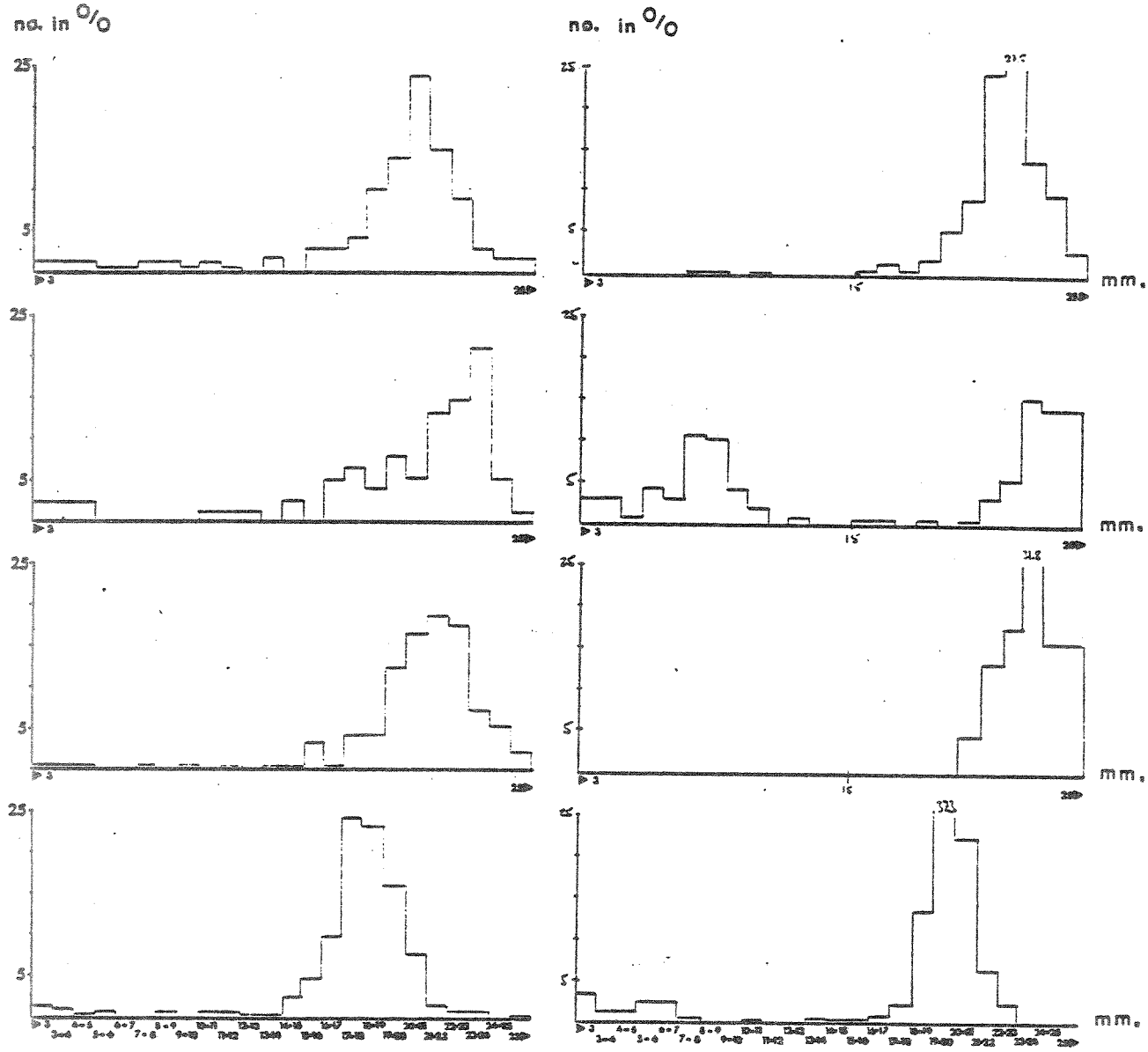

Fig. 6. A transect through positions 9-11 from all basins at 20/3-83 (left) and 20/9-83 (right) shows the size distribution of both marked and unmarked individuals.

Mortality.

To achieve an exact value of mortality two different methods are worked out. Within every sampling period we have searched for dead, marked winkles all over the basin bottoms (based on the assumption that L.l. from the stairs are washed to the bottom). This is the only practicable method for us to detect mortality of marked individuals and the approximately correct numbers. This method also give us seasonal variations of mortality. Statistics of mortality based on this method are in preparation and the number registred for each basin are shown in Tab. 2.

As an index of mortality we outline

$$\frac{\text{total no. marked ind.}}{\text{total no. dead marked ind. after 14 months}} = \text{M.index}$$

B1 and B3 are higher than C2 and C4. No comparison is made with CP due to lack of data. Deceased winkles are exposed to far higher degree of wave action at the pier than inside the basins. This effects the posibility of recapture in a negative way.

Tab. 2. Index of mortality Im for each basin. The enumerator is marked individuals and the denominator is dead marked individuals registred.

	Index of mortality I_m
B 1	$\frac{1970}{170} = 11.59$
C 2	$\frac{702}{28} = 25.07$
B 3	$\frac{246}{33} = 7.45$
C 4	$\frac{369}{29} = 12.27$

Another method for mortality estimations is analysis of sediment from all four basins. Two squares (42,5 x 42,5cm)

at two different positions in the southern half of all four basins were drained and the sediment thoroughly searched and all dead winkles measured with digital caliper (fig. 7).

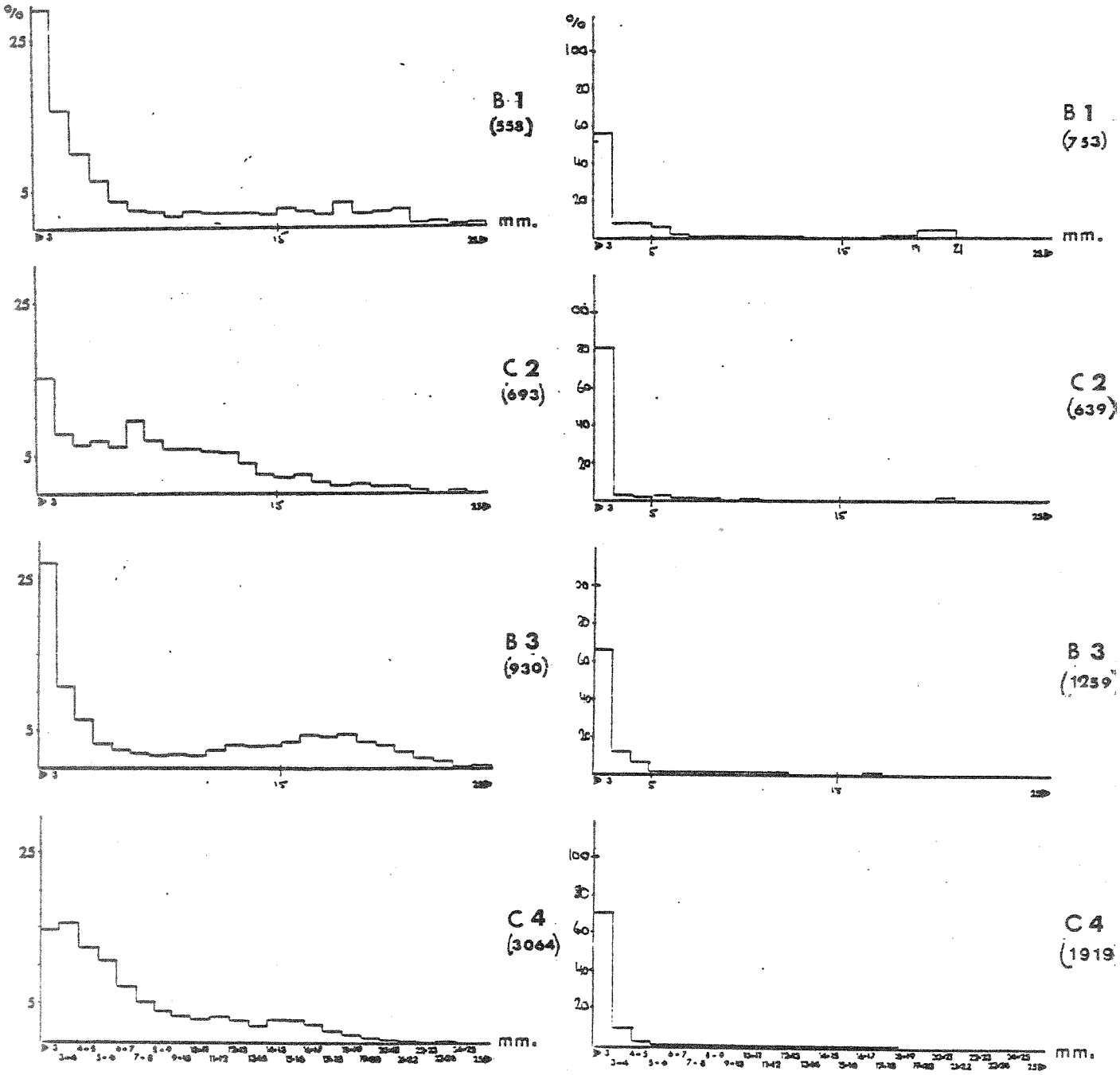

Fig. 7. Histograms showing size distribution of dead L.l. from sediment analysis (left 1/2-83 and right 1/10-83). Numbers in paranthesis refers to the number found and measured in each test. Sediment have a contagious distribution in the basins. For this reason these numbers have no authority.

The size-distribution histograms show a far higher mortality in B1 and B3 in Febr. 83. This applies especially to periwinkles up to 3-4mm long. As compared to the mortality index in Tab. 2. there is a similarity in B3 being a little higher than B1. No conclusions outlined with regard to mortality and oil contamination though.

Dry body weight

Periwinkles from all five localities have been sampled monthly since 20/I2-82 for dry body weight and HCN-analysis. Our technique, gently crushing the shell by using a vice, gives us the soft tissue without any loss. Destilled water is used for washing and to remove shell fragments. Drying lasted until constant weight was achieved (48 h. at 80 C^{o}).

A way of expressing the relation between dry body weight and lenght is given by,

$$\text{mean of } \log \frac{\text{length}}{\text{dry weight}} = Iw$$

were Iw is a value indicating the condition of the animals. High Iw gives low dry body weight in proportion to lenght and vice versa (Fig. 8)

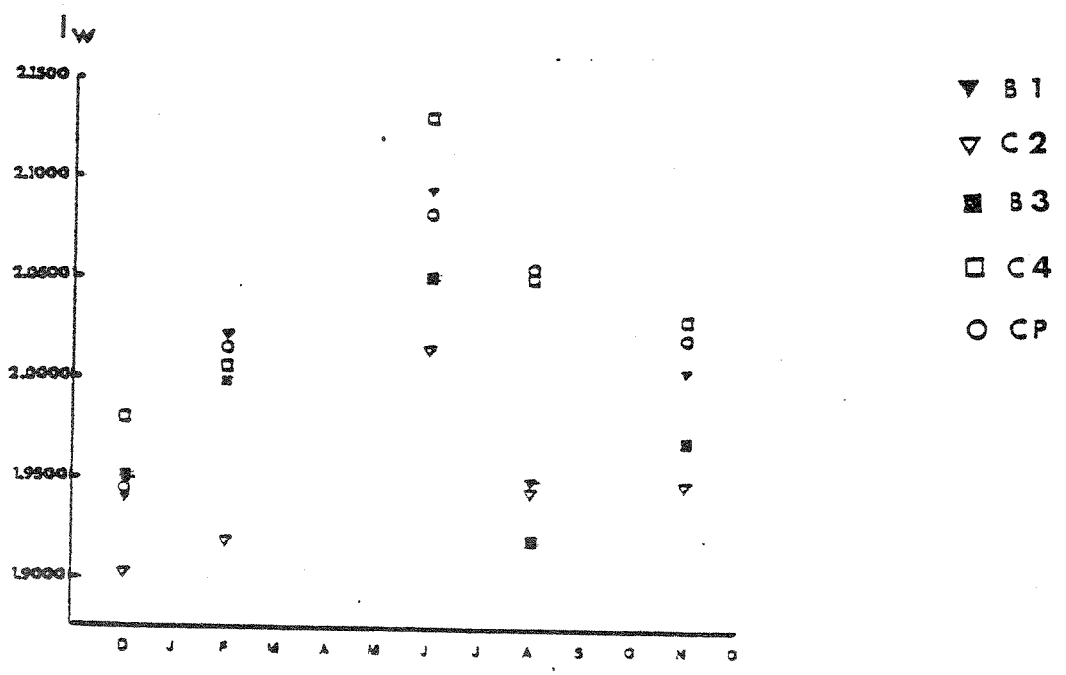

Fig. 8. Periodic variation of Iw based on the data in table 2.

The fluctuations of Iw during the year (Fig. 8) show similarities with the dry body weight analysis made by Grahame (1973) and Moore (1937).

Table 3. Values of Iw for the samples of 12-82, 02-83, 06-83, 08-83, 11-83, and the mean for each basin and period (n = 9 for each Iw-value).

Date	B1	C2	B3	C4	Cp	$\bar{x}$
12-82	1.9398	1.9027	1.9582	1.9796	1.9442	1.9444
02-83	2.0195	1.9175	1.9992	2.0065	2.0178	1.9921
06-83	2.0946	2.0147	2.0511	2.1317	2.0835	2.0751
08-83	1.9496	1.9463	1.9207	2.0519	2.0536	1.9844
11-83	2.0253	1.9692	1.9258	1.9914	2.0142	1.9842
$\bar{x}$	2.0058	1.9491	1.9706	2.0321	2.0227	1.9960

Two way annova and Student - Neumann - Keul tests gives significance at the level of p 0.05 for variation both during the year, and between the basins at the same date, except for C4 and CP which shows no significance.

The comparison of the relation between dry body weight and shell length, Iw, is based on the assumption that if Iw is high (low dry body weight in proportion to shell length), the animals are in poor condition. The optimal condition is high dry body weight in proportion to shell length, i.e. low Iw. On this assumption, the situation based on the data in Tab.3, is as follows.The Iw value of C2 is low and indicates L.1 in best condition compared to the other basins. The Iw value of B3 is of slightly higher value than Iw of C2 but not as high as the Iw value of B1. I.e. the L.1. in B3 is in a healthier state than in B1, but not as good as in C2. The Iw value of C4 and CP (show no significance) are the highest, and indicates L.1 in the poorest condition compared to the other basins. This ranking based on comparison of the Iw value of all basins and CP, correlates fairly well to the growth curves in Fig. 4 and 5.

Recruitment

The appropriate method to estimate recruitment is to count all L.1. below a certain length (which determine the animal to this years offspring) on the upper stairs in each basin. Fig. 6 shows recruitment in C2 & C4 but none in B1 & B3. These transects lies in the middle position of the basin and suppress the fact that there are small L.1. in the corners of the basin. A method to express the relation between the exact number of offspring and the mature population within each basin, is in preparation. At this stage we acertain that there is recruitmentin all basins, their origins are more doubtful though.

HCN analysis has not been possible to run due to frequent abruptions in analyser funktions. Hydrocarbon analysis of the tissue of L.1. winkles are carried out during December at SI.

Discussion

The size-frequency distribution of all five populations gives support to the idea that C4 and CPdiffer from the other populations in having a low mean length and more narrow spread in size-freqency (though not tested for significance). The comparisontests seem to be appropriate between B1, C2 and B3. (The growth of interval 15-18mm. (Fig.3 and Tab. 1) gives a picture of the situation.) As earlier mentioned, the C2 population shows highest growth and has the highest value of Iw in addition to the highest Im value. In respect to the test methods (growth, dry body weight and mortality comparison), it seems to be distinct that both populations of the oil contaminated basins show an aggravated condition.
The situation seems to be more complex taken into consideration the values of C4 and CP. This gives rise to a new serie of questions. Which other effects depress the growth in C4 below C1 level and make the pattern more like CP ? Are CP significant to other Oslofjord populations described by Eidnes (1982)?
Do the basins differ so much with each other at the starting point that C2 and C4 cannot be used as control basins? Are our methods valid for monitoring the situation at Solbergstrand?
Some assumptions may be outlined in near future. (We have just succeeded in running our data programs (Jacobsen 1982) which gives timesharing help with a high number of possibilities.)

01-12-83. Einar Lystad and Kjell Moe

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MARINE RESEARCH STATION SOLBERGSTRAND

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12. ENERGY BALANCE in *Littorina littorea*.

T. Bakke

H. Juelsen
E.Ø. Sahlqvist

Aim and purpose:

- to investigate the energy uptake and loss status of the four basin populations of *L. littorea* plus one wild population at two months' intervals during the period of oil dosing and if necessary during a recovery phase

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

- to link the individual energy budget considerations to the measurements of individual growth and mortality (Lystad & Moe) and to reproductive success (Bayne's group) in the species with and without oil stress. Attempts will also be made to link the feeding intensity of *L. littorea* to structure and development of the substrate microlayer at which *L. littorea* is grazing (Bokn & Pedersen)

- to link effects on energy utilization in *L. littorea* to tissue levels of oil hdyrocarbons.

Methods:

The investigation is based on measurements on 10 individuals of average size from each population on each occation. The same individuals are subjected to all of the following tests:

Food uptake.

Two types of feeding experiments are performed, one with a food source of microalgae and macroalgal sporelings grown on vertical glass slides in one control basin. The animals have been incubated for periods of 12 to 48 hours in the appropriate basin in individual plankton-net closed chambers (200 ml) supplied with one algae-covered glass slide. For each group of 10 individuals 4 empty chambers with slides are immersed as controls. At the end of incubation the remaining algal material of each slide has been transferred quantitatively to GF/C-filters, and rinsed quickly with destilled water, for determination of dry weight (DW) and

ash-free dry weight (AFD).

In the other type of feeding experiment the animals are incubated in individual chambers (80 ml) in the appropriate basin, with pieces of *Ulva lactuca* as food. For each set-up with 10 individuals two chambers with only *Ulva* are used as controls. The pieces of *Ulva* are preweighed to the nearest 0.1 mg after removal of water with blotting paper and drying in the air (20°C) for 10 min. The snails are allowed to feed on the *Ulva* for 48 to 72 hours (depending on season), after which the pieces again are weighed after blotting and drying.

Respiration

Aquatic oxygen consumption is estimated on individual snails in closed chambers (60 ml) supplied with a magnetic stirrer and an YSI mod. 5331 oxygen probe, connected to a pen recorder. The chamber is immersed in a water bath with the current basin temperature.

Aerial oxygen consumption has been estimated only on a few occations in a pilot experiment with the use of the same type probe and a very small chamber (3-5 ml).

Ammonia excretion

Individual snails are transferred to beakers with 70,0 ml of GF/C filtered control basin water, and incubated at the current basin temperature for 2 hours. The water samples are then fixed with 1% 8N H_2SO_4 prior to analysis of NH_4^+ concentration. Analysis have been performed at NIVA after the Solorzano method (Koroleff 1970). Two control beakers with water, but no animal, are always applied.

Faecal production

This has been estimated on groups of 50 individuals transferred directly from a basin to 1 l GF/C filtered sea water. Incubation has been 2-3 hours either in the appropriate basin, or in a water bath. The faecal material produced is transferred quantitatively to GF/C filters, and rinsed with distilled water for analyses of DW and AFD.

Assimilation efficiency

Attempts to estimate the percent food assimilated have been made by use of the AFD/DW ratio of the faecal material and of the microalgal food material grown on the feeding slides, according to Conover (1966). Attempts have also been made to use the AFD/DW ratio of the algal growth on the granite chips used by Bokn and Pedersen in antoher subproject.

O:N ratio

The atomic ratio of oxygen consumed to nitrogen excreted have been computed from the individual aquatic respiration and excretion rates.

Individual size and pre-history

All the individuals (except for the wild population) have been selected from the marked fraction of the basin populations. The shell height of

each snail is measured for comparison to previous measurements and growth rates according to Lystad and Moe. On some occations the individuals have been killed at the end of the physiological measurements, by quickly immersing them in boiling sea water. The soft tissue part with operculum have been stored for DW and AFD determination. On occasions cooccurrent with the samplings of Lystad and Moe, the snails have been returned to the basins, since the relation shell length to DW is expected to be derived from Lystad & Moe.

Results and discussion

The following periods of measurements have been performed:

15 - 31 May
26 June - 8 July
5 - 9 September
(12 - 19 December)

Due to late arrival of analytical balance and other tasks demanding priority, much of the material frozen for AFD and DW determination is still in the freezer. Hence the feeding experiments of July are only partly worked up and those of September (and December) not worked up.

Feeding rates

Microalgae

In May the feeding rates of the High Oil (HO) population was significantly reduced compared to the other populations (Table 1). The control basin 4 (C4) population had significantly higher consumption rate than the Low Oil (LO) and the control basin 2 (C2) populations. The figures shown in Table 1 are basic values not corrected for snail size.

In July (only partly worked up) the HO population had significantly higher consumption rate than the C2 population (Table 1). The extreme low C4 consumption shown in the table is an obvious underestimation since the snails had grazed away all the microalgae before incubation ended. This population was repeated but the filters are not yet analysed. Generally the July series is of doubtful value since the consumption rates were so high as to nearly remove all food material even at 12 hours incubation.

The technique applied has several serious disadvantages. Both food selection of the snails and their ploughing effect on the substrate as they move over it, makes it difficult to judge how much of the algal layer is really consumed and how much is detached and washed away. The ash content of the control surfaces was lower than of the grazed surfaces in July, indicating food selection of the snails, but this was not found in May. Also when recovered after incubation only a small fraction of the individuals were found actually on the food surface indicating a haphazardons crawling in the chamber. How much of the time which is actually spent on the food surface is therefore not known, but this behaviour obviously results in an underestimation of the real feeding rate occuring in nature where the snails move continously over a food substrate. A preliminary comparision of the energy intake with the energy

used in respiration also shows that especially in May the estimated energy uptake accounts for only about 50% of the respiration loss, indicating underestimation of the food uptake.

If one assumes that the snails have no preference to stay at food substrate, then the consumption rates should be multiplied by the ratio of food slide to chamber surface (which is 208/17.5 = 11.9). In any case the values derived at must be considered to be relative, but they can still be used to compare the populations.

Ulva lactuca

The use of *Ulva* as appropriate food for Littorina has been reported several times (cf Graham 1973 with references). *Ulva* was applied in this project from July, but we then had to do the weighings at NIVA. This process led to erroneous results (f.i. negative consumption) since the wet weight of *Ulva* is not at all stable. Figure 1 shows the weight decrease with time in air after removal of excess water by blotting.

The figure indicates that in order to obtain a stable wet weight the algae will have to be dried nearly to dry weight. For the September and December series I have tried to standardize the weighing process by weighing twice and always relating back to the control pieces. The data for these series have not been worked up yet.

Faecal production and assimilation efficiency.

The natural faecal production rates in May and July show no clear or common trend (Table 2.) Generally the greatest difference was found between the C2 and C4 populations.

The ratio of AFD to DW of the faeces was highest in the C2, C4 and W-populations both in May and June, which is surprising since it seems to indicate a better food utilization in the oiled populations. On the other hand, if the organic content of the faeces is a funtion of the organic content in the food, it might indicate either a better ability to select the organic fraction of the food in the control populations or a higher organic content in the microalgal layers of the C2 and C4 basins compared to the HO and LO. The granite chip data (cf Bokn) do not indicate such a difference in the microlayers. Hence the results may indicate an effect of oil on the food selection ability.

Assimilation efficiency has been calculated on basis of the ratios above and the ratio of AFD to DW of the slides used in feeding experiments. By this use of a common AFD/DW food ratio and assuming no difference in food selection, the oiled populations appear to have the highest efficiencies. As this is highly unlikely, it seems most appropriate to estimate the assimilation efficiency from the faecal material produced after feeding on a defined food such as *Ulva*. This will be done in the 1984 series.

Respiration

In May the HO population had significantly reduced oxygen consumption compared to the LO and C2 populations (Figure 3) which were not different.

The lowest respiration was found in the C4 animals but these had been caged for 2 months prior to analysis (with *Ulva* as food). A new set of C4 animals (4 only) showed a mean respiration of 92.73 ± 7.1 µl O_2/h (asterisk in Figure 2).

In July the difference between HO and the other populations had disappeared, but the difference between the populations was still significant ($p < 0.01$ analysis of variance). t-tests showed the W respiration to be significantly lower than all the others and also that C4 was significantly lower than HO, LO and C2. HO did not differ from LO and C2.

In September much of the same trend was found as in July. Highest respiration was found in HO. LO and C2 did not differ, but were both significantly lower than HO. The lowest respiration was found in C4 and W, but only the former differed significantly from LO and C2. One should, however, note that these values are not yet adjusted for snail size, and the C4 and W animals have always been 2-4 mm smaller in mean than the other populations.

Ammonia excretion.

In May and July the mean excretion rates of the populations differed significantly ($p < 0.05$, analyses of variance), but with no genereal trend (Figure 3). In May the populations were ranked as LO > C4 > HO > C2 with respect to excretion. Ranking in July was HO > C2 > LO > W > C4. In September the population means were not significantly different.

Elemental O:N ratios.

All populations showed gradual increase in this ratio from May to September, reflecting utilization of the summer food supply. In May the oiled populations showed the lowest ratios, hence enchanced protein catabolism compared to the controls. In July this trend had disappeared. The lowest ratio was found in the HO population, the highest in the LO population. In September this was reversed with HO showing the highest ratio. The ranking of the three control populations was the same in July and September: C4 > C2 > W.

Future plans.

The general idea is to continue the measurements in 1984, and if significant oil effects emerge, also into the recovery period after dosing has terminated. Several improvements in the measuring techniques will have to be developed, especially tied to measurements of food uptake and utilization. Emphasis will be put more strongly on the use of *Ulva* as a well defined food, but this also demands a better means of determining the wet weight of the alga. Drying for 2 hours will be tried, and especially how this affects loss of material during subsequent incubation for 1-3 days in the basins. The possibility of weighing the damp pieces within closed tared bags to prevent evaporation will also be tried. The use of a microalgal food should continue as this reflects more closely the natural food, but incubation should be in the laboratory to ensure

recovery of all material not eaten by the snail. In any case no more measurement periods will be done until all the 1983 material is worked up. This also implies relating the various processes to the individual size and prehistory of growth.

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

Graham, J., (1973). Assimilation efficiency of *Littorina littorea* (L.) (Gastropoda : Prosobranchiata). J. Anim. Ecol. 42, 383-389.

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

Table 1. Relative feeding rates of L. littorea on a diet of natural basin microalgae. Mean individual energy uptake (J/day) ± S.E.

Population	May	July	Sept.
H 0	5.73 ± 1.04	167.21 ± 5.24	n.a
L 0	22.15 ± 1.32	n.a.	n.a
C 2	21.70 ± 1.06	118.31 ± 1.28	n.a
C 4	29.40 ± 1.01	34.97 ± 0.80*	n.a
W	n.t.	n.a	n.a
Significance of popul. difference	p <<0.01		

n.t. not tested

n.a. tested, but data not yet analysed

* The value understimates the real feeding rate.
New test was performed but data not yet analysed.

Table 2. Faecal production and assimilation efficiency based on the AFD/DW ratio of potential food grown on vertical surfaces in basin water and the AFD/DW ratio of faecal pellets produced from natural basin feeding.

Popul.	Ratio AFD/DW Food	Ratio AFD/DW Faeces	mg DW/ind.d. Faecal production	% assimi-lation
May				
H 0	0.444	0.150	8.4	77.9
L 0		0.112	12.9	84.2
C 2		0.255	7.7	56.6
C 4		0.462	11.4	neg.
W		n.t.	n.t.	n.t.
July				
H 0	0.558	0.264	10.9	71.6
L 0		0.209	16.6	79.1
C 2		0.325	20.1	61.9
C 4		0.295	6.2	66.9
W		0.454	8.0	34.1

Table 3. Ratio of elemental oxygen consumed to ammonia-nitrogen excreted based on population means.

Population	May	July	September
H O	15.5	26.0	58.2
L O	20.0	47.7	41.2
C 2	29.1	30.6	43.5
C 4	23.1*	40.0	54.4
W	n.t.	30.0	37.5

* The respiration value is based on individuals not previously caged (cf respiration chapter).

Figure 1. Loss of weight in two pieces of Ulva lactuca of different size, when kept at room temperature. The dry weights shown are after 24h drying at 70°C of the same pieces.

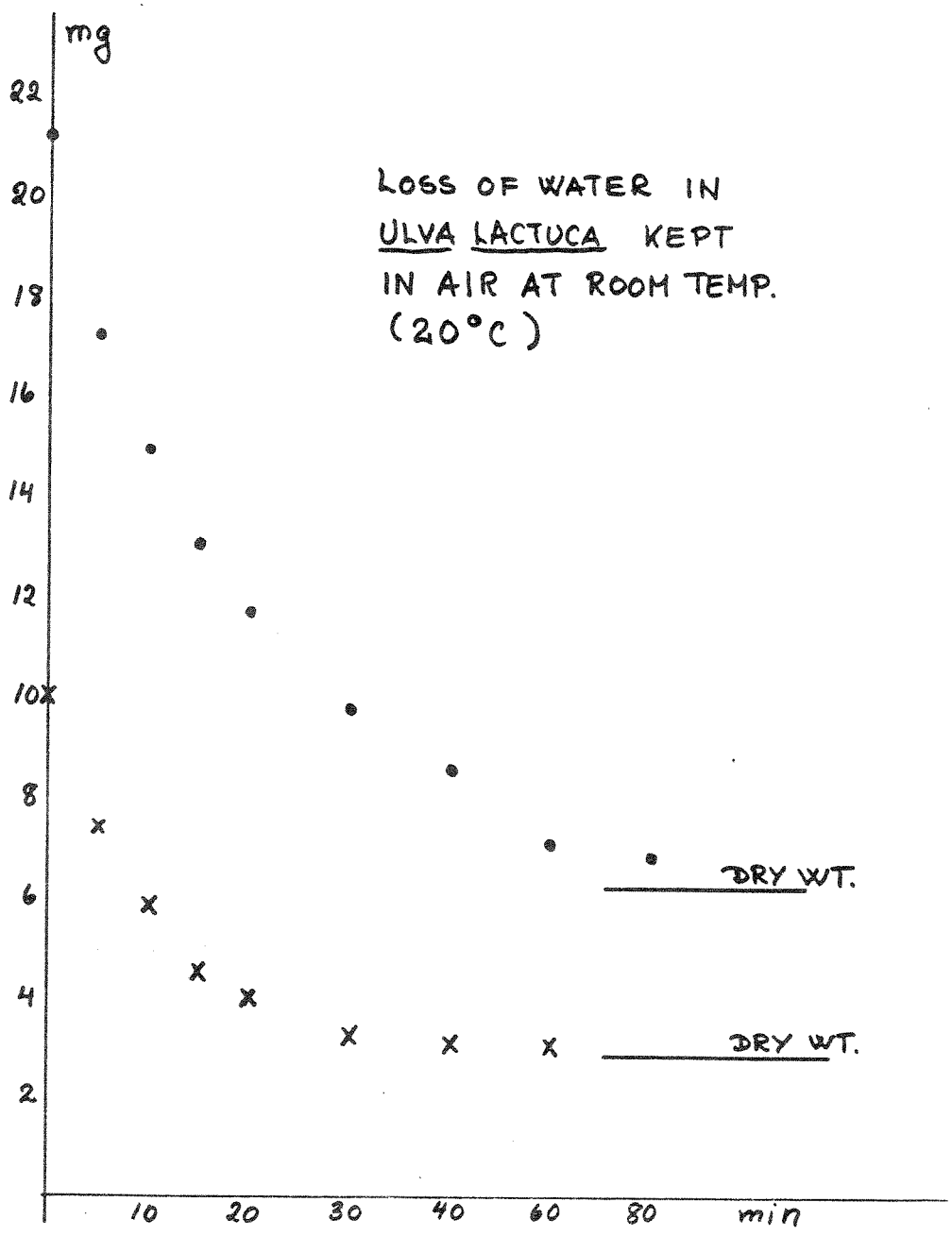


Figure 2. Oxygen consumption of basin and wild populations of Littorina littorea. The histograms show means of 10 individuals ± one standard error of the mean. The asterix of the May series is the mean of 4 uncaged individuals whereas the C 4-histogram shows the mean of 10 individuals having been caged for 2 months with Ulva lactuca as food.

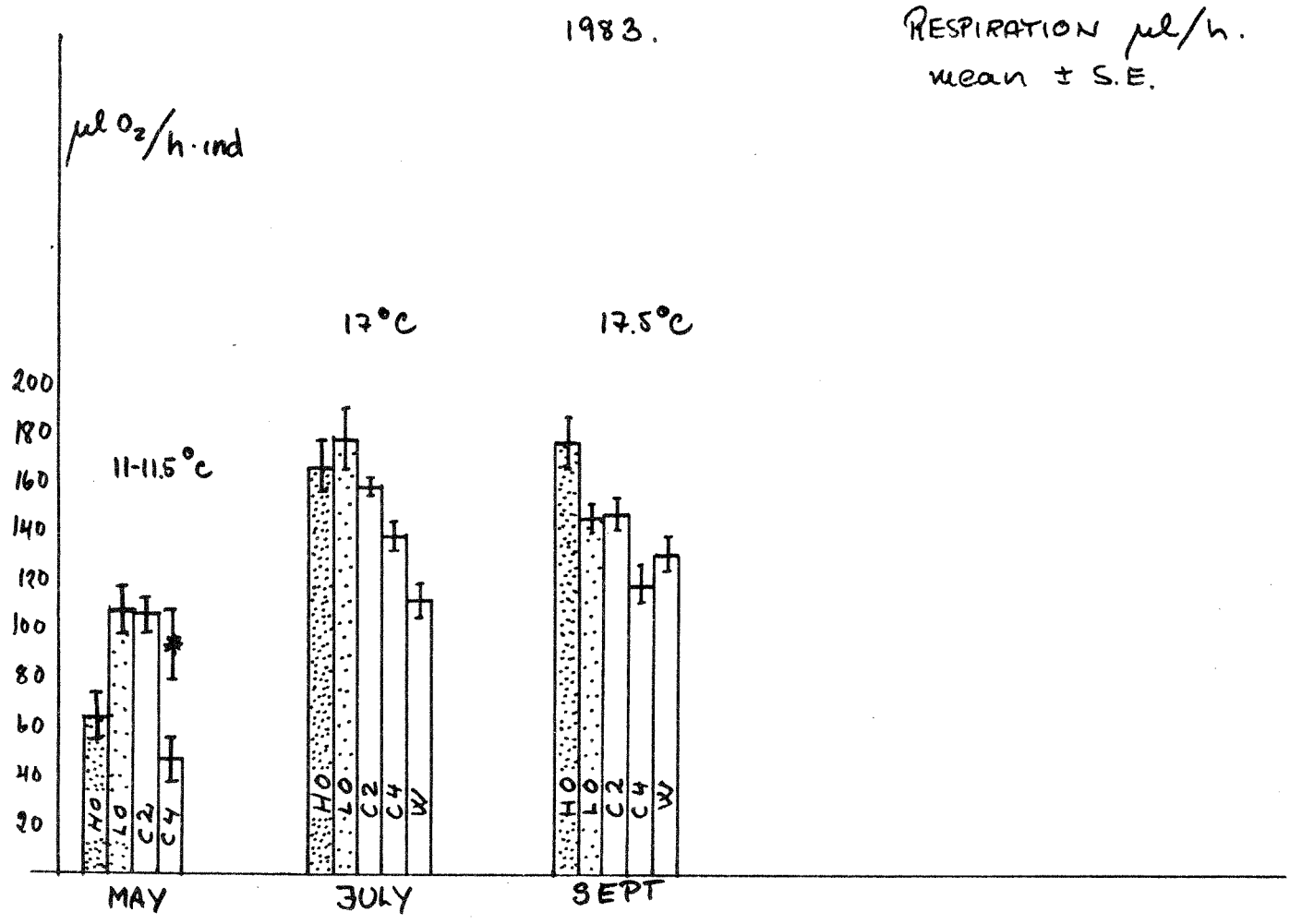
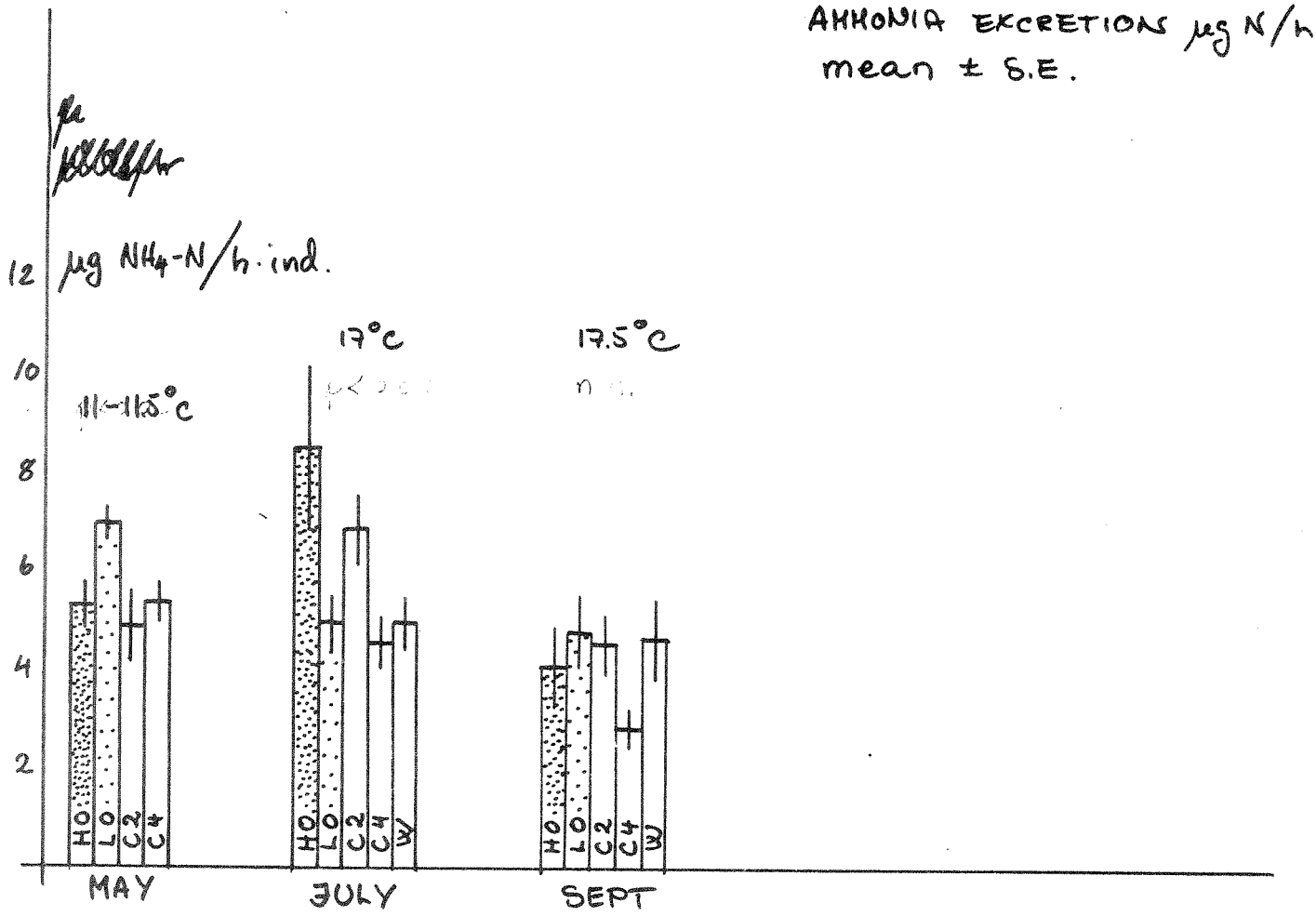


Figure 3. Excretion of ammonium nitrogen in basin and wild populations of Littorina littorea. The histogram show means of 10 individuals ± one standard error of the mean.



MARINE RESEARCH STATION SOLBERGSTRAND

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13. Sublethal Biological Effects and Short-Term Recovery of Mussels (Mytilus edulis) and Winkles (Littorina littorea) following chronic exposure to petroleum hydrocarbon

Authors: John Widdows, Michael Moore and David Lowe

Address: Institute for Marine Environmental Research, Prospect Place, The Hoe, Plymouth, PL1 3DH, England.

General Introduction

Previous laboratory studies at IMER have shown that physiological and cellular processes of Mytilus edulis are affected by chronic exposure to low concentrations of the water-accommodated fraction of North Sea crude oil (Widdows et al. 1982; Bayne et al. 1982). In addition, field studies have demonstrated cellular stress responses in Littorina littorea collected from sites near the Sullom Voe oil terminal (Moore et al. 1982).

The two main objectives of this study were:

1) to investigate the sublethal physiological and cellular responses of Mytilus edulis and Littorina littorea following chronic exposure to two concentrations of petroleum hydrocarbons, and
2) to study the short-term recovery of Mytilus and Littorina from the toxic effects of petroleum hydrocarbons.

Part 1: Physiological Responses - John Widdows

Material and Methods

The main experiments were carried out in May-June 1983. During this period the seawater temperatures in the Solbergstrand experimental basins were 11 to 13°C, salinities ranged from 17.5 to 12.5°/oo and suspended particulate load was 1.9mg l^{-1} (± 0.15 se) of which ~35% was particulate

organic matter (POM).

1) Effects of hydrocarbon exposure

Physiological responses, such as rates of feeding, food absorption efficiency, respiration, excretion and 'scope for growth' were determined for groups of Mytilus (n=12) and Littorina (n=8) collected from basin 1 ('high oil'; ∿140ppb), basin 3 ('low oil'; 30ppb) and basin 4 ('control'). Responses were measured using the techniques described by Widdows et al. (1982). The feeding rates of Littorina were determined gravimetrically by placing individuals in net-covered 100ml beakers and measuring the weight-loss of damp-dried pieces of Ulva lactuca over a period of 2 to 3 days. Faeces accumulated in the beakers were also sampled for assessment of absorption efficiency. All physiological responses were corrected to a 'standard size animal' using appropriate weight exponents (Mytilus mean dry weight = 0.25g; Littorina mean dry weight = 0.16g).

2) Recovery from hydrocarbon exposure

Groups of mussels were transferred and maintained in 'clean water' in basin 4 and their physiological responses were measured after 2½, 5 and 10 days of recovery.

3) Analysis of Tissues for Hydrocarbons

Tissue samples of Mytilus and Littorina were collected for hydrocarbon analysis. These have been stored at -30°C and await analysis by GC&HPLC.

Results and Discussion

A. Mytilus edulis

1. Effects of chronic hydrocarbon exposure

Mussels sampled from the three experimental basins had similar shell

lengths (32mm), but specimens from the 'high oil' basins had significantly lower dry tissue weights than those from the 'low oil' and 'control' basins (Table 1). In addition, there was a marked reduction in the relative size of the mentle (storage and gonadal tissue) in the 'high oil' group.

All measured physiological responses of Mytilus were affected by exposure to petroleum hydrocarbons (represented by Day 0 before recovery). A proportion of mussels from both 'high and low oil' conditions were partially closed and relatively inactive for extended periods of time (>4h) during physiological measurement, and the proportion increased with increasing hydrocarbon concentration (∿16% in 'low-oil' and ∿50% in 'high-oil' group; Fig. 1 Day 0). Therefore the results have been expressed in two forms,

i) mean ± s.e. of the total sample, n=12 (histograms), and

ii) mean ± s.e. of 'active individuals' (closed circles), in order to distinguish the effects of hydrocarbon exposure on basic processes from the indirect effect on valve closure and inactivity.

There was a marked effect of hydrocarbon exposure on the suspension-feeding (= clearance rate) of Mytilus edulis. High and low oil exposed mussels had significantly reduced clearance rates, 0.66 and 0.82 l h^{-1} respectively, compared to 1.55 lh^{-1} for the control mussels (Fig. 2, Day 0 - histograms). When the inactive specimens were omitted the clearance rate of the oil exposed mussels was still significantly lower (∿0.95 l h^{-1}; Fig 2 - closed circles).

The mean rate of oxygen consumption declined with increasing hydrocarbon concentration (Fig. 3, Day 0 - histograms) due to the increasing proportion of relatively inactive mussels. There was no significant effect of hydrocarbons on the oxygen uptake by 'active' individuals (Fig 3 - closed circles).

The rate of ammonia excretion was enhanced by hydrocarbon exposure, and this was significantly higher than the control when 'inactive' individuals were excluded (Fig 4, Day 0 - closed circles).

Food absorption efficiency (Table 2) was reduced by nearly 50% from 0.61 (controls) to 0.32 (oil exposed) and only small quantities of faecal material were produced by the 'high oil' mussels.

The physiological responses, feeding, digestion, respiration and excretion represent important components of the energy budget of the mussel. Conversion of these processes into energy equivalents enables the energy available for growth and reproduction, termed the scope for growth, to be calculated. Only mussels that were 'open' and respiring were used in the calculation of the mean scope for growth on Day 0 (Fig. 5). Mussels from both low and high oil basins showed a significant reduction in scope for growth, $\sim$ 1 J h^{-1} compared with 10 J h^{-1} for the control mussels. However, the exclusion of the high proportion of inactive individuals from the 'high oil' group (Fig 1, Day 0) is likely to lead to an overestimate of the scope for growth for this group.

2. Recovery from hydrocarbon exposure

Mussels from the high and low oil basins were placed in the control basin 4 and physiological responses were measured after 2½, 5 and 10 days.

The recovery of the 'high-oil' mussels was more rapid than the recovery of 'low-oil' mussels. Clearance rate of the high oil group recovered within 2½ days and by day 10 was higher than the controls (Fig. 2). In contrast, the clearance rates of the low-oil group recovered more slowly and were lower than the controls and high oil group after 10 days.

The mean rate of oxygen consumption by mussels from the high and low oil groups indicated recovery within 5 days, but this was mainly due to an increase in the proportion of 'active respiring' mussels.

The elevated rate of ammonia excretion by 'high oil' mussels recovered within 2½ days, whereas the 'low oil' group required 5 days to recover.

Food absorption efficiency showed no evidence of recovery during the 10 day period. The digestion and absorption efficiency in the control mussels was 2-fold greater than the oil exposed mussels (Table 2). These findings are in agreement with the observations on the cellular condition (structural and functional) of the digestive gland which show minimal recovery during 10-20 days in clean water (see Moore and Lowe).

The reduced scope for growth values for mussels exposed to oil were near near the maintenance level (= zero value). During the 10 day recovery period the 'low-oil' group showed little increase in scope for growth, whereas the 'high oil' group significantly increased scope for growth to ∿50% of the control value after 10 days (Fig. 5).

Therefore recovery of Mytilus edulis following chronic exposure to hydrocarbons appears to require more than 10 days. Feeding recovers within 5-10 days but digestive processes take longer than 20 days. This suggests that the reduction in feeding and digestive efficiency is not a direct effect of the concentration of hydrocarbons in the water, but is probably due to the concentration of hydrocarbons accumulated within the different tissues. Consequently the rate of recovery is likely to be a function of the rate of hydrocarbondepuration from the tissues. Laboratory studies at IMER have demonstrated that the rate of depuration of labelled naphthalene is 2 to 4 times faster in the gills than in the digestive gland. This may be a partial explanation for the more rapid recovery of clearance rate, a function of gill ciliary activity, compared with the slower recovery of digestive processes associated with the digestive gland.

There has been considerable mortality of mussels in basin 1 during 7 months of exposure to 140 ppb. Two aspects of the physiological responses of 'high-oil' mussels suggests that there may have been the selection of a more resistant phenotype in basin 1.

Firstly, there was no evidence of a simple dose-response relationship. The mean values of scope for growth for both high and low oil exposed groups on day O were similar and near zero. Those severely stressed 'high-oil' mussels with a negative scope for growth would have died within the 7 month exposure period. And secondly, the recovery of clearance rate, ammonia excretion and scope for growth was more rapid in the 'high oil' group than in the 'low oil' group.

B. Physiological responses of Littorina littorea to chronic hydrocarbon exposure

The feeding rate of Littorina littorea declined with increasing hydrocarbon concentration (Fig 6A). Absorption efficiency of food (Ulva) was high (85-90%) and not significantly affected by hydrocarbon concentration. Respiration rates were higher in the oil exposed individuals but only significantly enhanced in the 'low oil' group (Fig 6B). There were no significant differences between the ammonia excretion rates by Littorina from the three experimental conditions (Fig 6C). Scope for growth of Littorina declined with increasing hydrocarbon concentration (Fig 6d). The control group had a positive scope for growth (+ 1.9 J L^{-1}), the 'low oil' winkles had a scope for growth near zero, and the 'high oil' specimens had a negative scope for growth (-1.7 J L^{-1}). The generally low and negative scope for growth values obtained for both oil conditions suggest that the feeding rates may have been underestimated. Feeding rates may have been reduced in response to the change in diet, the time taken to locate the food, disturbance, or continual immersion; however, the relative effects of hydrocarbon exposure are still apparent.

Table 1. Shell Length and Tissue Weight Data for Mytilus edulis

Condition	Shell Length (mm)	Dry Mass (g)	% Mantle tissue
Control	31.9 ± .62	0.311 ± .026	17.9 ± 1.5
Low Oil	32.1 ± .57	0.272 ± .024	16.9 ± .024
High Oil	31.8 ± 1.15	0.165 ± .016	11.6 ± 1.2

Mean ± s.e. n=12

Table 2. Food absorbtion efficiencies of Mytilus edulis during recovery from oil exposure. Mean ± s.e.

Condition	Days			
	0	2.5	5	10
Control	0.61 ± 0.03	0.59 ± 0.03	0.55 ± 0.01	0.58 ± 0.03
Low Oil	0.32 ± 0.06	0.22 ± 0.06	0.30 ± 0.04	0.30 ± 0.03
High Oil	0.33	0.37 ± 0.03	0.24 ± 0.03	0.30 ± 0.03

Fig. 1.

FREQUENCY DISTRIBUTIONS OF ACTIVE MUSSELS BASED ON CLEARANCE RATES.

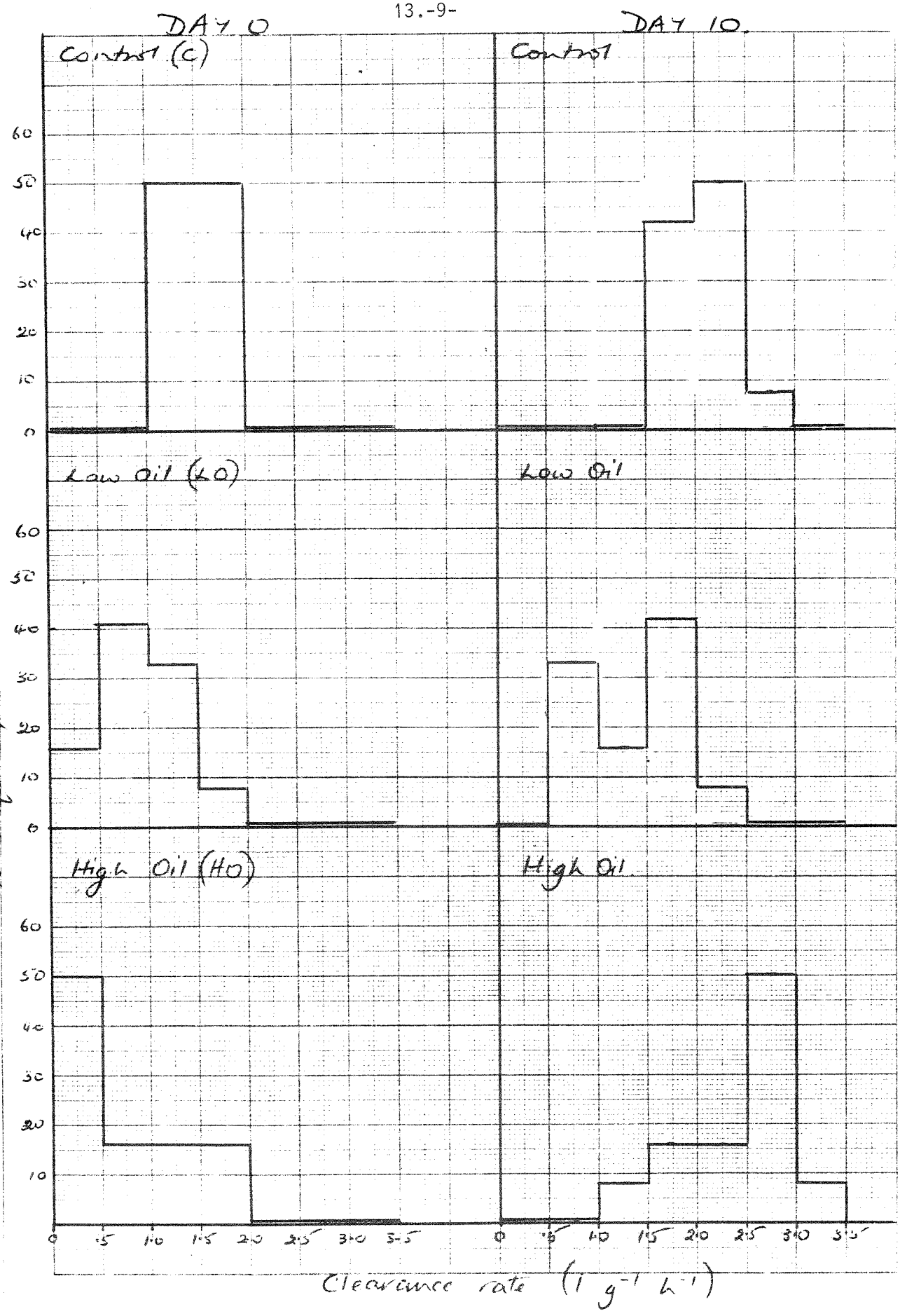


Fig. 2

CLEARANCE RATE BY MYTILUS EDULIS (Standard animal 0.025g)

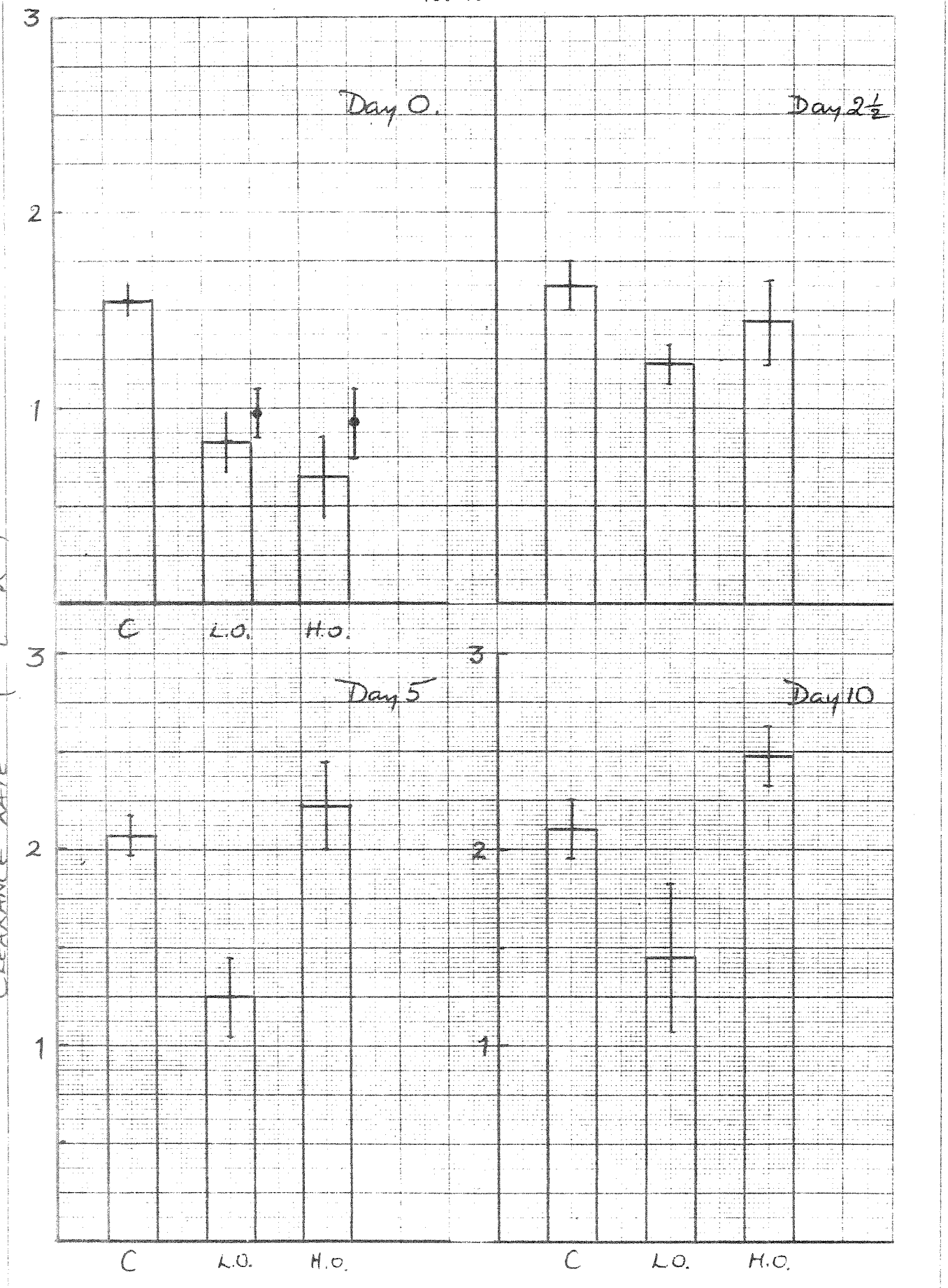


Fig. 3.

OXYGEN CONSUMPTION BY MYTILUS EDULIS

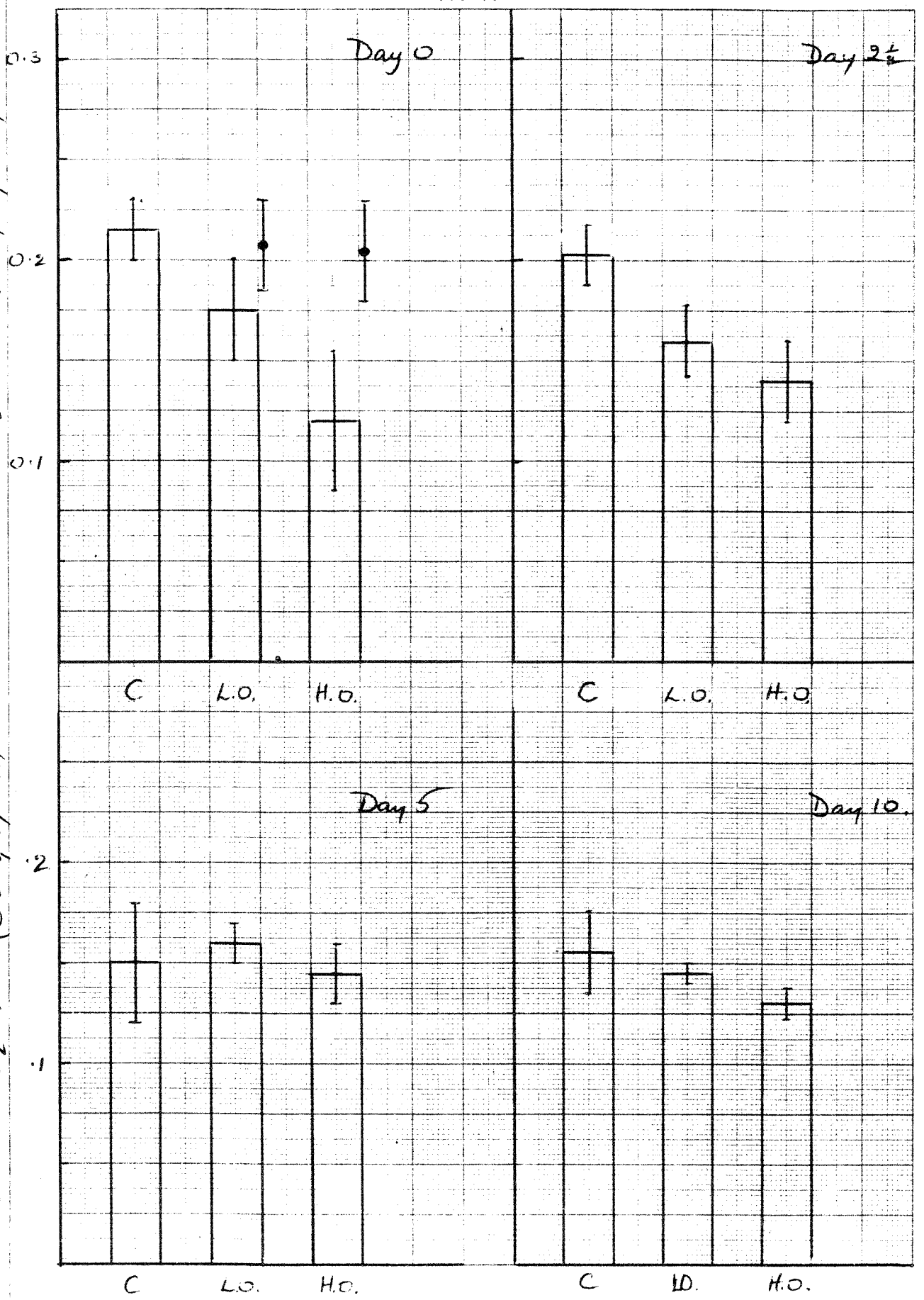
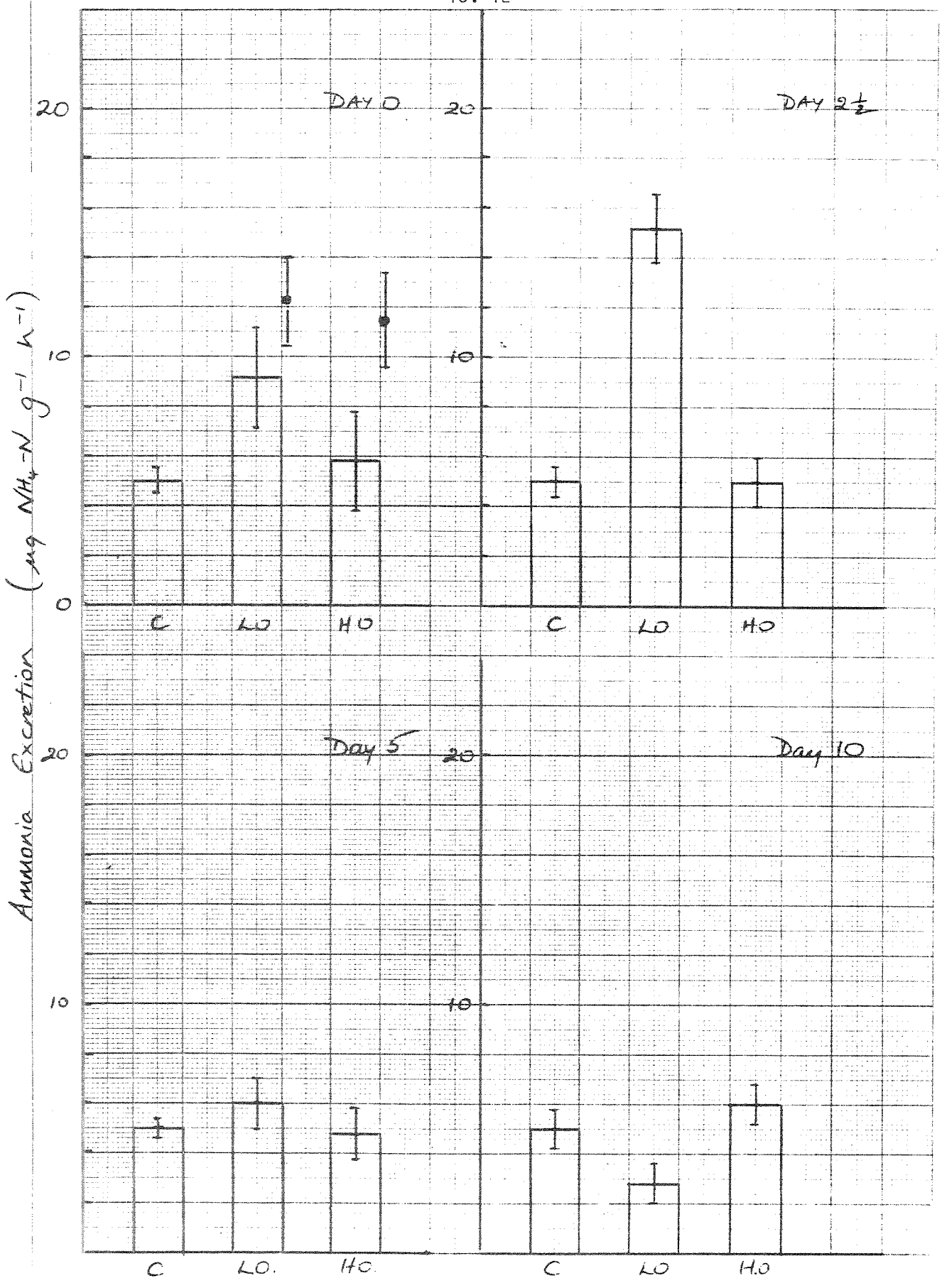


Fig. 4

AMMONIA EXCRETION BY MYTILUS EDULIS



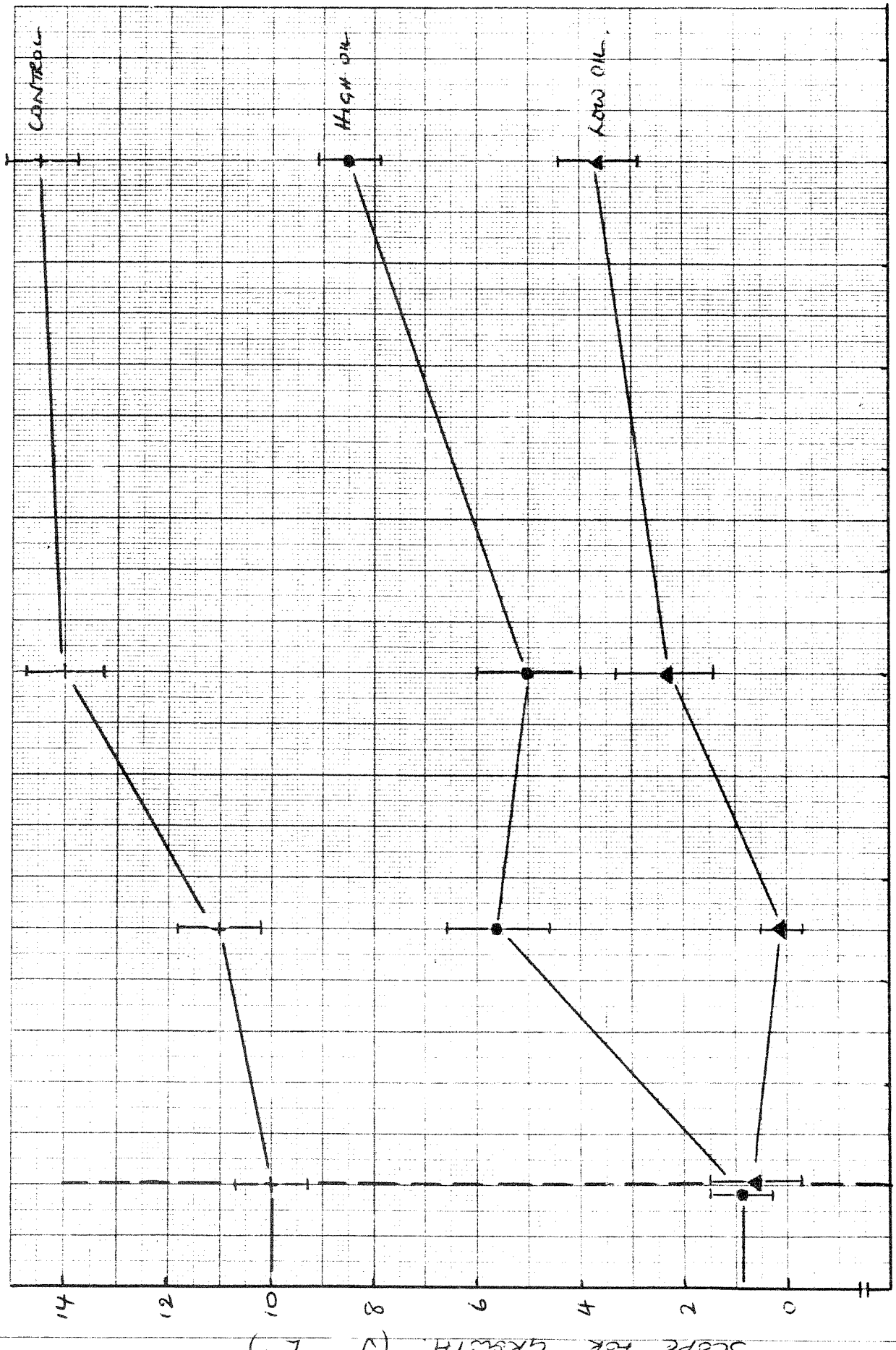
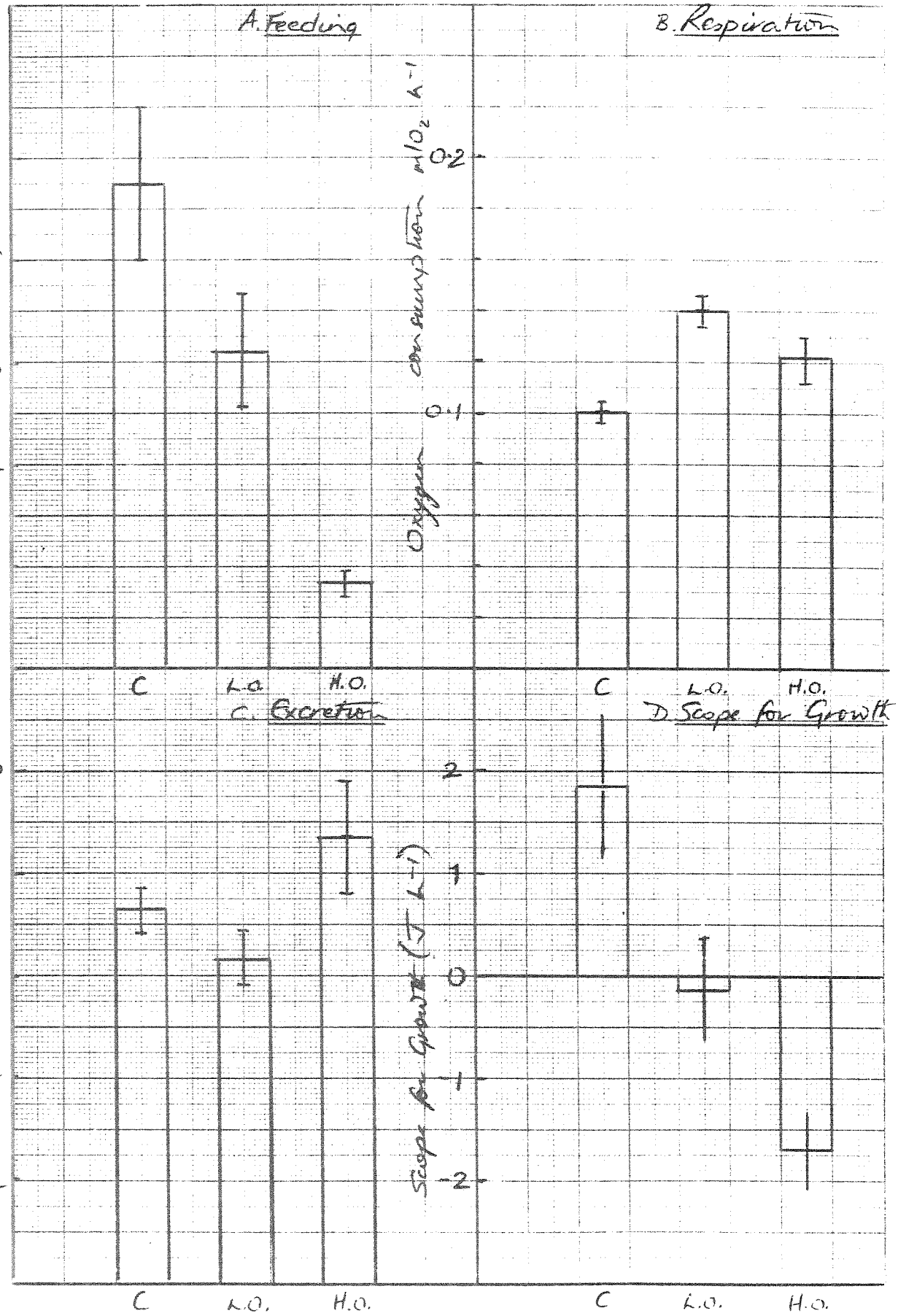


Fig. 5 Effect of oil on scope for growth of Mytilus edulis

Fig. 6.

Physiological Responses of Littorina littorea (Standard animal of 0.16g).

Mean ± S.E. n=8.



Part 2: Cellular reactions - David Lowe

Objectives

To investigate cellular reactions to petroleum hydrocarbons in the byssus gland and the reproductive and digestive tissues and characterise these using a quantitative approach to cellular pathology.

Materials and Methods

Material collected during the May-June visit to Solbergstrand Experimental Station did not yield particularly comprehensive results due principally to a shortage of suitably sized animals. It was decided therefore to set up mussels in cages in Basins 1, 3 and 4, thereby eliminating predation as much as possible, and use these specimens during subsequent visits. The bulk of the data discussed in this report therefore refers to mussels collected from the cages during the August visit to Solbergstrand.

When studying cellular aspects of gametogenesis in mussels it has become apparent, from studies at IMER Plymouth, that the size at which sexual maturity is attained is variable for different populations. Therefore, before any detailed analysis of the effects of oil exposure could be undertaken, it was first necessary to establish at what size sexual maturity is attained in Solbergstrand mussels. Analysis of specimens collected in May from both the Fjord and the basins indicated that sexual maturity was achieved in the size range 20-30 mm. It was therefore decided to base the studies during August on the size ranges 40-50 mm and 50-60mm as these represented animals typical of both somatic and germinal growth.

Cellular effects were considered in three separate, but related, experiments:

i) long term exposure to oil i.e. 80 days

ii) short term exposure to oil i.e. 5 days

iii) short term recovery i.e. 5 days.

For the short term exposure studies Fjord animals (size class 40-50 mm) were placed in netting socks in the cages in basins 1, 3 and 4 (Water Temp 18.4-19.5°C; Salinity 24-26°/oo). Similarly the short term recovery mussels (size range 40-60 mm), from the cages in basins 1 and 3, were placed in netting socks in the cage in control basin 4.

Following collection the animals were sacrificed and one mantle lobe dissected out and weighed following 12 hrs fixation in Bakers formol calcium (Bfc). The foot/byssus gland was also dissected out and this, with the remaining tissues, fixed in B.f.c. Fixed tissues were dehydrated through an ascending alcohol series, cleared in Histosol and embedded in Paraplast. Sections, 5µm, were cut and stained in Papanicoloau.

Analysis

A stereological analysis of mantle tissues was carried out at a magnification of x500 on 5 fields (271 x 274µm) per animal on 8-10 animals per treatment. Where possible, equal numbers of each sex were analysed and the data pooled per treatment. For the purposes of these studies the reproductive tissues were considered as follows:-

Storage tissues	Vesicular connective tissue cells	(VCT)
	Adipogranular cells	(AG)
Gametes	Ripe	
	Developing	
	Evacuated	

A third group of tissues, termed atretic, was also identified. This category includes any gametes undergoing resorption following spawning or any gametes breaking down due to disease.

Pathology

All tissues were examined for any evidence of growth disturbances or disease, including parasites.

Results and Discussion

1. Effects of hydrocarbon exposure: Mytilus edulis.

The blotted wet weight of fixed mantles indicated that the animals exposed to 30 ppb oil were approximately 66% of the control group and the 140 ppb oil exposed animals were 41% of the control. Fjord animals, which were spawned out, were similar in weight to the 30 ppb group.

Fixed Mantle Tissue wet weights of 40-50mm Mussels (gms)

Control Basin	30 ppb Oiled	140 ppb Oiled	Fjord Control
1.246 ± 0.136	0.837 ± 0.120	0.507 ± 0.085	0.818 ± 0.077

Reproductive Tissues

The results of the stereological analysis (Table 1) indicated that the Fjord reference mussels had all spawned and were entering a phase of nutrient storage. Data resulting from the experimental control group of mussels indicated that some had spawned and were entering a storage phase, whilst others exhibited a high gamete volume density (55-72%) and would have doubtless spawned shortly. The situation in the 30 ppb exposed mussels was less well defined than the experimental controls and many animals exhibited developing gametes and lower volume densities of ripe gametes. Of more significance, however, is the lack of A.G. cells in this group indicating very limited lipid and protein reserves (Lowe, Moore & Bayne 1982). Atretic gametes were also in greater abundance than in previous groups examined. The 140 ppb exposed animals exhibited considerable inter animal variability; however, the developing mean gamete volume density for both developing and ripe fractions was comparable with the 30 ppb group. The volume densities of both AG and VCT cells were similar to the 30 ppb group indicating once again a lack of lipid and protein reserves. Atretic gametes were present in many of the animals and many follicles exhibited areas void of gametes. This is not, however, taken to indicate spawning activity but rather resorption by the numerous brown cells present which have a phagocytic capability. The observation that the oil exposed groups of mussels are low in both gamete ripe and developing gamete and lipid and protein reserves, as indicated by low AG

cell volume density, is significant in that it is believed that AG cell resources play a role in gamete maturation. If this is the case then in order that these groups can produce a reasonable number of offspring, and in the absence of a phytoplanktonic bloom to boost nutrient throughput, it would be necessary to mobilize resources from elsewhere in the tissue mass. Lubet (pers comm) maintains that one method of achieving this is to selectively break down gametes and recycle the nutrient. The reason for the lack of A.G. cells is not apparent, however, they are additionally involved in maintenance metabolism and it may be that oil exposure has imposed a heavy demand such that this particular storage moiety has become severely depleted. Alternatively the cytotoxicity of the oil may have affected the digestive processes such that a normal storage phase was not possible. This hypothesis gains some support from the analysis of mussels taken at day 0 when the cages were first set up. The results indicated that the A.G. Cell volume density was almost zero at that time, a condition which appeared to be unchanged following 80 days exposure to oil.

Byssus Gland

Mussels exposed to 130 ppb oil did not appear to produce byssus thread and because of this do not attach to the netting socks and fall to the floor of the basin where they become easy prey to the numerous starfish and crabs. A preliminary investigation was undertaken to see if there was any cellular evidence to indicate oil cytotoxicity in the various glands that produce and tan the byssus. No obvious changes had occurred in any of the glands examined. It is stressed, however, that this is very much a preliminary investigation which will require more analytical histochemistry to rule out cytotoxicity altogether. The byssus cap is also being considered as a possible reason for unsuccessful byssus formation/attachment.

Digestive Gland

The technique used to analyse the digestive tract is currently under revision and a new analysis is presently being formulated which should give data

which can better relate to studies on digestive physiology. Nevertheless an examination of the digestive epithelium revealed consistent differences, in terms of epithelial cell atrophy, between the various treatment groups.

Tissue-associated granulocytic blood cell numbers were very high in many animals from all the treatment groups. However, sample numbers were too small to indicate if there were any significant differences between treatments. Mussels from the 140 ppb treatment group all contained large clumps of brown cells and degrading granulocytic blood cells in the epithelium of the intestinal tracts. Such cell clumps were not in the control animals and although present in the 30 ppb group, were greatly reduced in both size and numbers. In 140 ppb treatment mussels which had been allowed to recover, the clumps were greatly reduced and comparable to 30 ppb exposed mussels. A similar reduction in the size and incidence of these clumps was apparent in the 30 ppb group permitted to recover, although it was not reduced to control group level. These clusters of cells migrate through the epithelium and are voided into the intestinal lumen and probably represent some enhanced excretory activity resulting from xenobiotic exposure. Fjord mussels subjected to 5 days exposure to 30 and 140 ppb oil did not develop these cell clumps but tubule atrophy was widespread throughout the digestive gland.

Pathology

There was no evidence of any pathology in any of the treatment groups other than that discussed already. Whilst some encysted parasites were present, no particular group exhibited a higher incidence than any other. Some animals exhibited small granulocytomas but once again this was not particular to any one treatment.

Recovery from hydrocarbon exposure: Mytilus edulis.

A sample of mussels from both the 30 and 140 ppb basins were placed in the control basin for 5 days to assess recovery of the digestive cells and the storage cell matrix. Stereological analysis indicated that there was no effect

on gamete volume density, however, the low oil (30 ppb) mussels exhibited a significant increase in the volume density of the A.G. cells. This increase may be due to increased feeding activity following transfer to clean water and thereby the removal of any cytotoxic agents which may be present in the oil phase. Once again, it is hoped to clarify this when the digestive gland analysis is completed.

2. Effects of hydrocarbon exposure: Littorina littorea.

Control: The majority of the snails collected were ripe or nearly ripe and exhibited a highly granulated apical digestive epithelium with numerous small vacuoles in the basal region. The brush border of the digestive epithelium was well defined and the epithelial basophil cells contained occasional giant granules which appeared to be formed from the fusion of the numerous small granules present in the cytoplasm. These granules appeared to empty into the tubule lumen as in a goblet secretery cell.

30 ppb oil exposure: Exposure to oil at the above concentration did not appear to have affected gamete production and all the animals appeared ripe. The digestive epithelium, however, exhibited considerable disruption with large vacuoles developing and a considerable increase in the numbers of giant granules associated with basophil cells.

140 ppb oil exposure: As with the 30 ppb group, exposure to oil did not appear to have affected gamete production. However, the large vacuoles present in the 30 ppb snails were not present. Instead the basal region of the digestive epithelium contained numerous small vacuoles. The incidence of giant granules had increased, owing to an apparent increase in the numbers of basophil cells.

Recovery from hydrocarbon exposure

Recovery of the snails was considered over four time periods, 2.5, 5, 10 and 20 days. So far it has only been possible to examine the 2.5 day recovery group. The results are encouraging and indicated that even over such a short period, recovery had begun. Snails exposed to 30 ppb oil exhibited a general

reduction in both large vacuoles and giant granules to levels comparable with the control animals. Similarly the 140 ppb exposed snails exhibited a reduction in the incidence of giant granules with an associated reduction in basophil cell numbers.

The apparent increase in the numbers of basophil cell numbers with their associated giant secretery granules probably represents an adaptive response to meet the increased demands on the digestive secretory system resulting from the presence of oil. When time becomes available it is proposed to quantify the incidence of the basophil cells with a view to developing an index for future pollution studies and also as a measure of the adaptive responses and capabilities of the snail as it relates to fundamental digestive cell processes.

Table 1.

Volume densities of reproductive tissue components - caged animals plus Fjord control.

	Cytes		Ripe		A.G. Cells		V.C.T. Cells		Evacuated		Atretic	
	$\bar{x}$	Se	$\bar{x}$	Se	$\bar{x}$	Se	$\bar{x}$	Se	$\bar{x}$	Se	$\bar{x}$	Se
Basin Control	4.41	± 1.82	34.05	± 10.62	12.03	± 6.17	35.60	± 5.91	0	0	3.99	± 1.66
Low Oil	4.98	± 1.04	19.46	± 8.08	6.96	± 4.43	47.17	± 6.19	1.13	± 0.75	12.65	± 6.96
High Oil	8.30	± 3.45	16.94	± 7.65	3.54	± 2.01	42.11	± 7.12	9.73	± 4.22	8.10	± 6.16
Fjord Control	2.04	± 0.84	2.62	± 2.23	28.59	± 8.13	51.22	± 3.71	1.01	± 0.74	1.96	± 1.03

Part 3: SUBCELLULAR REACTIONS TO CELL INJURY - Michael Moore

Materials and Methods

The experiments were carried out in May/June 1983 and August 1983. During these periods the seawater temperatures in the experimental basins were 11-13°C (May/June) and 18.4-19.5°C (August). Salinities were 12.5-17.5°/oo (May/June) and 24-26°/oo (August).

Effects of hydrocarbon exposure

Subcellular reactions to cell injury included the cytochemical determination of lysosomal stability and the relative activity of microsomal NADPH-neotetrazolium reductase (NADPH-NTR) in the digestive cells of *Mytilus edulis* (n=5) and *Littorina littorea* (n=5) collected from basin 1 (∿140ppb), basin 3 (∿30ppb) and basins 2 and 4 (control). The cytochemical reactions were measured as described by Moore (1976, 1979) and Moore *et al.* (1982). NADPH-NTR activity was assessed by a nominal grading system based on a linear estimate of intensity of coloured reaction product in the digestive cells. Mussels sampled were 20-25mm shell length (May/June) and 45-55mm shell length (August). This discrepancy arose due to the lack of mussels in the basins and the mussels used in the August sample had been collected from the Fjord, placed in large cages and transferred to the basins for 80 days. Littorinids sampled were 20-25mm shell height on both occasions.

Recovery from hydrocarbon exposure

Mussels were taken from the 30 and 140ppb hydrocarbon conditions, placed in mesh bags and transferred to the control basins for 5 days (August), and 10 and 20 days (May/June). Littorinids were likewise transferred and held in cages (May/June) or polythene bottles (August), in which had been cut numerous holes to allow free passage of water. These cages or bottles

were supplied with Ulva lactuca and control snails were also held in this manner during the recovery period. Snails were sampled after 2.5, 5, 10 and 20 days (May/June) and 5 days (August).

Cytochemical measurements were made on all samples of mussels and snails.

Results and Discussion

Mussels - effects of hydrocarbon exposure and recovery

Latency of lysosomal β-hexosaminidase was significantly reduced in the digestive cells of hydrocarbon exposed mussels (Tables 1 and 2), indicating a reduction in lysosomal stability. The reductions at both hydrocarbon concentrations were not significantly different from each other, (Mann-Whitney U-test) and were not significantly different between the May/June and August samples (Mann-Whitney U-test, Tables 1 and 2).

Transfer of exposed mussels to the control condition did not lead to any increase in the lysosomal stability indicating that there was no evidence of recovery (Tables 1 and 2). This situation prevailed at both sampling periods. There was a slight but significant decrease ($P<0.05$, U-test) in lysosomal stability in the 30ppb condition after 10 days of recovery period (Table 1).

NADPH-NTR, which is a measure of the xenobiotic-inducible microsomal respiratory chain terminating in Cytochrome P-450, could not be detected in the May/June samples. This was probably a consequence of their reproductive state, as this particular test often fails when the animals are approaching a ripe condition or have recently spawned. There was significant elevation of NADPH-NTR activity in the digestive cells of mussels from the 140ppb condition sampled during August but not from the 30ppb condition (Table 3). After a recovery period of 5 days the levels of NADPH-NTR were

not significantly different from the controls (U-test; Table 3), indicating a recovery of this system.

Littorinids - effects of hydrocarbon exposure and recovery

Latency of lysosomal β-glucuronidase was significantly reduced in the digestive cells of hydrocarbon exposed snails (Tables 4 and 5) indicating a reduction in lysosomal stability. The reductions at both hydrocarbon concentrations were not significantly different from each other (U-test) and were not significantly different between the two sampling periods (U-test; Tables 4 and 5). These findings are similar to those reported for the mussels, and show encouraging reproducibility.

Transfer of snails to the control condition resulted in significant recoveries ($P<0.05$, U-test) in lysosomal stability within 10 days for the May/June experiment and 5 days for the August experiment. In the May/June experiment the 140ppb snails were not significantly different from the controls (U-test) after 20 days of the recovery period.

These findings are obviously different from those in the mussels and appear to indicate a greater capacity for recovery of lysosomal function in the snails.

NADPH-NTR activity was significantly elevated in the 30ppb condition (August only) and in the 140ppb condition (May/June and August) as shown in Tables 6 and 7. In the May/June recovery experiment the 30ppb condition did show a significant increase over the control after 2.5 days (Table 6) but not at the other sample times. Activity remained elevated in the 140ppb condition throughout the 20 day recovery period. In the August experiment both hydrocarbon exposed conditions retained an elevated level of activity after a 5 day recovery period (Table 7).

Conclusions

1. Both mussels and snails show evidence of perturbation of lysosomal function in the digestive cells, which would probably impair intracellular digestion.

2. The snails demonstrate a capacity for recovery of lysosomal function which is not present in the mussels within the duration of the experiments.

3. There is evidence of induction of the microsomal detoxication system (NADPH-NTR) in both mussels and snails. This elevated activity is maintained in the snails for up to 20 days of a recovery period.

4. Measurable effects have been shown which can be related to cell injury and these appear to be reproducible for 2 samples in time. There are no indications of dose-related reactions.

Other Work

Research is still in progress on the ultrastructure of the digestive cells of mussels and snails from Solbergstrand. Preliminary data is also available on the absence of any evidence of hydrocarbon-induced lipid peroxidation of lysosomes in either snails or mussels, although there is evidence of a pathological accumulation of lipid within the lysosomes of hydrocarbon treated animals.

These data will be fully reported at a later date.

Table 1. Labilisation periods of latent lysosomal β-hexosaminidase in M. edulis digestive cells: effects of hydrocarbons and recovery (May 1983).

Treatment	Baseline	Recovery Period	
		10 days	20 days
Control	100[A] 92[B]	-	92
∿30 ppb	80	60 **	52
∿140 ppb	49.6 **	48 **	56 **

A. All data presented as a percentage of baseline control (basin 4).

B. Baseline control from basin 2.

** $P \leq 0.01$, U-test comparing with control and based on untransformed data. Only the mean value is shown for reasons of clarity.

Table 2. Labilisation periods of latent lysosomal β-hexosaminidase in M. edulis digestive cells: effects of hydrocarbons and recovery (August 1983).

Treatment	Baseline	Recovery Period 5 days
Control	100[A]	
∿30 ppb	34.3 *	23.8 **
∿140 ppb	23.8 **	18.5 **

A. All data presented as a percentage of baseline control.

* $P \leq 0.05$, U-test comparing with control and based on untransformed data. Only the mean value is shown for reasons of clarity.

** $P \leq 0.01$.

Table 3. Activity of NADPH-NTR in the digestive cells of M. edulis: effects of hydrocarbons and recovery (August 1983).

Treatment	Baseline	Recovery Period 5 days
Control	52.6[A]	-
∿30 ppb	94.7	63.2
∿140 ppb	100 *	84.2

A. All data presented as a percentage of baseline ∿ 140 ppb value.

* $P \leq 0.05$, U-test comparing with control and based on untransformed data. Only the mean value is shown for reasons of clarity.

Table 4. Labilisation periods of latent lysosomal β-glucuronidase in L. littorea digestive cells: effects of hydrocarbons and recovery (May 1983).

Treatment	Baseline	Recovery Period			
		2.5 days	5 days	10 days	20 days
Control	100[A] 88[B]	92	-	96	96
∿30 ppb	40 **	60 **	56 **	60 **	64 *
∿140 ppb	29.6 **	40 **	36 **	52 **	68

A. All data presented as a percentage of baseline control (basin 4).

B. Baseline control from basin 2.

* $P \leq 0.05$, U-test comparing with control and based on untransformed data. Only the mean value is shown for reasons of clarity.

** $P \leq 0.01$.

Table 5. Labilisation periods of latent lysosomal β-glucuronidase in L. littorea digestive cells: effects of hydrocarbons and recovery (August 1983).

Treatment	Baseline	Recovery Period 5 days
Control	100[A]	106.7
∿30 ppb	29.3 **	93.3
∿140 ppb	38.7 **	100

A. All data presented as a percentage of baseline control (basin 4).

** $P \leq 0.01$, U-test comparing with control and based on untransformed data. Only the mean value is shown for reasons of clarity.

Table 6. Activity of NADPH-NTR in the digestive cells of L. littorea: effects of hydrocarbons and recovery (May 1983).

Treatment	Baseline	Recovery Period			
		2.5 days	5 days	10 days	20 days
Control	0[A]	12.5	-	12.5	12.5
∿30 ppb	62.5	100 *	12.5	50	0
∿140 ppb	100 **	163 *	137.5 *	100 *	93.8 *

A. All data presented as a percentage of baseline ∿140 ppb value.

B. Baseline control from basin 2.

* $P \leq 0.05$, U-test comparing with control and based on untransformed data. Only the mean value is shown for reasons of clarity.

** $P \leq 0.01$.

Table 7. Activity of NADPH-NTR in the digestive cells of L. littorea: effects of hydrocarbons and recovery (August 1983).

Treatment	Baseline	Recovery Period 5 days
Control	37.5[A]	45.8
∿30 ppb	108.3 **	79.2 *
∿140 ppb	100 *	83.3 *

A. All data presented as a percentage of baseline ∿140 ppb value.

* $P \leq 0.05$, U-test comparing with control and based on untransformed data. Only the mean value is shown for reasons of clarity.

** $P \leq 0.01$.

MARINE RESEARCH STATION SOLBERGSTRAND

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14. A COMPARISON BETWEEN AN ARTIFICIAL LOCALITY AT SOLBERGSTRAND FIELDSTATION (BASIN II) AND A NATURAL LOCALITY WITH RESPECT TO SETTLEMENT AND GROWTH. A SUMMARY

by Odd - Arne Follum

University of Oslo
Inst. of marine biology and limnology.
Dep. of marinezoology and -chemistry.

November 1983.

CONTENTS

INTRODUCTION

This report is a summary of work done at Solbergstrand Field Station between June -81 and November -82.
A part of my research was to compare the settling and growth of organisms in basin II (control basin) with a natural locality in the fjord outside the station.

In all work done in one or several of the basins, it is important to know how the results from these are corresponding to the real, natural conditions in the wild. There is reason to believe that results from semi-closed localities like the basins present are suffering from defaults which can be correlated to the afterall artificial environment which the basins represent.

This report shows how different settling and growth can be in two localities which at first were believed to be very much alike and "natural". Some of the reasons why such differences are believed to occur are discussed, in an attempt to clarify the obscure term "basin effect".

MATERIALS AND METHODS

Paving-stones were chosen as a substrate for the organisms to settle on. These have a rough surface, believed to be an advantage for settling of marine organisms (Barnes et al. 1950, Crisp 1974). The stones are heavy (meas. ca. 15x17x25cm and weight ca. 12kg), not easily carried away by the sea or by people. They are also made of granite, and very much like the naturally occuring substrate in the surroundings.

The stones were placed at three different depths in basin II. The uppermost level was totally submerged at high tide and totally exposed to air at low tide. The two deeper levels were placed about 0.5m and 1m below on the stairs in the basin. There were three replicates at each level.

The same arrangement was used in the fjord locality which was about 60 - 70m south of the river outlet by the station. A "canal - iron" was plugged in the sand with two wooden sticks, and the stones fastened to it with two bolts and nuts. Three replicate stones were fastened to each iron at the same depths as in the basin.

This was an extremely successful arrangement since the heavy ice - scouring which took place during the late winter -82 did not affect the framework at all.

These stones,"Series - A", remained in place during the whole research period.

In June -82, a year after the start, two other series were set out. A "Series - B" was placed at the two localities in the same way as described above, with two stones at each depth, one free as before and one in a cage of stainless steel wire netting, 8 meshes per inch. This netting is reported not to prevent settlement (Connel 1961ab).

The aim of this series was to look at the effect of controlling the predation, observed last autumn, mainly by Asterias rubens on barnacles and Littorina littorea on the green algae.

A last series, "Series - C", was set out at the same time. Two stones were replaced every month to compare the "primary settling potential" and the "actual settling success" (Harms & Anger 1983). There were only two stones at each locality, standing on a platform about 0.5m over the sea-bed, and at a depth of about 0.5m under the surface. This arrangement was intended to prevent predators from reaching the stones.

All the stones were analyzed every 4th - 6th week by a nondestructive method. They were loosened from the frame, carried inside the lab in a bucket of seawater and placed under a microscope. A transparent plastic sheet with 100 evenly distributed points was placed over one side at the time, and percent cover was counted for every species. The species were placed in two categories; primary growth and canopy.

The stones were then carried back and placed in the same position as before. In this way, I was able to follow the development of the community at both localities and three

depths on five different sides(west-,south-,east-,north- and topside).

At the same time, some effort was made to find the causes of the obvious differences in community development at the two localities:

a) Plankton hauls from the surface in the fjord were compared with hauls at the basin inlet to check if the input of organisms to the basin was reduced or changed by the pumping-system or by other causes.

b) Analyses of the nutrient content of the water at both localities were carried out to see if some accumulation took place in the basin, or if the riverwater nearby had any influence on the localities.

c) The light was measured over a periode of time to see if one of the localities received more light than the other.

d) A primitive wave-exposure measurement was made by comparing the wave-heights in the fjord with wind-direction and -strength.

RESULTS

SERIES - A

Species

Every stones was followed for 17 months, and the history and development of the community for each side has been recorded (Follum in prep.). This is summarised in Table 1. The results for all sides have been pooled for each depth during the whole periode to give a semi-quantitative comparison.

One cross in the table means that the species has been observed having less than ten percent cover all the time. Two crosses means that the species has been observed having between 10 and 30 percent cover one or several times in the periode.

The dominant algae in the fjord are the encrusting brown-alga Ralfsia sp. and the filamentous green alga Chaetomorpha linum. Monostroma grevillei has been observed mostly at 1m depth in the spring and early summer. Dumontia incrassata and Nemalion helmintoides had a blooming in late summer and autumn.

In the basin, Enteromorpha spp. dominate during the first autumn. This is mainly E. intestinalis, but also a proption of E.compressa; the two species are difficult to separate in the field when they are growing close together.

The second autumn, Ulva lactuca completly dominated the substrate in basin II. Ceramium rubrum and C. stricta grew in clusters through the whole period at the deeper levels Some blooming of Ulothrix sp. occured in early spring in the upper level, and Ectocarpus sp. at the deeper levels. Polysiphonia spp. occured in the last autumn, also in the deeper part of the basin.

The dominant animal in the fjord was always Balanus improvisus. Heavy settlement in the summer made this barnacle extremely abundant from July to November - December. B. balanoides appeared in late spring and early summer, and shared the substrate with B. improvisus in the uppermost level.

Table 1. A semi-quantitative survey of series-A. All sides put together. See text for examples.
Key: -:not found, + : 10%, ++: 10-30%, +++: 30-60%, ++++: 60-100%

	FJORD			BASIN II		
SPECIES	0 m	0.5m	1 m	0 m	0.5m	1 m
Rivularia atra	+	-	-	+	-	-
Enteromorpha spp.	++++	-	-	++++	++++	++++
Ulva lactuca	-	++	-	++	++++	++++
Chaetomorpha linum f.aerea	+++	++++	+++	-	+	+
Ulothrix sp.	-	-	-	++++	-	-
Monostroma grevillei	+	++	++++	-	++	++
Cladophora sp.	+	+	++	+	-	-
Fucus spp.	+	-	++	+	+	+
Laminaria sp.	-	+	+	-	++	++
Ralfsia sp.	++++	++	++	++	++	++
Ectocarpus sp.	-	+	+	++	+++	++++
Petalonia fascia	-	+	+	+	-	-
Scytosiphon lomentaria	-	++	++	+	++	+
Dichtyosiphon sp.	-	-	+	-	-	-
Ceramium spp.	+	+	+	++	++	+++
Dumontia incrassata	+	+++	++	-	+	-
Nemalion helmintoides	-	++++	+++	-	-	-
Polysiphonia spp.	-	+	+	-	+++	+++
Balanus improvisus	+++	++	++	+	+	+
Balanus balanoides	++	+	+	+	+	-
Laomedea spp.	++	+	++++	+	+	++++
Laomedea gracilis	-	-	-	-	-	+++
Bougainvillia ramosa	+	+	+	-	+	+
Clava multicornis	-	+	-	-	+	+
Pomatoceros triqueter	-	+	+	-	+	+
Metridium senile	-	-	-	-	+	+
Electra pilosa	-	-	+	-	+	++
Membranipora membranacea	-	-	-	-	+	++
Mytilus edulis	-	-	+++	-	-	-

Mytilus edulis settled at 1m during the summer, but never managed to develop a dense cover and disappeared later in the autumn.

Some animals occured only in the basin; Metridium senile, occurred only on the lowest part of the stones in the lower levels where they were protected from "algae washing". Laomedea gracilis was abundant in the lowest level early in the autumn, and the bryozoans also occurred at the lower levels in protected aereas.

Community

To compare community development for the two localities, a dendrogram was drawn from a clusteranalysis, using the DECK - 10 computer at the University of Oslo, and the data package CLUSTAN (Wishart 1978).

All species with percent cover of ten or more, one or several times during the research period were put in a matrix with sides over time. This made up 12species x 90 sides for the autumn -81 (5sides x 3depths x 3months x 2 localities) and 22 species x 120 sides(4 months) in 1982.

The dendrograms were made using "Bray - Curtis dissimilarity index" and "group-average sorting".

Fig. 1 shows the results from the three months in 1981, September, October and December, and Fig. 2 the results from April, May, September and November in 1982.

Taking first the -81 data, the dendrogram consists mainly of two very distinct clusters. Cluster a is made up only of the fjord-sides and cluster b is made up of all the basin-sides. This leads to the conclusion that all the basin-sides are more alike eachother than any of the fjord-sides.

Drawing, arbitrarily, a line at the dissimilarity level 0,4 makes several clusters. In Fig. 3,I have tried to present the results so they are more available. The "boxes" with the same number represent sides which are very much alike

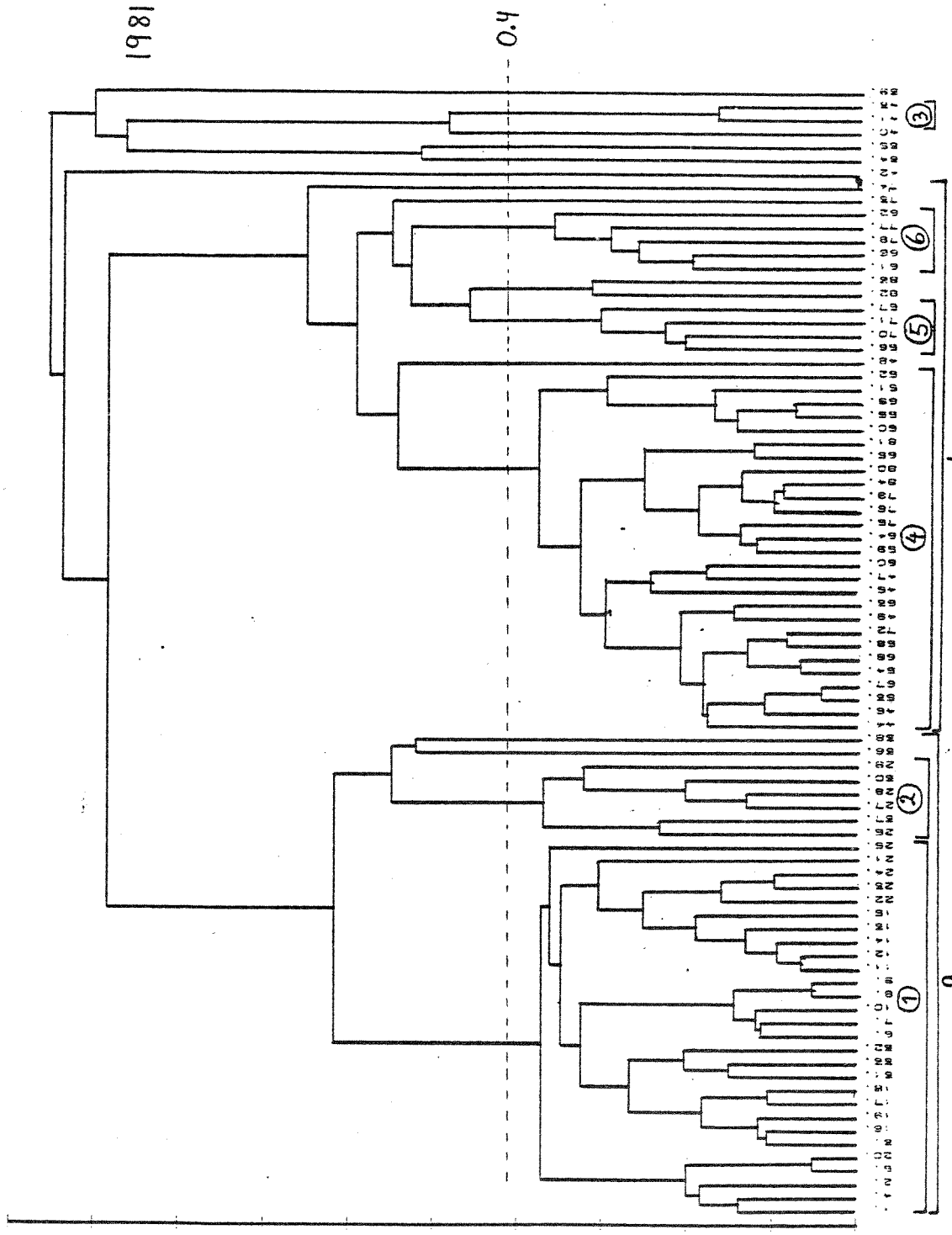


Fig. 1. Dendrogram of percent cover for 12 species at both localities all depths and sides for three months in 1981. Vertical axis is Bray-Curtis dissimilarity index. See text for further explanation.

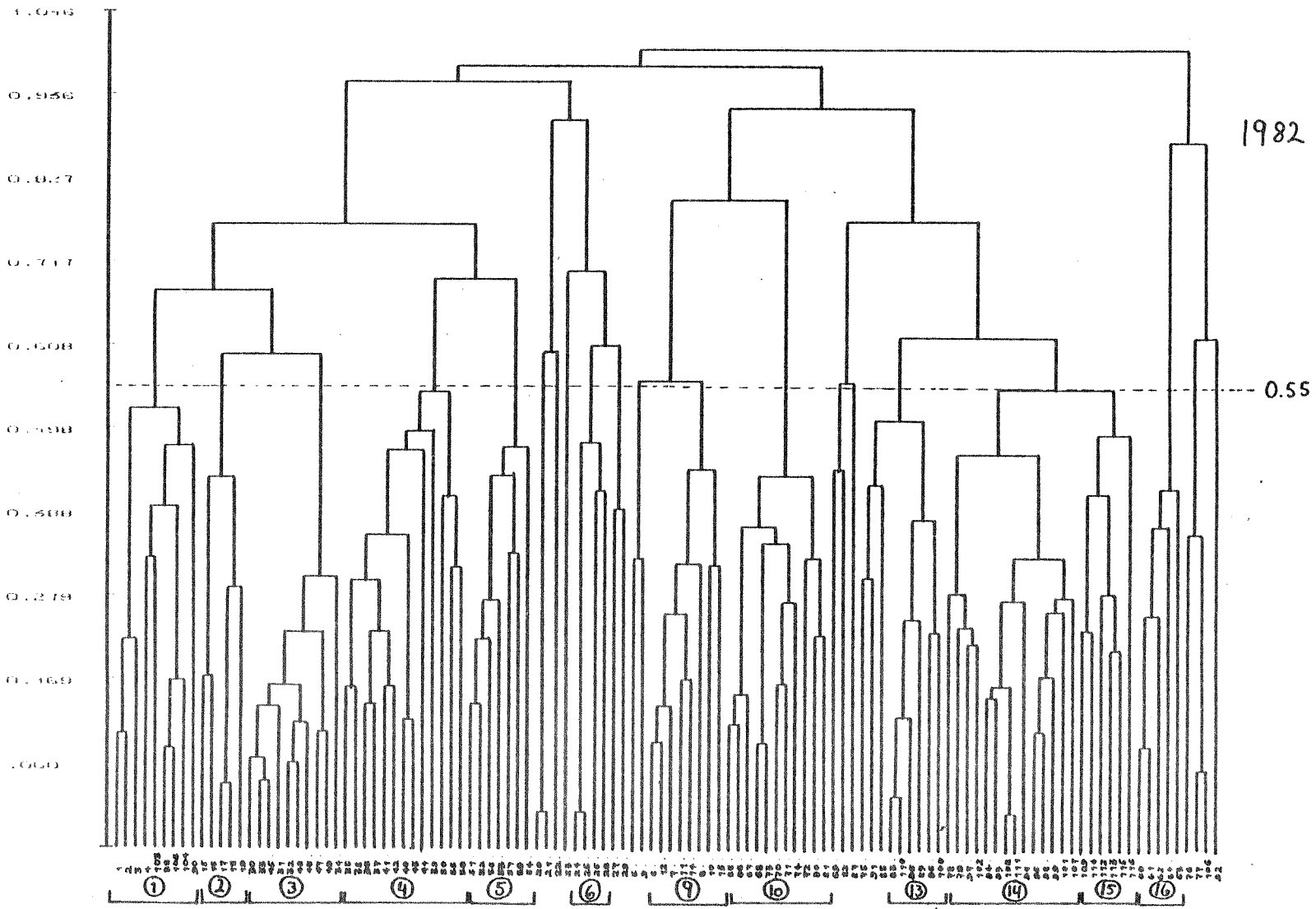
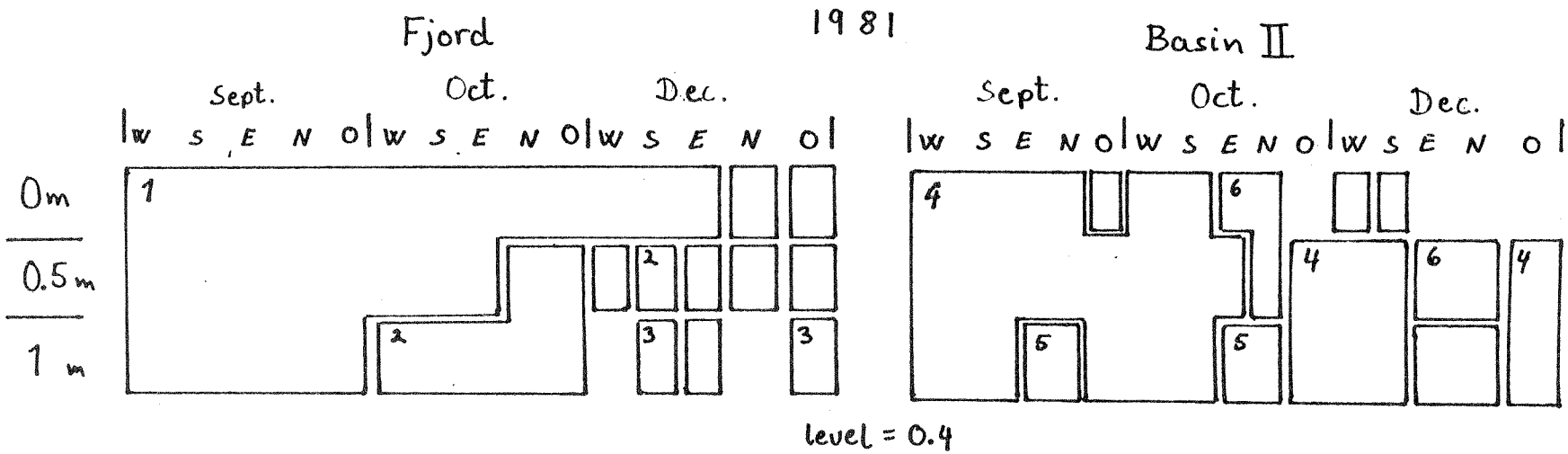


Fig. 2. Dendrogram of percent cover for 22 species at both localities, all depths and sides for four months in 1982. Vertical axis is Bray-Curtis dissimilarity index. See text for further explanation.



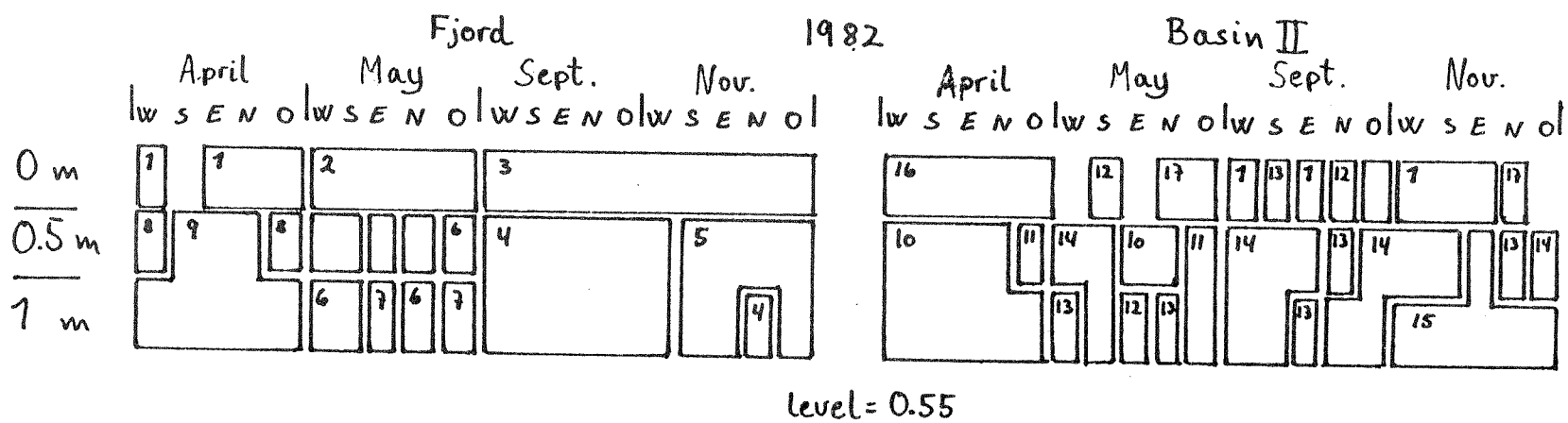


Fig. 3. "Box-diagram". Each box corresponds to clusters in Fig.1 and 2. All sides inside a box are similar in species-composition and percent cover.

according to the dendrogram. For 1981, the box no. 1, which corresponds to cluster 1, dominates the fjord-sides. This means that all sides of the stones at 0m in the fjord have the same sort of community from September to December except north- and oversides in December, and are similar to 0.5m and 1m sides until October, except for 0.5m north- and oversides the last month.

The reason for the deeper levels first being unlike the upper, is that Asterias rubens first preys on B.improvisus at the deepest parts.

In spite of doubt that Asterias actually do prey on B.improvisus (L. Vadas and W. Syratt in pers. comm. at Solbergstrand sept.-82) I have shown in lab experiments that this is possible when other food such as Mytilus edulis is available (Follum in prep.).

Unnumbered boxes represent sides that are not similar to any others. Open spaces in the diagram are sides taken out of the analyses because there was no settlement at all. Zero values for all species made it impossible to draw the dendrograms.

For the basin, sides heavily covered with Enteromorpha the first autumn are placed in cluster 4. At the end of the year, the algae disappeared, probably because of decreasing temperature, first in the uppermost level.

The oversides at this level probably suffer from desiccation and thermal stress, because the settlement and growth are minimal. The north- and eastsides, especially at the 1m level differ from the other sides mainly because they are standing too close to the stairs, and therefore in the shade.

For the 1982 data, the dendrogram became more complex. Ten more species and another month made the picture far more difficult to understand. The general view that fjord- and basinsides are divided in two clusters does not fit the analyses any longer, although a trend is showing up when not being too restrictiv in looking at the dendrogram.

Drawing, again arbitrarily, a line at the 0.55 level, gives many more clusters than the year before. Cluster 1 consists of sides with very little settlement and growth. The cover in April in the uppermost level in the fjord was mainly made from surviving Ralfsia sp. from the previous year. These sides are very much like the uppermost sides in the basin in September and November, since Ralfsia sp. here was almost the only species surviving the summer.

The uppermost level in the fjord in May was dominated by B.balanoides and Ralfsia sp.. In the last months, B.improvisus shared the substrate with the other two.

The results from the basin are not easily available. The system here, if any, is very complex and difficult to discuss any further at the moment.

Primary growth and canopy

Fig. 4a and b give the percent cover of the two categories primary growth and canopy at both the localities. All sides at each level are put together and drawn for September to December (Fig. 4a) and April to November (Fig. 4b). Unfortunately the data for July at 0m in the basin are missing.

As stated before, the primary growth in the fjord the first autumn was dominated by B.improvisus and Ralfsia sp. The barnacle level dropped to zero in December at 1m because of predation by the starfish.

The primary growth of basin II consisted of a very light cover of B .improvisus and Ralfsia sp.. In the uppermost level there were small thalli of Enteromorpha sp. which formed a dense cover in September and seemed to prevent further settling by other organisms. In the deepest level, bryozoans and Metridium senile made up the primary growth.

Generally speaking, the primary growth in the fjord was much denser than in the basin. On the other hand, the canopy in basin II made up a much denser cover than in the fjord, and this consisted mainly of Enteromorpha spp. in the upper level and Enteromorpha spp. and Ceramium spp. in the lower parts.

In the fjord, the canopy cover consisted mainly of Chaetomorpha linum.

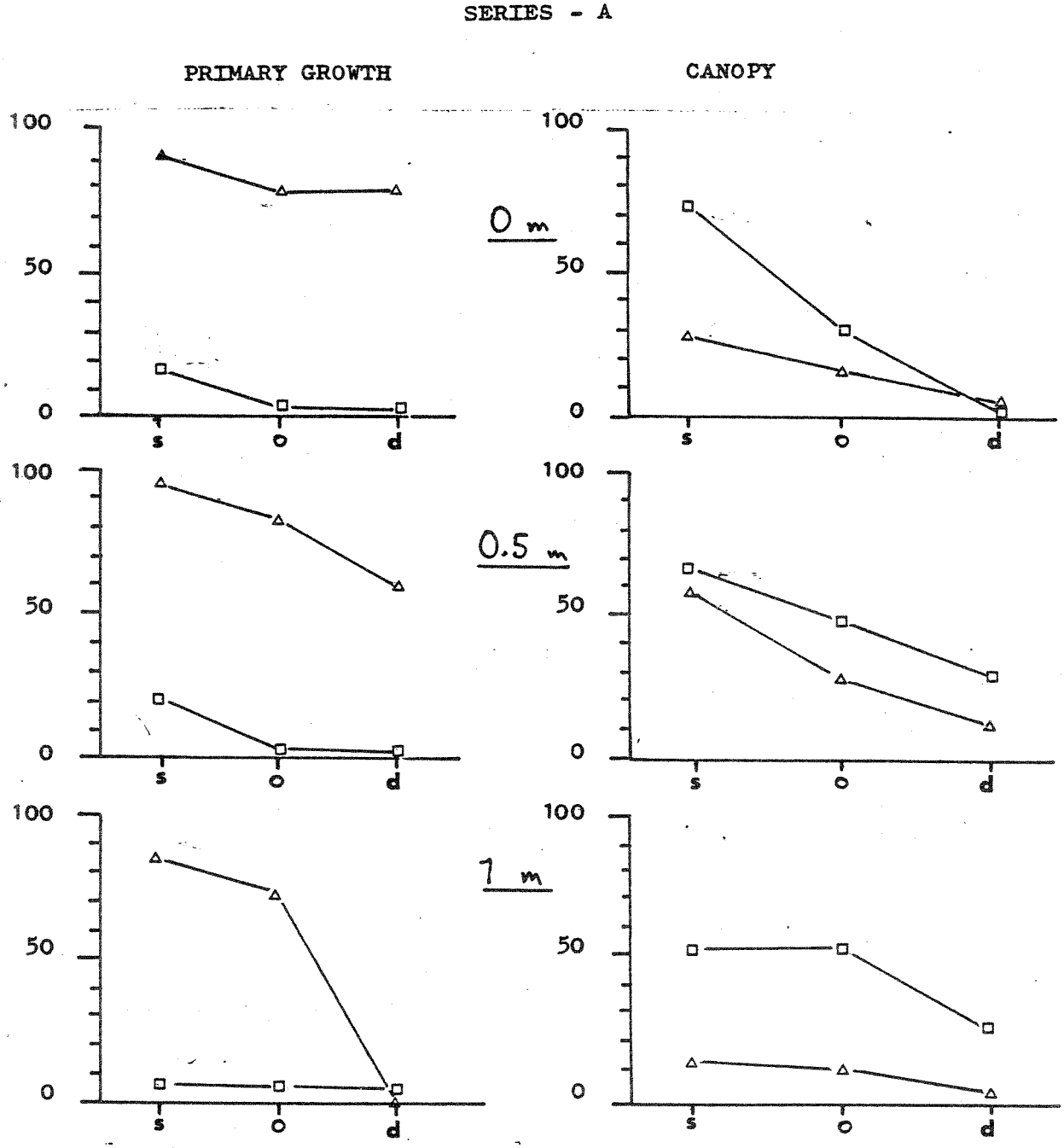


Fig. 4a. Percent cover of primary growth and canopy in series-A, September, October and December 1981. □——□ Basin II, △——△ fjord.

SERIES - A

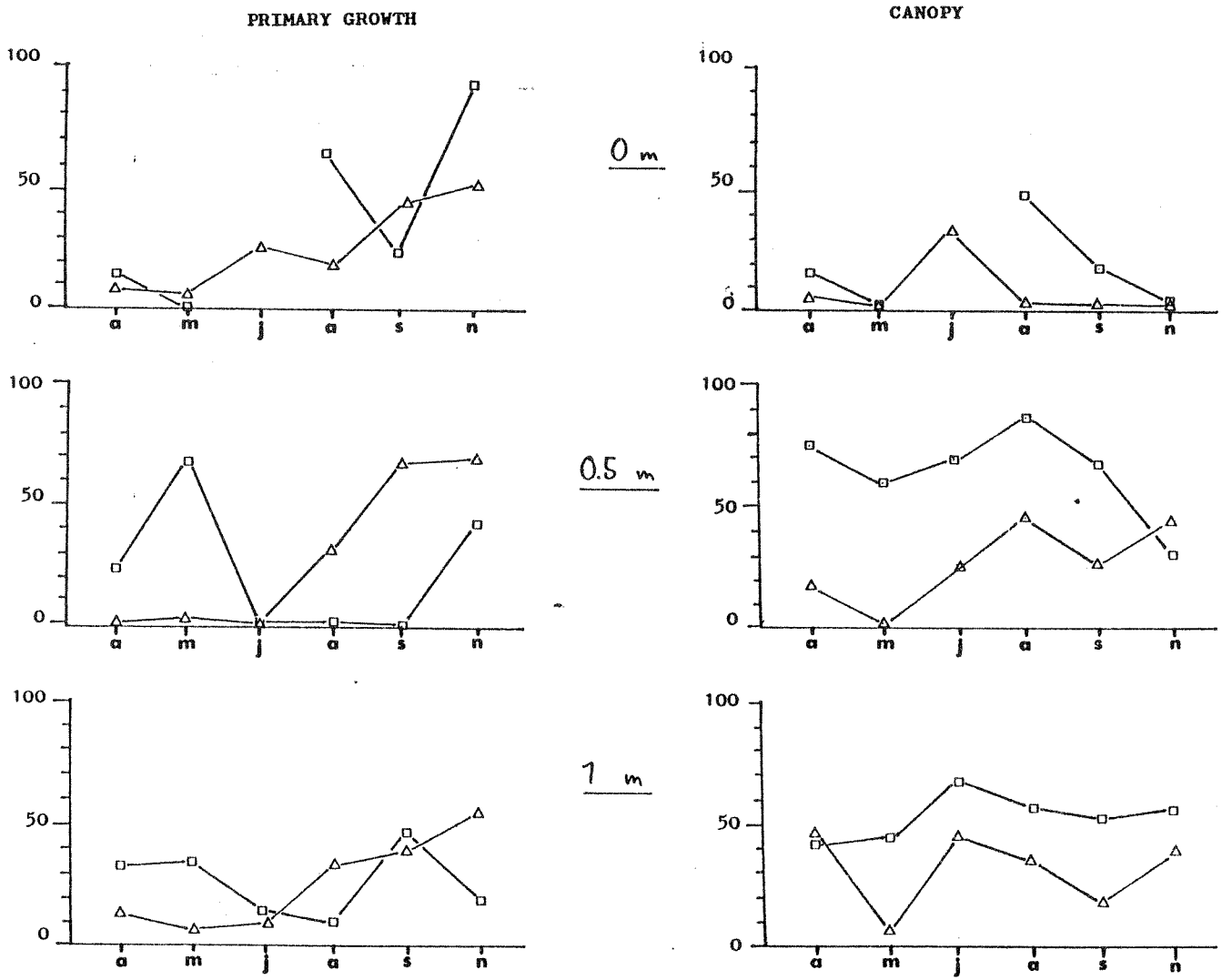


Fig. 4b. Percent cover of primary growth and canopy in series-A, April, May, July, August, September and November in 1982. □——□ Basin II △——△ fjord.

During the winter, most of the cover died because of thermal stress and ice-scouring. The only surviving species in the spring was Ralfsia sp. in the fjord. The increasing primary growth which was then recorded was due to this surviving alga, B.balanoides coming in at 0m in May and B.improvisus in August and September. 0.5m and 1m lacked B.balanoides but increased rapidly from July with the heavy settlement of B.improvisus .

In the basin, Ulothrix sp. bloomed in April at 0m and made a very dense cover, preventing further settlement. In August and September, Enteromorpha spp. did the same. Algal thalli of 1 - 2 cm made up a very dense mat and have to be considered as primary growth. At the end of the year, this "primary growth" consisted of a dense cover of detritus and benthic diatoms which will be discussed later on.

The canopy in the fjord was again Chaetomorpha linum, but also Laomedea flexuosa and Enteromorpha spp. in the spring and Nemalion helmintoides in the autumn.

In the basin Ulva lactuca formed a dense canopy most of the time, but also Enteromorpha spp. and Ectocarpus sp. were present.

SERIES - B

Unfortunately the experiments with cages were not a sucess. It seems very difficult to keep predators out of the cages all of the time, which I had been warned about(L.Vadas in pers. comm. at Solbergstrand sept.-82). This seems to be a problem in all experiments involving cages(Connel 1961b, Menge 1976, Underwood 1980).

It was impossible to keep out Asterias rubens which always shows a remarkeble ability to get in or out of "impossible" prisons(Kvalvågnæs 1972).

Fig. 5 shows the fjord-data for the three depths, all sides put together. The last settlement of B.balnoides the second year appeared only inside the cage in July. They possibly escaped predation here at first, but were

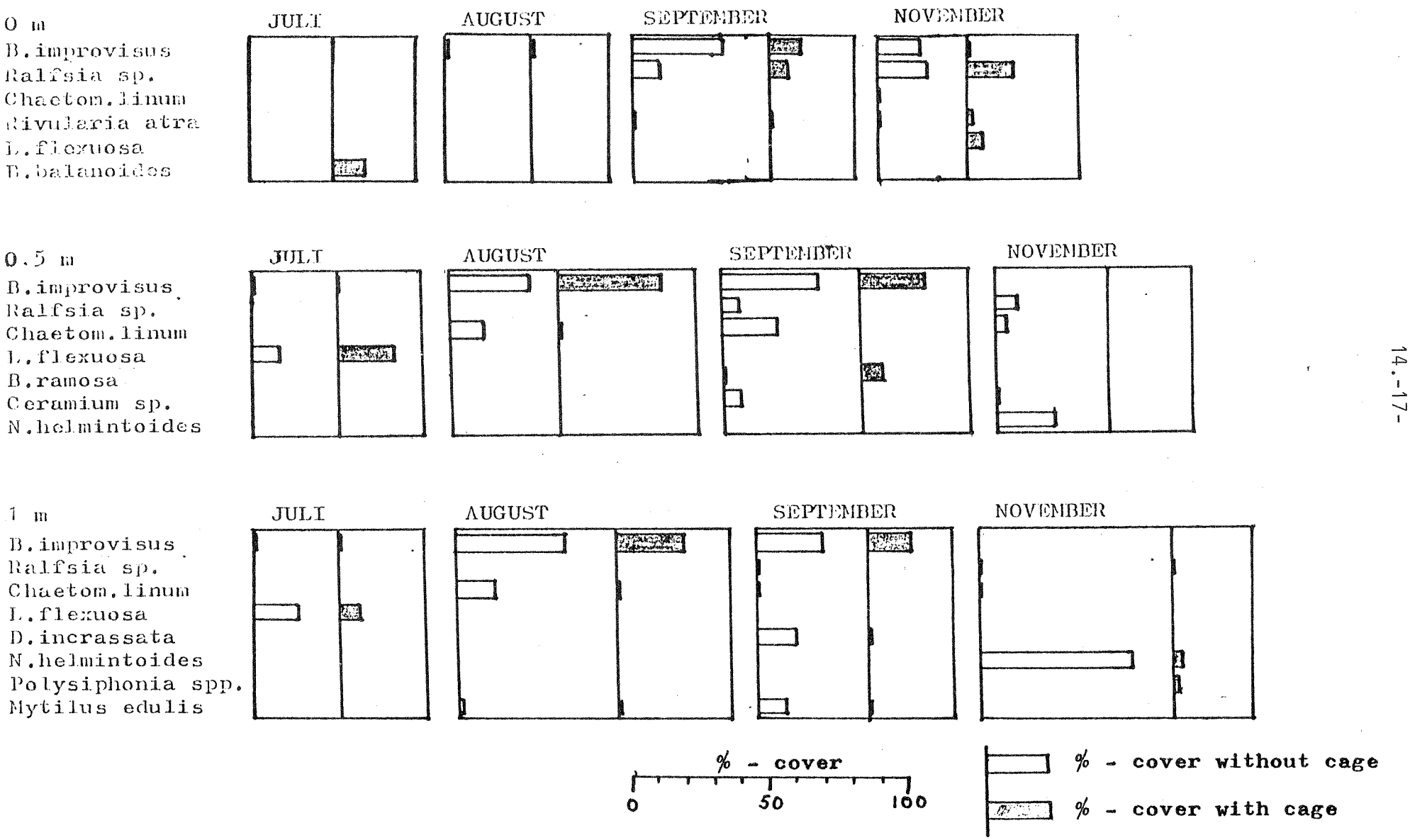


Fig. 5. Percent cover of series-B, pavementstones with and without cages. Fjordlocality.

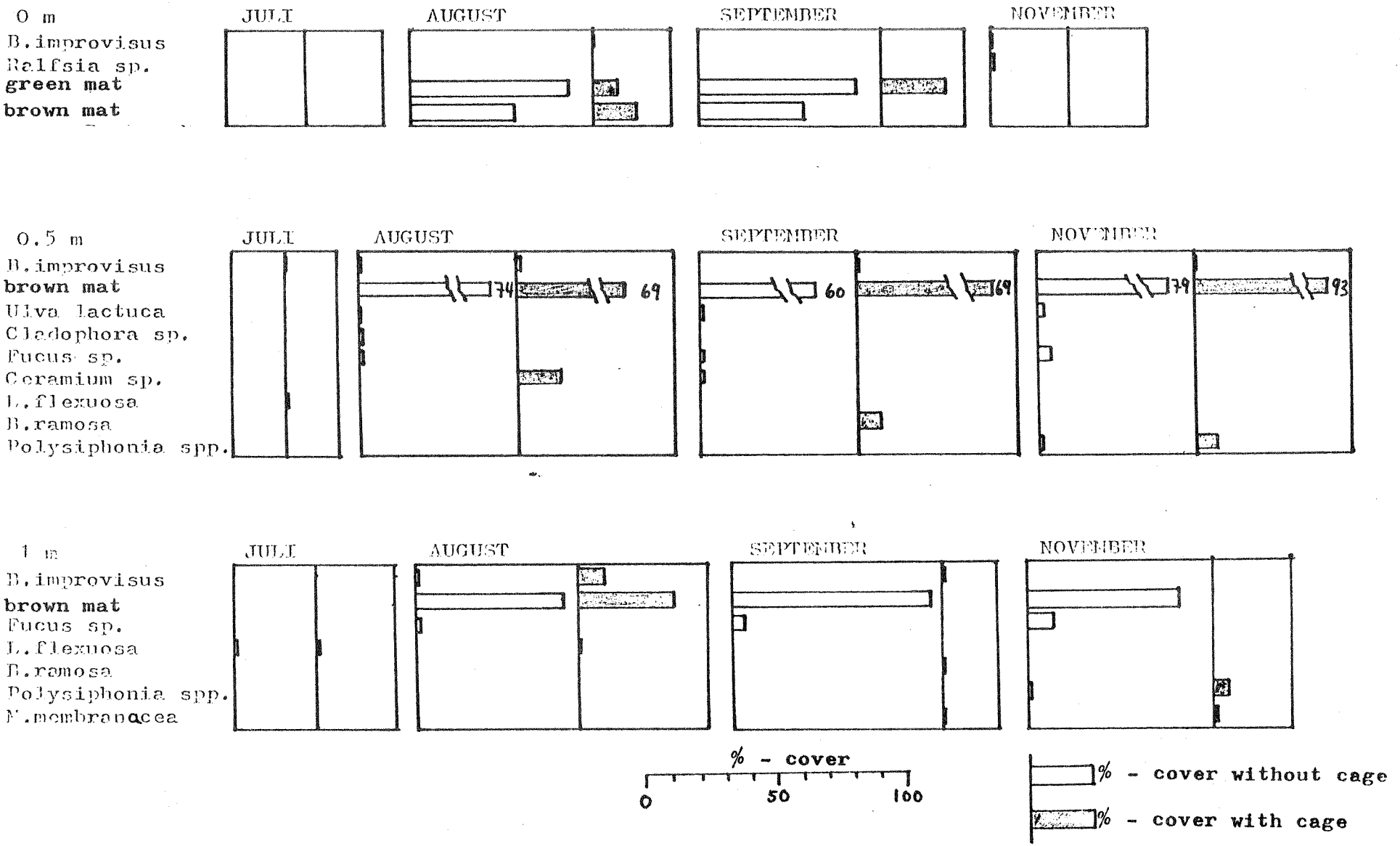


Fig. 6. Percent cover of series - B, pavementstones with and without cages. Basin II.

gone the following month when Asterias managed to get inside. The same thing seems to happen with B.improvisus which had less growth inside the cages.

It seems that all stones without a cage were more attractive substrates for all the species. Another factor is the light, which was reduced to about 50% inside the cages. This will certainly affect the algal growth inside.

It seems that Chaetomorpha linum is unable to grow inside the cages, though it settled willingly upon the cage itself, on the outside.

Growth and clogging of the netting in the summer was also a problem. Denley & Underwood(1979) claim that mesh with of 2.5mm reduces water current under the cage and therefor reduces settlement. Since clogging and growth on the netting did occur, it seems possible that it could reduce the current and then settlement and growth because of lack of particles reaching the substrate. It is a fact that surviving barnacles never grew to what can be said "a normal size" inside the cages.

The extreme lack of growth in November at 0.5m is certainly due to predation. The heavy settlement of N. helmintoides at 1m is believed to be due to the substratum which then was densely covered with empty barnacle shells. This seems to be an attractive substrate for the alga, either because of its texture (Paine 1974) or because the predatory periwinkles are not able to move over it (Menge 1976).

There was a complete different picture in basin II (Fig 6). There was an extremely light settlement of all species. A dense cover of detritus and benthic diatoms and probably some algaesporlings dominated the stones. This is probably due to the small wave-exposure, and perhaps to less dense population of periwinkles in the basin.

The clogging of the cages was a tremendous problem here, and they needed to be cleaned twice a week to keep them as free of dirt as wanted.

Fig. 7 gives the total cover of the two localities with and without the cages. The dashed lines indicate the

SERIES - B

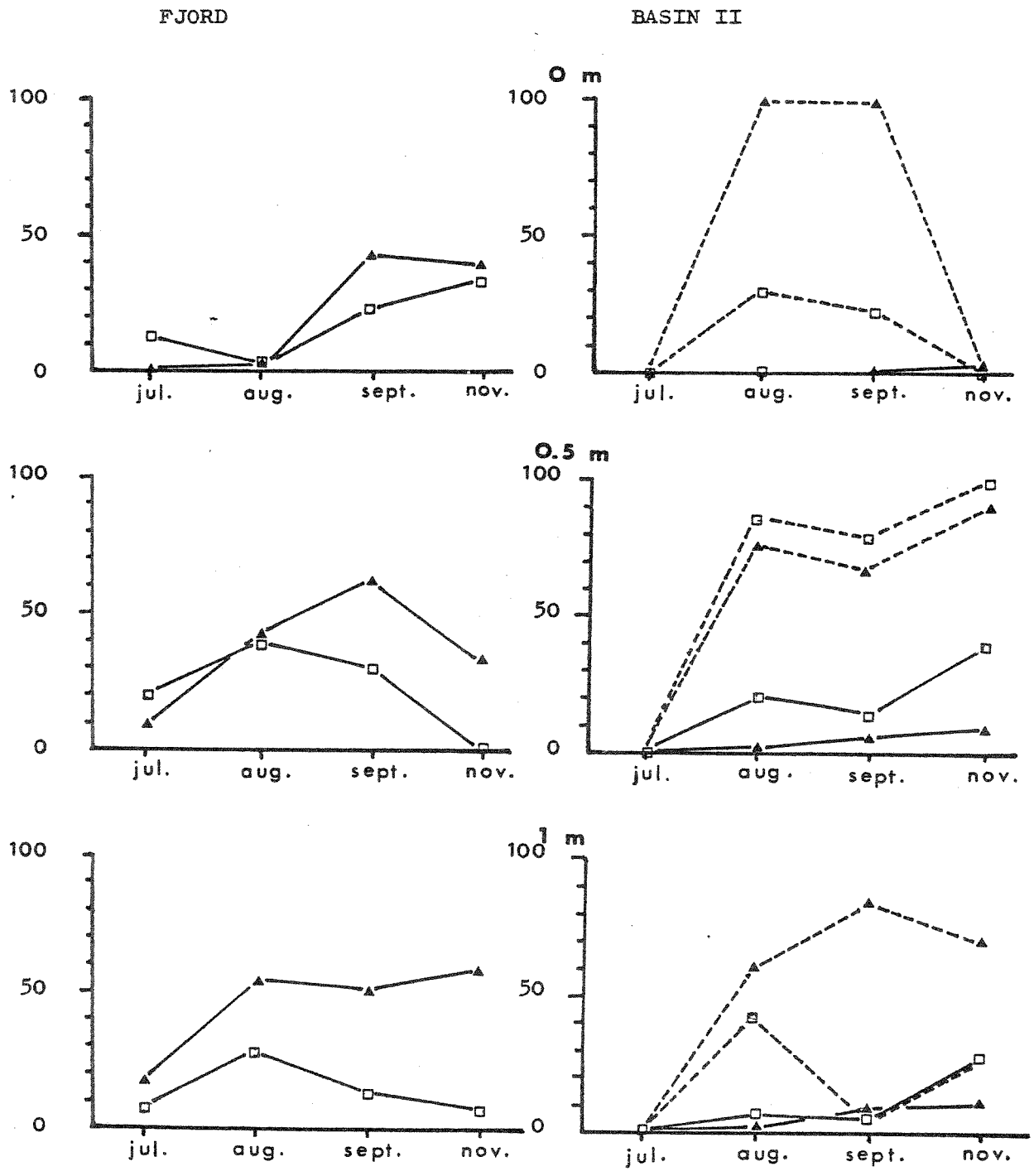


Fig. 7. Percent cover of series-B, totalgrowth of both localities.

▲——▲ w.out cages } without the
□——□ with cages } green/brown mat

▲----▲ w.out cages } with the
□----□ with cages } green/brown mat

total cover with the detritus mat. This probably reduced settlement very greatly. It was like a thick carpet, completely covering the contours of the stones.

SERIES - C

The last series clearly showed the differences in settlement at the two localities(Fig. 8). The first month from June to July was almost the same for both, with some B.improvisus and L.flexuosa. From July to August, barnacles dominated the fjord completely, with about ten percent cover of Cladophora sp. in addition. In the basin, only a small number of barnacles will settle during the month.

The next two months a lot of species will settle on the fjordstones, but from beginning of August and the rest of the year, there was no settlement in the basin at all.

COMPARING THE THREE SERIES

Fjord

In Fig. 9 we can see that in the two first months, the barnacles were not able to settle in the "old" substrate to the same extent as the "new" one. This was probably due to the algal cover which is believed to prevent settlement.

In September, algae settled on series - C. It is not clear whether the algae can settle because of a decline in the numbers of cypris larvae and therefore reduced barnacle settlement, or wether barnacle settlement is in fact reduced by algal settlement.

What is clear, is that more species are looking for a place to settle in the early autumn than in the hot summer, and competition for free space become significant.

The hydroid Bougainvillia ramosa only grew on the new substrate, and Fucus sp.(prob. F.serratus) which is believed

SERIES - C

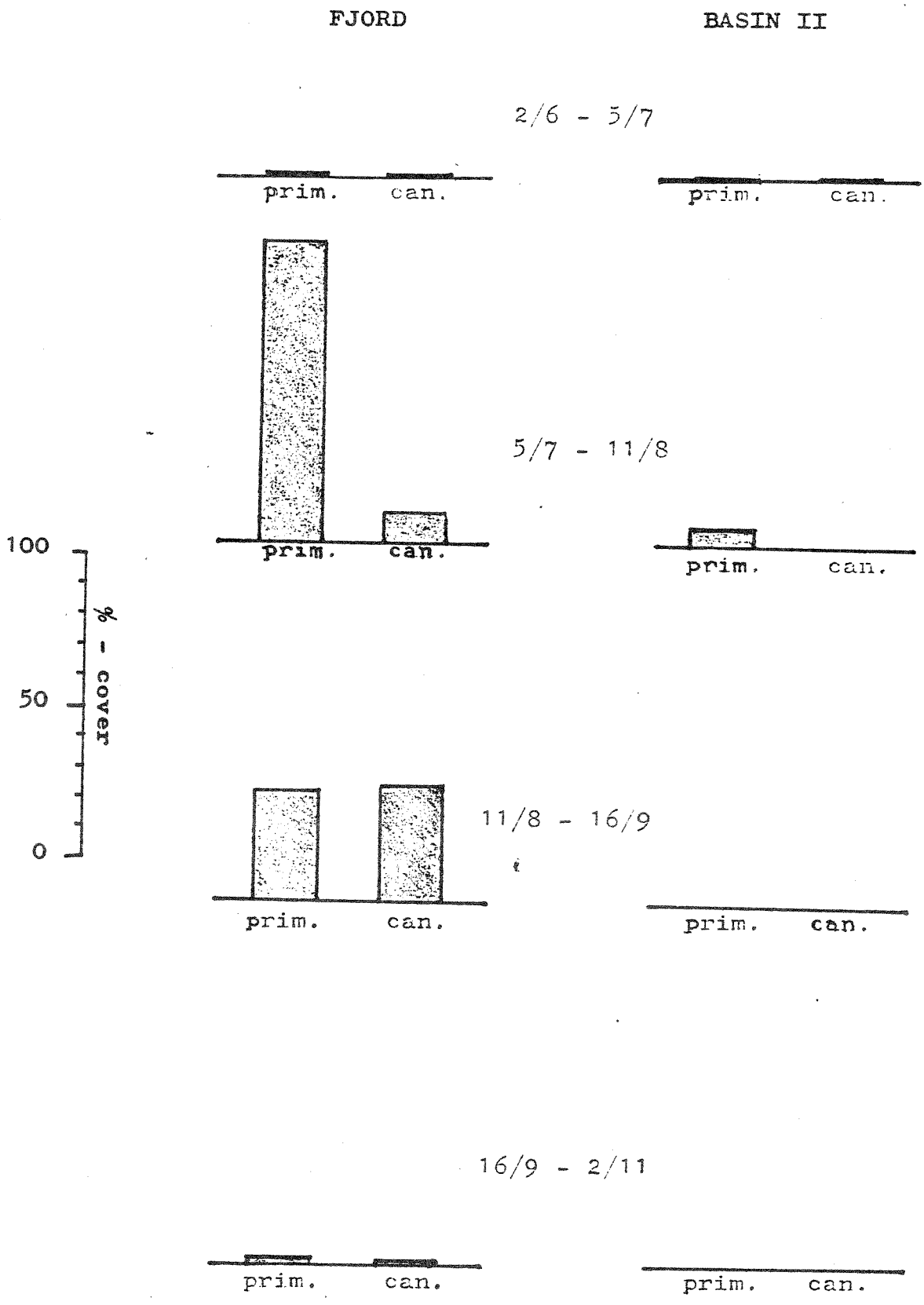


Fig. 8. Percent cover for series-C, comparing settlement for the two localities in primary-growth and canopy.

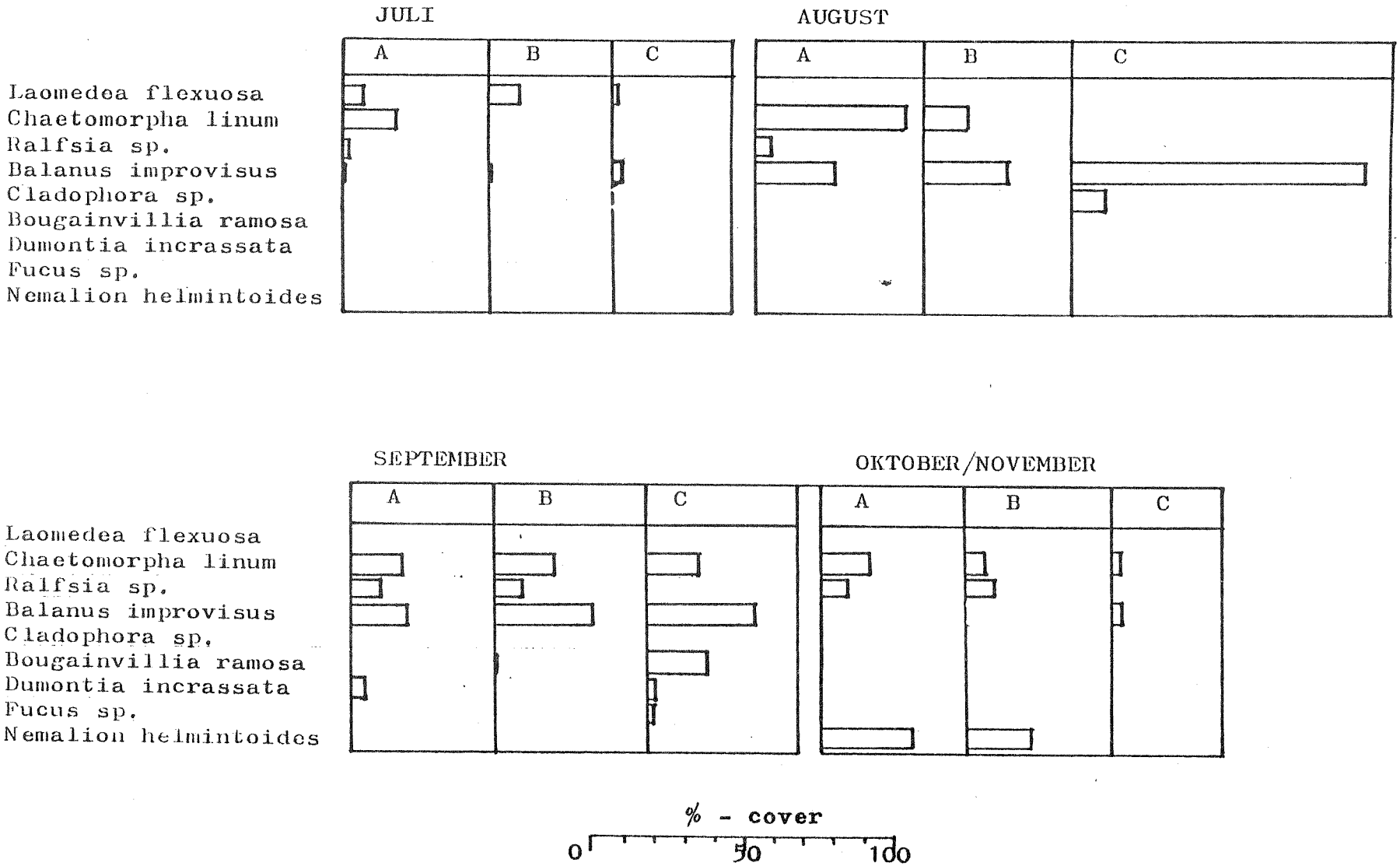


Fig. 9. Comparing the percent cover of all three series in the fjordlocality.

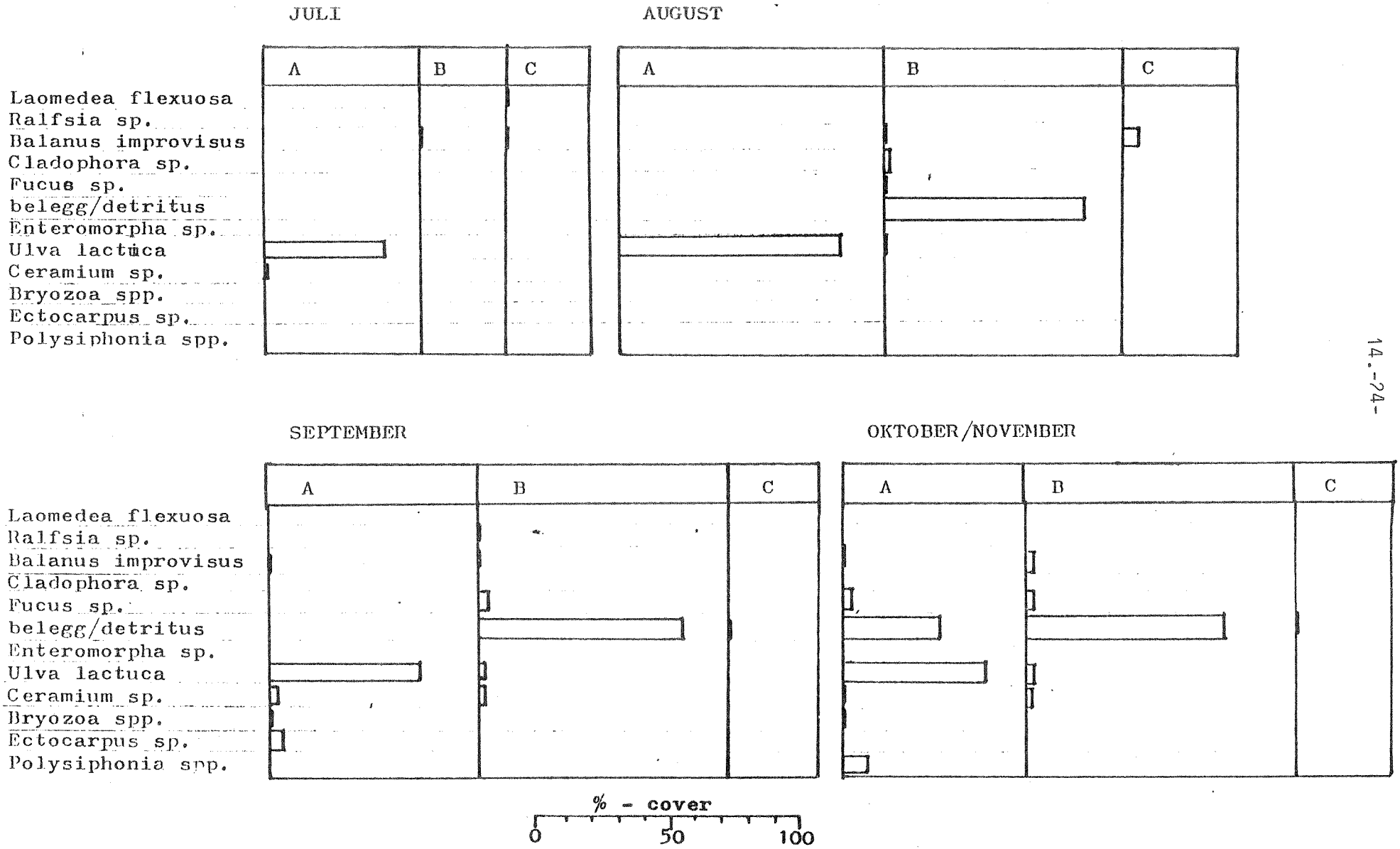


Fig. 10. Comparing the percent cover of all three series in Basin II.

to choose a K-selected strategy appeared on the new substrate and not on the old one.

Late in the autumn the number of organisms settling is of course very much reduced. Only N.helmintoides bloomed on the old substrate, probably for reasons stated before(p.9).

Basin II

Ulva lactuca (Fig. 10) dominated the oldest substrate all the time, preventing further settling until September/ October when water temperature or age decreased its growth and allowed other species to settle. Detritus and clogging is a problem on the B-series as stated before, and no settlement occurred on the new substrates.

PLANKTON HAULS

The figures for the basin and fjord in Table 2 should not be compared directly. The aim of the plankton hauls was to find differences in the species composition, due to the pumpingsystem. The amount of water filtered in the two localities will not be the same, but the amount filtered from time to time in one locality should be the same, so the figures for one of them will be comparable through time.

The only organisms not found in the basin are the most fragile ones. Medusae and comb-jellies were never recognised here. They are believed to be destroyed by the pump. But, surprisingly, relatively fragile organisms like the barnacle nauplii and egg capsules of Littorina littorea pass through the pumping system without any visible damage.

The most significant difference between the hauls was in the colour, and therefore the phytoplankton content of the samples. The fjord samples were dominated by a thick, brown soup of Ceratium spp.in the summer. The basin samples on the other hand were colourless and transparent, containing only few specimens of phytoplankton, and when they do, large species like Coscinodiscus sp. were found.

Table 2. Planktonhauls from the two localities 1982. 5min. hauls from the intake of Basin II and surface hauls from the fjord outside the station.

BASIN II

	12/3	11/4	15/5	19/6	21/7	28/8	5/10
Bivalve, larvae	4	6	1129	3250	270	265	19
L. littorea, capsules	1	16	1041	164	10	3	0
Opistobranch, larvae	0	4	230	4	29	29	32
Prosobranch, larvae	0	0	2	1360	45	79	2
Cirripednauplius	93	6	11	2	26	13	0
Cyprislarvae	0	0	12	14	289	36	1
dead cyprislarvae	0	0	0	0	371	0	0
Spionid, larvae	3	5	173	12	1	25	9
Cyphonauteslarvae	0	0	0	0	11	7	5

FJORD

	12/3	11/4	15/5	19/6	21/7	28/8	5/10
Bivalve, larvae	2	2	2440	1545	1880	2450	110
L. littorea, capsules	0	51	3780	145	340	0	0
Opistobranch, larvae	0	6	1710	35	60	20	65
Prosobranch, larvae	0	0	10	520	2040	1550	35
Cirripednauplius	48	7	0	5	140	140	5
Cyprislarvae	0	1	0	15	720	440	5
dead cyprislarvae	0	0	0	0	360	20	0
Spionid, larvae	0	60	360	10	60	350	25
Cyphonauteslarvae	0	1	0	0	0	80	35
Obelia spp.	2	13	180	25	480	290	10
Lizzia blondina	0	0	0	0	1240	160	715

This I believe can be a factor explaining the reduced growth of barnacles in the basin, because of lack of food.

ANALYSES OF NUTRIENTS

Water samples were collected at 0m and 1m at three localities; near the stones in the fjord (F) south of the river outlet, at the water intake (I) in the fjord about 10m north of the outlet and inside the basin II (B).

Table 3 gives the consentration in µmol for the nutrients. These data give no reason to believe that nutrients are accumulating in the basin.

In spite of the comparison of these data with wind-direction, tide and amount of water in the river, I can see no correlation, except maybe for April values. Most of the nutrients have greater values at this time in the fjord. This is probably due to the river water being blown soth-ward over the locality and influencing the nutrient concentrations in the water.

For the other dates, I can see no correlation and have to conclude that nutrient concentrations in the water do not explain differences in settlement and growth.

LIGHT

The incoming light for the two localities was measured for 22 hours to see if one of them received more light than the other.

Two cosinus-collectors were placed at 1m depth at both localities, one in the middle of the basin, and one hanging about 5m out from the shore in the fjord. They were both connected to a recorder which gave a continual picture of the incoming light between 400 - 700nm.

Data from the same time were picked out of the curves

Tab. 3. Nutrientanalysis. Watersamples collected at Basin II (B), intake of water to basins by the pier (I) and fjordlocality about 70m south of the river-outlet (F). The concentrations are given in µM.

	17/4				15/5				19/6			
	NO_2	NO_3	NH_4	PO_4	NO_2	NO_3	NH_4	PO_4	NO_2	NO_3	NH_4	PO_4
0m B	0.18	7.27	0.18	0.35	0.34	5.12	1.46	0.14	0.10	0.89	2.34	0.21
1m B	0.16	7.16	0.18	-	0.34	4.36	1.19	0.28	0.10	0.53	0.88	0.21
0m I	-	-	-	-	0.20	10.66	1.10	0.31	0.05	1.11	1.28	0.14
1m I	-	-	-	-	0.34	6.90	1.19	0.14	0.05	0.44	0.64	0.73
0m F	0.35	27.93	2.56	0.29	0.34	4.67	1.10	0.07	0.05	2.67	0.51	0.21
1m F	0.33	19.16	2.56	0.21	0.34	5.56	1.64	0.14	0.05	0.76	1.37	0.21

	21/7				28/8				5/10			
	NO_2	NO_3	NH_4	PO_4	NO_2	NO_3	NH_4	PO_4	NO_2	NO_3	NH_4	PO_4
0m B	0.0	0.22	0.73	0.46	0.0	0.0	0.55	0.27	0.20	5.26	1.46	0.36
1m B	0.0	0.89	1.64	0.36	0.68	3.08	0.37	0.14	0.10	2.24	0.91	0.14
0m I	0.0	0.44	0.91	0.27	0.0	0.53	0.55	0.27	0.51	32.37	2.01	0.86
1m I	0.0	0.67	0.55	0.55	0.0	0.26	0.55	0.14	0.16	4.13	1.10	0.54
0m F	0.0	0.0	1.0	0.46	0.0	0.79	0.91	0.18	0.25	6.32	1.37	0.68
1m F	0.0	0.89	1.37	0.41	0.0	0.45	0.91	0.18	0.20	6.05	1.28	0.46

and translated to be directly comparable, using the correlationfactor in each cells certificate. The immersio-factor was set to 1.4.

The two curves are shown in Fig. 11. The anomalies at about 0830 are due to some algal thalli flouting around in the basin, and the shadow of the gallow in which the collector was hanging in the fjord.

It seems that the basin receives less light than the fjord locality even though there are no physical hindrances for light such as trees, mountains, buildings etc.

The sky was completely cloudless all the time, and it was blowing a normal summer breeze due to the difference of heating on land and sea (wind from south-east, Beaufort's scale: 3).

A possible explanation for this difference in light must be that the general environment in the basin is darker. This is because of the walls, the dark wave-machine and the many large brownalgae on the stairs. Even though the angle of total reflection (48.6^{o}) is believed to be exceeded before light hits the walls, the amount of light which can be reflected from the bottom and the surroundings via the water surface and down to the collector is lower than in the fjord where surroundings are mainly very bright sand.

A 2pi-collector which I think is more convenient for measuring light input for the benthos would probably show an even greater difference in incoming light.
Unfortunately two collectors of this kind were not available.

WAVE - EXPOSURE

The wave heights were measured at the fjord locality with a stick, marked at 10cm intervals,which was placed in the same position every time. Wave heights were measured in different weather conditions, and the average wave height of about 15 waves was compared with the wind direction and strenght.

The waves in the basin never exceeded 10cm in height. Using Beaufort's scale, southerly winds at force 2 and more

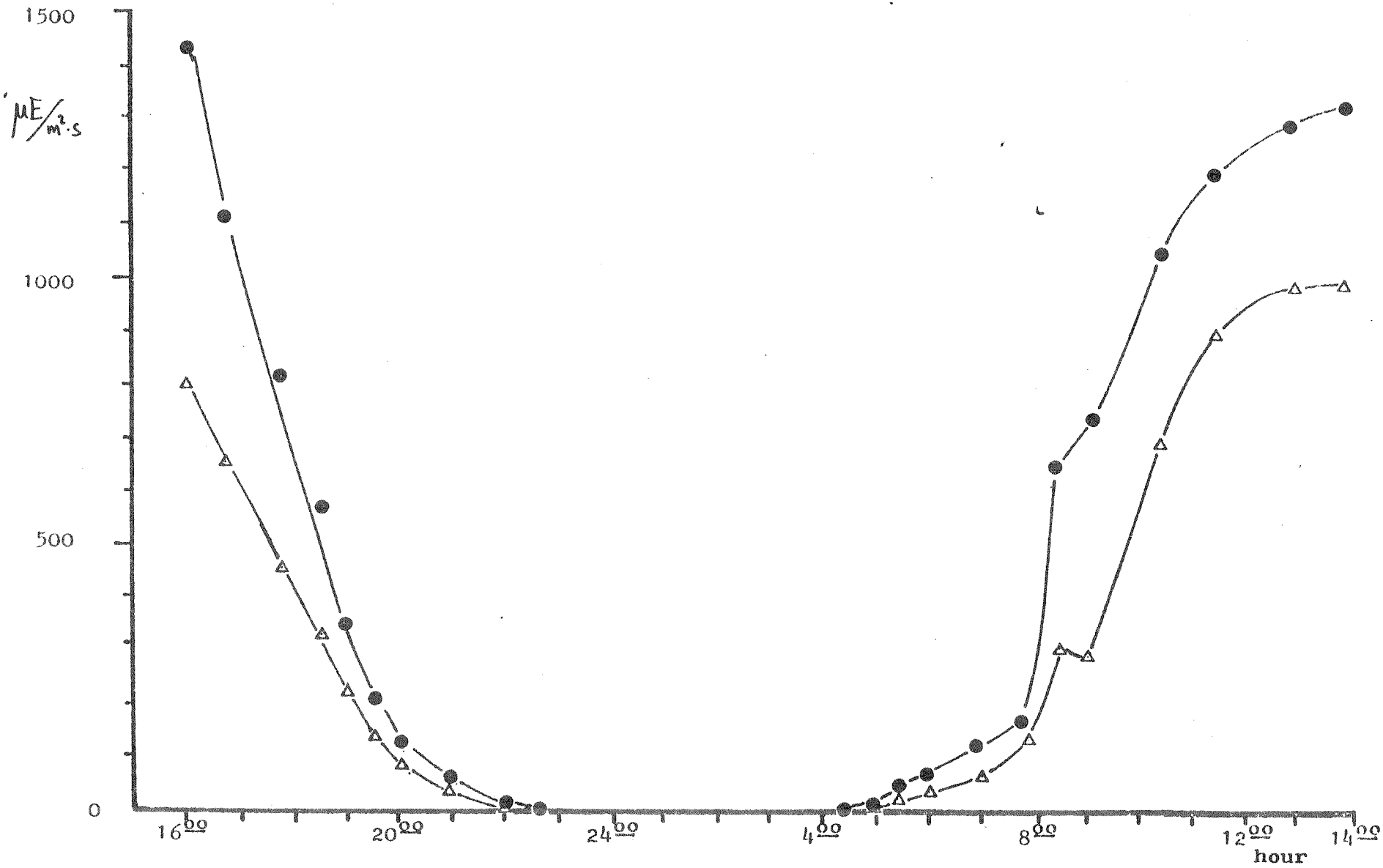


Fig. 11. Variation in incoming light at 1m depth at the two localities for 22 hours.
●——● fjord △——△ Basin II

and northerly winds at force 3 or more gave wave heights of 10cm or more in the fjord.

Winds from these directions and strengths blew for nearly 50% of the research time. In addition there were westerly winds (which I have no wave measurements for) and the many passages of ferries, tankers and private vessels every day.

Since the wave machine was turned off during work and investigation at the basin, the amount of exposure and water movement is considerably less in the basin than in the fjord locality.

This fact must surely influence the settlement and growth of organisms in the basin.

DISCUSSION AND A SUGGESTION FOR FUTURE MONITORING

There is a clear difference in settlement and growth between the control basin and the natural locality. This can be caused either by an altered input of organisms from the pipelines and the pump, or because the environment in the basin itself is unfavowrable for some organisms or of a completely different character from the fjord.

The lesser growth of the sessile animals in the basin can be due to a lower supply of livivng food particles such as phytoplankton. The distribution of plankton in the water is certainly patchy. If the current outside the pier is not in any way able to lead the plankton masses to the inlet as expected, this could be a reason for a reduced amount of phytoplankton in the basin.

However, this is not a good explanation because the plankton hauls from the basin II contain a lot of small zooplankton not expected to have a very strong swimming ability compared with the current speed.

It is also difficult to imagine a vertical distribution of the phytoplankton in such thin layers that they are

unavailable for the pump at a depth of 1m.

Another problem to consider is the pumping system itself with the pipes. There is an extremely high density of filterfeeders inside all the pipes. This population seems always to have enough clean water and probably enough food. It is possible for these animals to filter away a lot of plankton before the water reaches the basins. The current inside the pipes will surely be turbulent because of the rough walls covered by living animals.

Another problem is how the organisms are distributed to each basin. Is it possible for most of the plankton to be pushed out in the first basin, leaving nothing for the rest, or will the strong current in the pipes take the lighter particles and move them to the last basin or perhaps to the overflow? I think this is still an open question.

Another important reason for differences between basin and fjord is wave exposure and water movement. There are a lot of symptoms of little water movement in the basin. The clogging and smothering from sediment and detritus is a great problem. Less growth and death is reported (Lewis 1968, Ryland 1972) when heavy smothering occurres.

The basin probably works as a sediment trap. The waves stir the sediment in the fjord and make it easily available for the pump. Inside the basin, most of the sediment will settle because there is less water movement.

The reduced water movement is also evident from formation of hydrogen sulphide under very thin layers of sediment (1 - 2mm).

Another source of particles could be from the population of Mytilus edulis in the basin. They are reported (Tsuchiya 1980) to produce large amounts of faeces and pseudo faeces during a year.

The stones in the deepest part of the basin did indeed suffer from smothering by sediment and detritus. Primary growth was seldom present here, and never on horizontal surfaces.

In addition to this, the water movement itself seems important to settlement and growth (Barnes 1955,1970, Barnes & Powell 1953, Crisp 1955). Algal"washing" will be a problem even in sheltered water (Grant 1977) and was a problem for some of the stones facing big macroalgae such as Laminaria saccharina and Ascophyllum nodusum. A heavy algal growth will also prevent settlement and feeding activity (Barnes 1955).

The thick layers of diatoms will also prevent settlement (Bastida et al. 1971), and a large population of grazers would be needed to have any effect on this diatom carpet (Castenholz 1961). The population of L.littorea is considerably smaller in the basin II than in the fjord (Lystad & Moe pers. comm.).

The species composition of the diatom layers seems to be differnt in the fjord and basin II. Only one sample was collected, but the diatoms in the sample from the basin were mobile, pennate ones, wheras those in the fjord sample were covered with mucus and fastened to the surface with a mucus string.

I am aware of the great fluctuations in settlement and growth in the littoral zone from year to year, and that the picture this report has been given may change during time. But, the fact is that there are great differences from an artificial to a natural locality. I think it is possible to find most of the sources of these problems by a careful monitoring of the basins and all the time compare them with the wild conditions.

I suggest the following as a future monitoring program for the basins :

a) Plankton hauls once a week or fortnight at the inlet of every basin and in the fjord. This may show differences in distribution of organisms to the basins. It will also be possible to see if organisms in the fjord are selected by

the pumping system in one way or another, and it will give an excellent record of natural cycles in the fjord, useful for many of scientists working here.

To diminish the sources of errors in plankton distribution because of possible vertical zonation, the hauls should be taken at similar tide- (or better water-) heights every time.

b) Settling plates in every basin should be compared with each other and with settling plates in the fjord e.g. once a month. This will give an idea of which organisms prefer the different localities, and will give the opportunity to ask why this is so. The plates should be compared with the plankton hauls.

To standardize all the settling experiments, a float could be permanently placed in the fjord outside the station, and all the settling plates, collectors etc. could be handeled from here.

c) The monitoring of the sessile basin community should continue in all the basins and a control, similar in shape and structure, should be built in the fjord.
A"basin" with stairs could be built at the pier, open to the sea. This would give an opportunity to follow carefully a complete natural littoral community to compare with the basins.

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MARINE RESEARCH STATION SOLBERGSTRAND

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15. SOFT BOTTOM EXPERIMENTS

Status report pr December 1, 1983.

by John A. Berge, The University of Oslo

Indoor basins

The construction of the indoor basins were finished in April 1983. Figure 1 - 3 gives a brief description of the basins. Each of the two basins has a surface area of 120 m^2 and a maximum water depth of 1.6 m. Each of the basins are divided into three sections by removable vertical walls (figure 2). The basins are supplyed with sea water from 42 m depth in the fjord. During the last seven months the turnover time in the basins has been approximately four days and the salinity and temperature range has been 30.5 - 33.4 ‰S and 6.8 - 13° C respectivly.

Initially each section of the basins was supplyed with sea water from to points on the bottom and drained from two overflows (se figure 2).

The first attempt to establish a subtidal community in the basins was done in the period 25/4 - 29/4-83. Sediment was taken from 30 m depth at "Bjørnehodebukta" 5 km north of Solbergstrand. The sediment was sampled with a Day grab (1/10 m^2). The sediment was placed in 48 boxes with a surface area of 0.5 m^2 (8 grabs in each box) and in 16 boxes with a surface area of 1 m^2 (16 grabs in each box) giving a sediment depth of approximatly 20 cm and leaving the sediment surface approximatly 10 cm below the top of each box. All the boxes were placed on the bottom inside the basins. The intention was to leave the boxes untoutched for 6 months before starting any experiments.

In July and the beginning of August the surface of some of the boxes turned black indicating ironsulfid. The smaller boxes were more effected than the bigger ones, probably reflecting a lower surface area / distance from sediment surface to the top of the box ratio. Measurements of oxygen concentration in the basins indicated that the concentration of oxygen 5 cm above the sediment in the boxes with black sediment were in the range 1.4 - 1.8 ppm and in the boxes with grey sediment 2.2 -4.5 ppm whereas the oxygen concentration in the rest of the basin water was 6.4 - 7 ppm.

Measurements of current velocetie in the basins have been attempted. However, the current measurement device had a lower detection limit of 1.5 cm/s and current velocetie in the basins never excceeded this limit apart from in the immediat vicinity of outlets and inlets. It is belived that the currents in the basins are not high enough to supply oxygen to the water volume immediatly above the sediment in quanteties sufficient to compensate for the oxygen consumption in the sediment. In order to increase the supply of O_2-rich water to the sediment a new system for supplying water has been built in one of the sections (se figure 1). Along one side of the section a tube with a diameter of 10.5 cm was placed horisontaly 15 cm above the level of the top of the boxes. Along this tube holes with a diameter of 2.5 cm was drilled at 5 cm intervalls and the water to the section is supplied through this pipe. At the opposit side of the basin a similar system is placed with a overflow to drain the water out of the section. This system has yet only been built in one section. Before building a similar system in the other sections we want to performe a detailed mapping of water currents so that information for necessary improvements can be found.

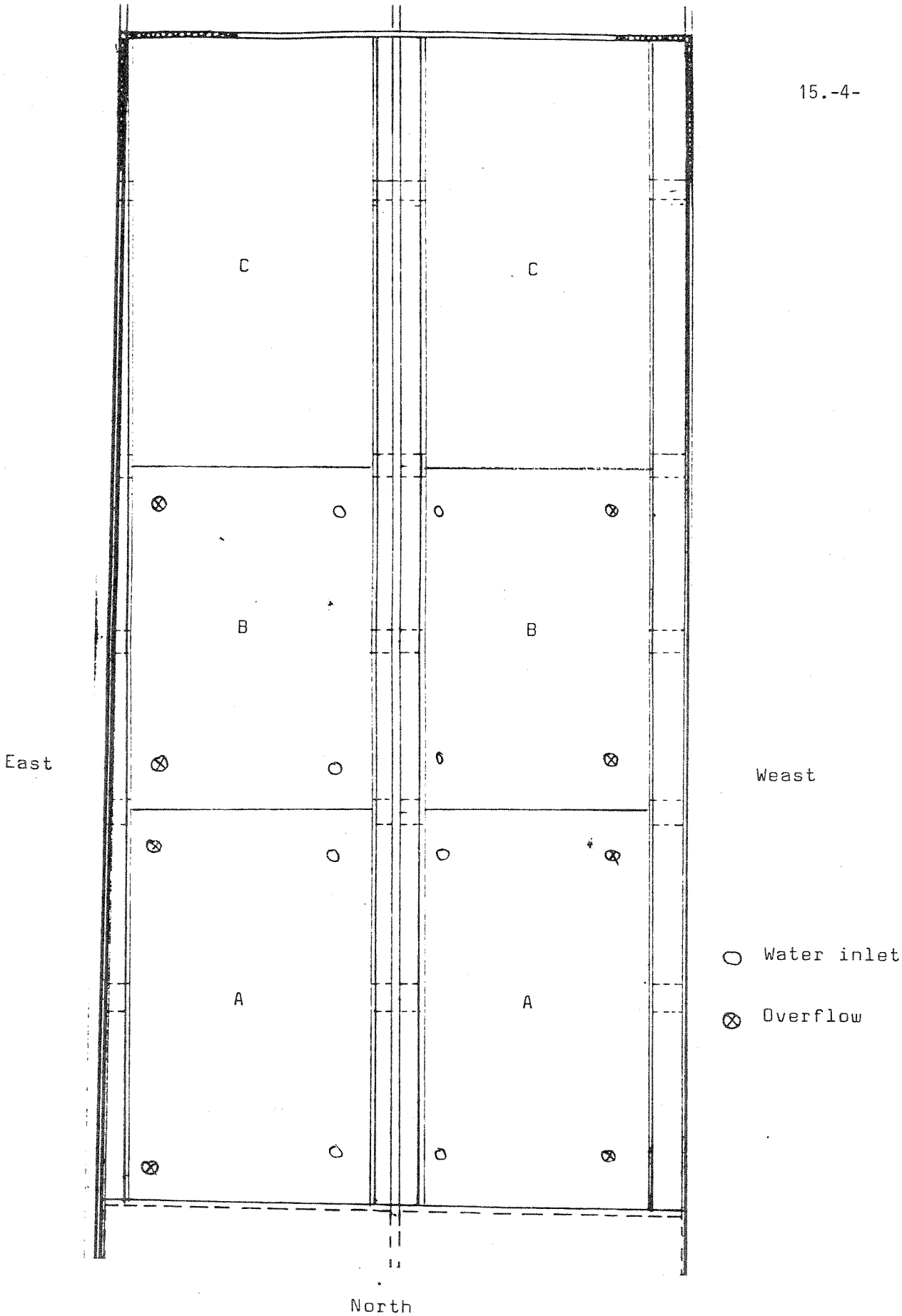
Unfortunatly there has also been some technical problems with the soft bottom system. First we have had problems with a leakage in one of the basins. Secondly it seems that the chemical environment in the soft bottom plant has been unexpecedly severe. This has resulted in signs of corrosion on all iron constructions and the wooden constructions have been infected with fungi. This forced us to treat all wooden constructions with a fungicid and repaint both wooden and iron constructions inside the plant. This woork was finished by the end of November 1983.

Most of the boxes were black sediment has been observed will be replaced with boxes with new sediment. The sediment will be sampled with a box corer (1/4 m^2). This woork will be finished by the end of December 83.

Experiments in the sea outside Solbergstrand

Previous experimental woork in the Oslofjord and on the west coast of Norway has shown that 4000 ppm (wet weight) of Ekofisk crude oil in the top 3 cm of the sediment has a clear effect on

benthic recruitment in a uneutrophicated situation, whereas the same concentration has a little effect in a eutrophicated situation. In order to establish a crude " dose respons curve" for settling of benthic fauna on oil contaminated sediment, we have placed sediment filled boxes contaminated with diffrent concentrations of crude oil on the bottom at 18 m depth outside Solbergstrand. The concentration used are 4000 ppm, 400 ppm, 100 ppm and untreated sediment. The boxes will be left submerged for 1 year and will then be brought to the surface for faunal analysis.



m=1:100

Figure 2. Softbottom plant seen from above.
At present only section A and B are used.

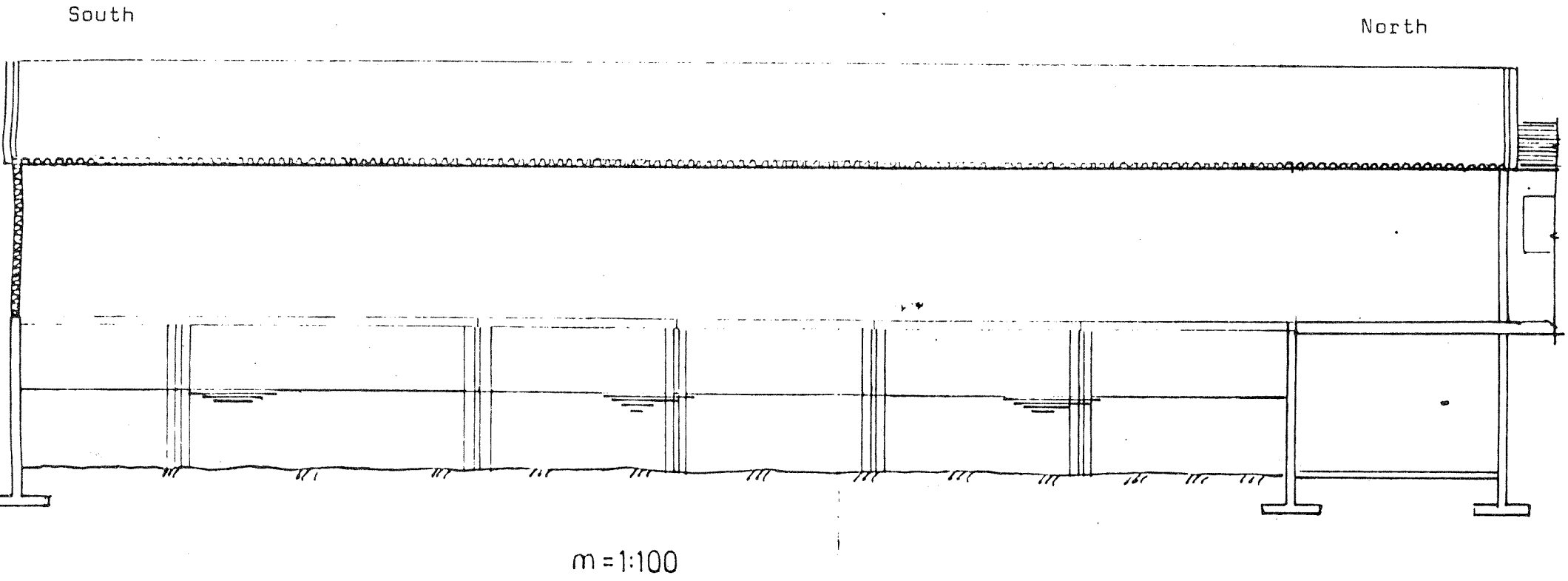


Figure 3 . Softbottom plant seen from East.

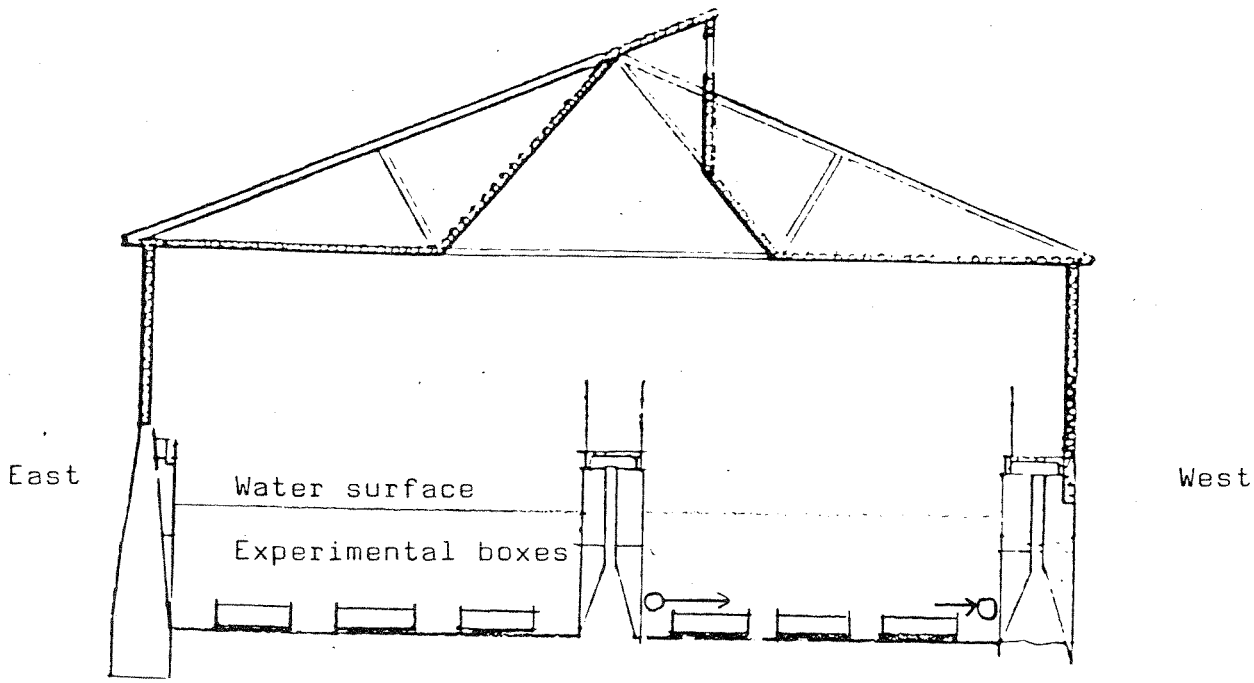


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Figure 1. Softbottom plant seen from North. Arrow in East basin indicate flow of water from new water supplying system built in one of the sections in the West basin.