NIVA – REPORT

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The report describes the various research elements of the rocky shore community project at Solbergstrand, and their status until 30 June 1983.

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SOLBERGSTRAND EXPERIMENTAL STATION, DRØBAK

Long term effects of oil on marine benthic communities in enclosures

LITTORAL ROCK COMMUNITY PROJECT

PROGRESS REPORT NO 3

June 1983

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1. FOREWORD

This document is the third Progress Report from the rocky shore community project of the BP/NIVA/UiO Research Programme on long term effects of oil on marine benthic communities.

The report compiles contributions from all participating scientists and describes in short terms the work that has been performed in the period 1 March 1983 to 30 June 1983.

All formal enquiries about the report or the sub-projects should be addressed to the Programme Manager. For a more complete description of the Programme we refer to "0-82007 SOLBERGSTRAND EXPERIMENTAL STATION. Long term effects of oil on marine benthic communities in enclosures. Research Programme May 25 1982".

Oslo, 31 July 1983

Torgeir Bakke
Programme manager

INNHOLDSFORTEGNELSE:

SOLBERGSTRAND EXPERIMENTAL STATION

Progress report No 3 Period: April - June 1983

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2. Project: Routine Monitoring of dosed hydrocarbons by fluorescence spectrometry

Torgeir Bakke, Kai Sørensen, Unni Efraimsen and Håkon Juelsen.

Purpose:

- to perform frequent estimates of the concentration of oil hydrocarbons in the water/oil emulsions (water accomodated fraction, WAF) produced for dosing into the test basins, by use of fluorescence techniques;

- to improve the fluorescence technique for routine monitoring of oil in experimental set-ups of the present type;

- to utilize the analysis results for routine adjustment to keep a stable level of exposure in each test basin;

- to correlate the fluorescence results with monthly average analysis of the same WAF by high resolution GC or GC/MS (cf the SI programme) and with analysis performed with an IR continuous oil monitor.

The sampling and analysis have followed the scheme outlined earlier. An attemt to store hexane extracts frozen for later analysis has proven successful.

Analysis progress is satisfactory. There are still problems in getting constant WAF concentration.

Figures 1 and 2 show the weekly and monthly mean nominal concentration of total hydrocarbons in the inflowing water to basins 1 and 3 based on WAF concentrations and WAF feeding rates to the header tanks.

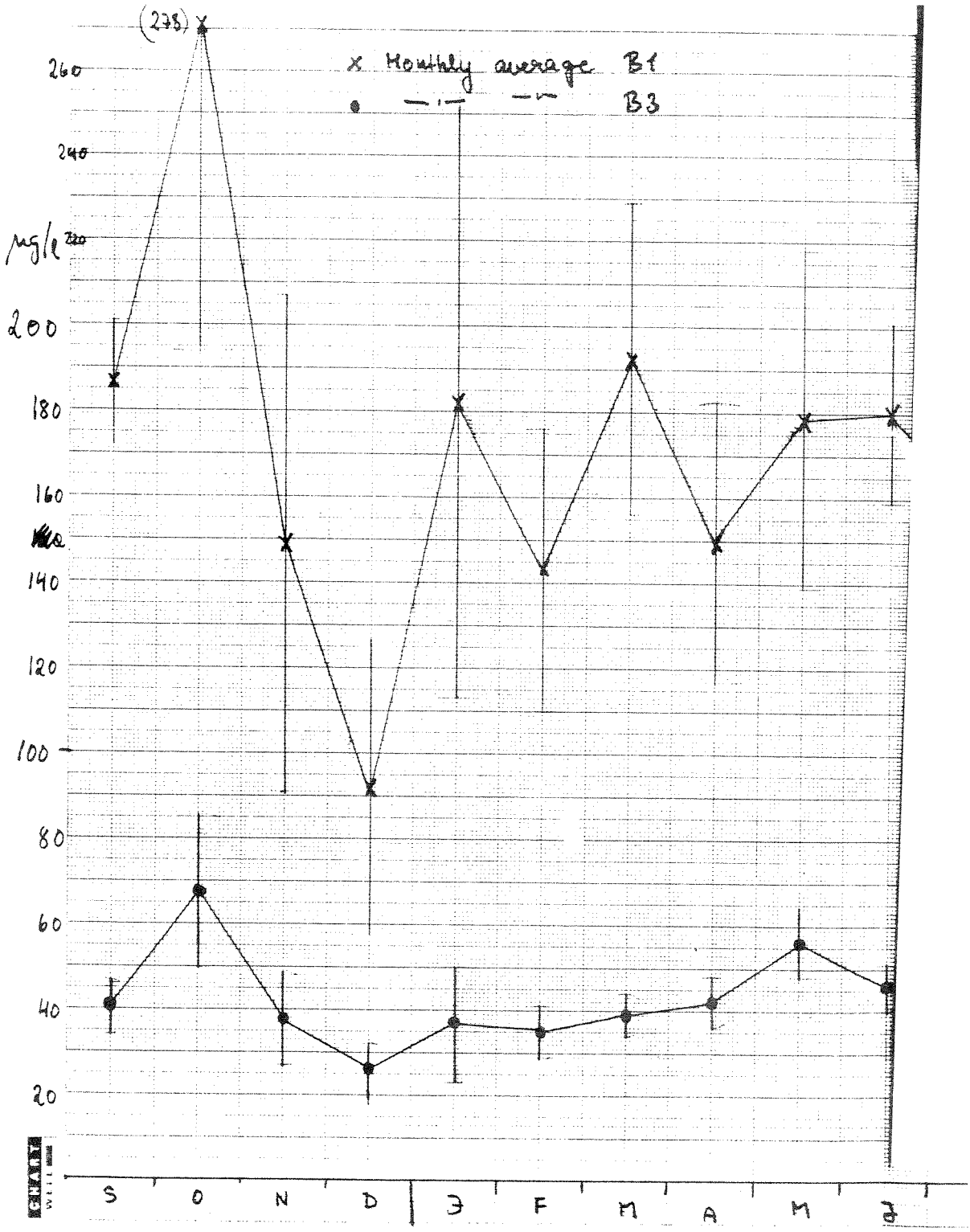

Fig. 1. Monthly mean nominal concentration of total hydrocarbons in the inflowing water to basins 1 and 3, based on WAF concentrations and WAF feeding rates to the header tanks.

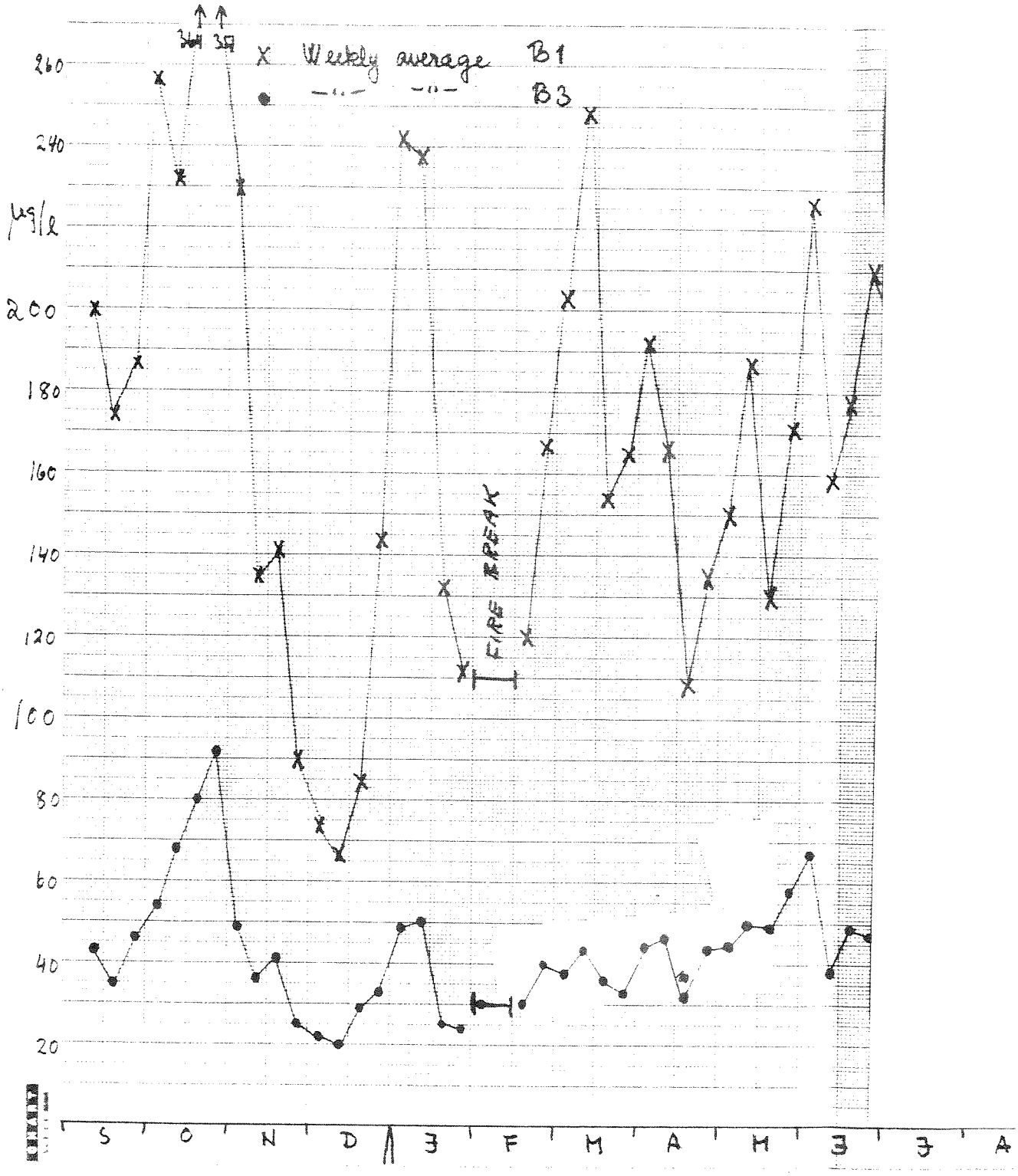

Fig. 2 Weekly mean nominal concentration of total hydrocarbons in the inflowing water to basins 1 and 3, based on WAF concentrations and WAF feeding rates to the header tanks.

Fortcoming quarter

Analysis will proceed as now. Attempts to do fluorescence analysis on the basin waters will be tried out.

Torgeir Bakke

Project leader

4. COMMUNITY STRUCTURE

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

Aim of the study:

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 detect any community changes and deviations between oil exposed basins and controls.

Description of the work:

The monitoring since March 1983 was performed during 21 March - 15 April and 30 May - 17 June. Preliminary results have shown some normal annual changes and some significant reduction of *Fucus distichus ssp. edentatus* and *Laminaria saccharina* associations in the heavily oiled basin as well as in one control basin. The more lightly oiled basin and its control has not shown a similar pattern. *Mytilus edulis* has more or less disappeared in the heavily oiled basin (B1), while there is no observed reduction in the control basin (B2). Reduction of *Asterias rubens* is found in both basins in question. The number of *Carcinus maenas* has varied in B1, but seems to have been reduced, while the number in B2 is more stable. The behavior of *Littorina littorea* in B1 has been extraordinary during the months after the oil exposure started.

The growth is documented by photos immediately after the monitorings.

5. RECOLONIZATION AND POPULATION STRUCTURE

Participant: T. Bokn and J. Knutzen (NIVA)

Aim of the study:

To see if diesel-oil has any effects on gametes, zygotes, larvae and/or germlings, granite chips in two basins (one oiled/one control) will be studied every month during three years.

Description of the work:

Harvesting of chips (short time study) was performed 18 April, 16/18 May and 16 June. Chips for long time study were sampled 16/18 May. Organisms have been identified to a certain degree (genera). Results gained during the start of the project to the May sampling were presented at the XIth International Seaweed Symposium, 19-25 June this year in Qingdao, China. The conclusion of this paper was that all the predominating species in the oil exposed basin have been diatoms, and none has played any important role in the flora in the control basin with very few exceptions. Included in the predominating species of the control basin have been two annuals of Phaeo- and Chlorophyceae, which never showed the same appearance in the oil exposed basin. The present data of ash free dry weight are inconclusive as to whether the diesel oil improves or inhibits the growth on the chips.

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6. INDIVIDUAL ASPECTS - GROWTH OF BENTHIC ALGAE

Participant: T. Bokn (NIVA)

Aim of the study:
To check if petroleum hydrocarbons have effect on overall growth of macroalgae, linear growth is measured in selected species.

Description of the work:
Tagged tips of *Ascophyllum nodosum* - knobbed wrack - have been measured 22 April. Since the measuring started during June last year 50 - 70% of the tagged tips in B1, B2 and B3 have been lost. In B4 every tip has been torned away. Preliminary data do not show any differences between the oiled and control basins. *Laminaria digitata* and *L. saccharina* were measured 25 April and 12 June. No significant growth difference was shown during that period. The ratio between fertile and sterile tips of *F. vesiculosus* plants was estimated 23 May. The data is not processed yet.

Eleven samples of *Ascophyllum nodosum* (knobbed wrack), *Fucus vesiculosus* (bladder wrack) and *F. serratus* (serrated wrack) were harvested 1 May. 16/18 May two samples (B1 and B2) were harvested from the long term chips. Analysis of hydrocarbons are not performed and the samples are still kept in the freezer.

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7. COMMUNITY METABOLISM

Are Pedersen (NIVA)

1. Purpose of the work

The aim of this sub-project is to determine if oil will effect the production and/or respiration of newly settled flora and fauna.

2. Sampling

The following servey program has been followed:

Survey no.	date	Parameters	Long term chips	Short term chips	Awa	CN	P	Metabolism
5.	1983-03	16-17	x	x	x	x	x	x
6.	1983-04	14-15		x	x	x	x	x
7.	1983-05	13-16	x	x	x	x	x	x
8.	1983-06			x	x	x	x	x *)

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

- All samples were taken according to the procedures described in Progress report No 1, with some modifications. Samples for ID and countings were included in Tor Bokn's sampling program.

The three short term chips were used as follows: The first chip was used for metabolism recordings and afterwards the chip was sampled for chl.a and phosphorous. The other two chips were used for CN and parallel chl.a determinations providing two parallels

of CN samples and three parallels of chl.a samples. The single long term chip was first measured for metabolism and then sampled to give three parallels of CN samples, two chl.a samples and one phosphorous sample.

The sampling program has proceeded according to schedule.

FURTHER WORK

This autumn the metabolism rates will be measured in a respirometer (Progress Report No 1). The instrument is not yet available due to unforseen shipping delays, but should arrive by September/October.

RESULTS

Some of the results from the chlorophyll sampling have been obtained. The chlorophyll content is higher in the non oiled basin, but the results require further examination and camparison with the measurements.

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Svein E Fevolden Susan P Garner

8. GENETICS SECTION

Progress report for the population genetics section

Littorina littorea and Mytilus edulis collected in August and November, 1982, respectively, continue to be run with the horizontal starch gel electrophoresis technique. By the end of this quarter (June '83), 288 Littorina and 312 Mytilus have been completed. For Littorina 24 enzymes are being tested on five different buffer systems, and 17 enzymes on six different buffer systems for Mytilus. A total of 43 loci have been scored for Littorina, 39 for Mytilus. Quite a few of these loci were scorable for only a rather small number of animals and will probably not be included in the statistical analyses of the data.

When the data for all four basins is pooled together Littorina littorea shows a frequency of heterozygotes of approximately 1.5 per cent which is a very low number for marine invertebrates in general. At this time the frequency of loci with more than one allele is 28 per cent and the most polymorphic locus is **6-phosphogluconate dehydrogenase** (6PGDH) with an avereage heterozygosity among scored animals of 48.4 per cent. The remaining polymorphic loci have a frequency of heterozygotes of less than 2.5 per cent.

Mytilus edulis has a more pronounced genetic variability. Average heterozygosity is 5.5 per cent (that is an animal is, on the average, polymorphic for 5.5 per cent of the loci). The frequency of loci with more than one allele is 31 per cent. The most polymorphic loci are (average heterozygosities in parentheses): **Phosphoglucose isomerase** (PGI; 0.573), **Isocitrate**

dehydrogenase (IDH; 0.538), Leucine aminopeptidase-3 (LAP; 0.469), LAP-2 (0.199), and Phosphoglucomutase (PGM; 0.176).

Ninety-six Littorina littorea from the sea just outside Solbergstrand have been run for the most polymorphic loci to compare with the basin animals. No significant differences have been discerned, although further statistical testing is needed.

By the end of this quarter no new recruitment of this year's brood of either Littorina or Mytilus has been detected in the four basins. The study of potential allelic selection among successful recuiters in the oil-exposed basins is therefore delayed. However, 100 specimens of the last survivors of Mytilus in the basin with the highest oil concentration were collected and will be run for selection studies. In addition, fast-growing and slow-growing Littorina from the oil-exposed basins will be collected (mid-fall) and compared for allelic differences.

Svein E. Fevolden
Susan P. Garner

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Progress report No 3. Period: March 1983 - June 1983

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9. *Balanus improvisus*
POPULATION ASPECTS Odd A. Frydenberg

Measurement periods

The measurement periods described in the first report have been changed. The work has proceeded as planned during the period. The measurement periods have been:

15 - 20 March
5 - 10 May
5 - 10 July
5 - 10 September

Results

From start in September 82 to the middle of March, the mortality has been almost equal in all three basins. From the middel of March to the beginning of May the mortality was quite different between the heavily oiled basin (B1) and the control basin (B4). In the lightly oiled basin (B3), the mortality was almost as in B4 in this period. The mortalities at the sea station B4 were about equal in this period. The growth is also different between the heavily oiled basin and the other three locations.

Statistical treatment of the data remains to be done.

Next generation

At 21st of June sampling of good larvae for study of the next generation was done. I have sampled larvae from 2-3 animals from each basin from the sea station. The larvae are reared in beakers (3 litres), one beaker for each basin, with replenishment of water once a day, and food (Skeletonema) three times a day. This experiment will go on into the next report period.

Odd A. Frydenberg

SOLBERGSTRAND EXPERIMENTAL STATION

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10. *Mytilus edulis* Pål Thome Mats Walday

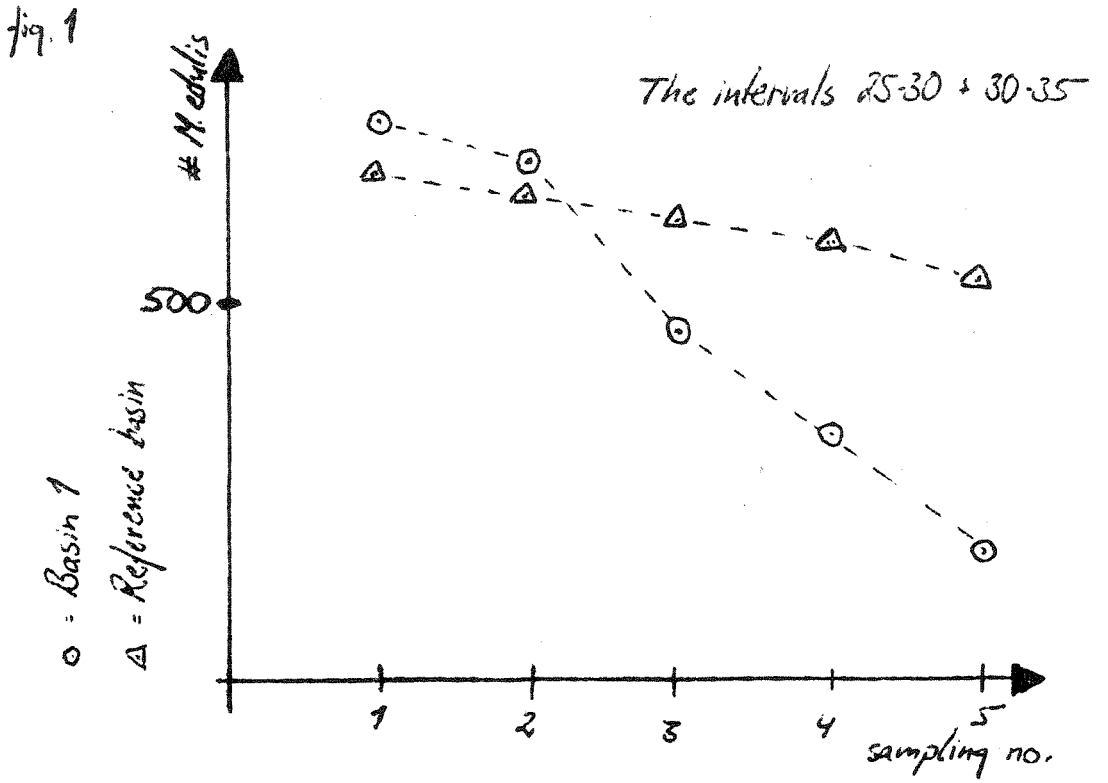
Sampling no. 5 was done in the first week of May. The mortality in basin 1 was still high (fig 1), so we decided to get the most out of what was left of livivng animals.
Dr. B Bayne used the interval 15-20 (142 animals) and in the beginning of June, Svein Fevolden got the rest of the intervals 20-25 and 25-30 for genetic analysis.

Since the 9th of June, we have been looking for recruitment in the basins and out in the fjord.
We are hanging out special nets and counting no. of M. edulis settling on them.

Our last sampling takes place in the middle of Sept.

Pål Thome Mats Walday

fig. 1



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11. *Littorina littorea*

Population Structures and Dynamics Einar Lystad og Kjell Moe

Recapture have occured according to schedule for the period. In addition to the random sampling (six squares from each level), a three squared broad (127.5 cm) transect in the middle, tranverse position of each basin was sampled, and all the individuals were measured (Fig. 1a). Same method was used in the pier area (Fig. 1b). This was the most appropriate method to incorporate the small and the unmarked individuals and get a complete view of each population.

Sediment sampling for mortality analysis took part in 1st. of February. Two random squares (42.5 cm. x 42.5 cm.) at the bottom, in areas where there was visible sediment, were drained and all the dead L.l. were measured (Fig. 2). No conclusions are outlined from the values in Fig. 1 a,b and 2 at this stage.

An alternative method for recruition measures (egg capsules no. from intake water – egg capsules no. from outlet water) was tried out in a three days period from 19 to 23 of april (spawning time). The samples have not yet been examined completely.

The progress of our work so far has been satisfactory and we have achieved some neat recapture values for all the basins and the pier area.

Further Plans

Our sampling periods will go according to schedule (Prog. Report no. 1) except for a few alterations. Extending of the sampling period so it will be at least one overlapping growth period taken note of, i.e. last recapture will occur in september which also is the time for the last sediment sampling. Monthly, every 1st and 20th there will be sampled 20, respectively 10 individuals from each basin (and pier) for CN analysis, Hydrocarbon contents of tissue, Dryweight and Gonaddevelopment analysis.

Within each population (each basin) there are some fast and some slow-growing individuals. We (it is hoped Fevolden too) will take interest in running some electrophoretic analysis of this event. So what's left then is a few more things to work out in the laboratory.

Einar Lystad and Kjell Moe.

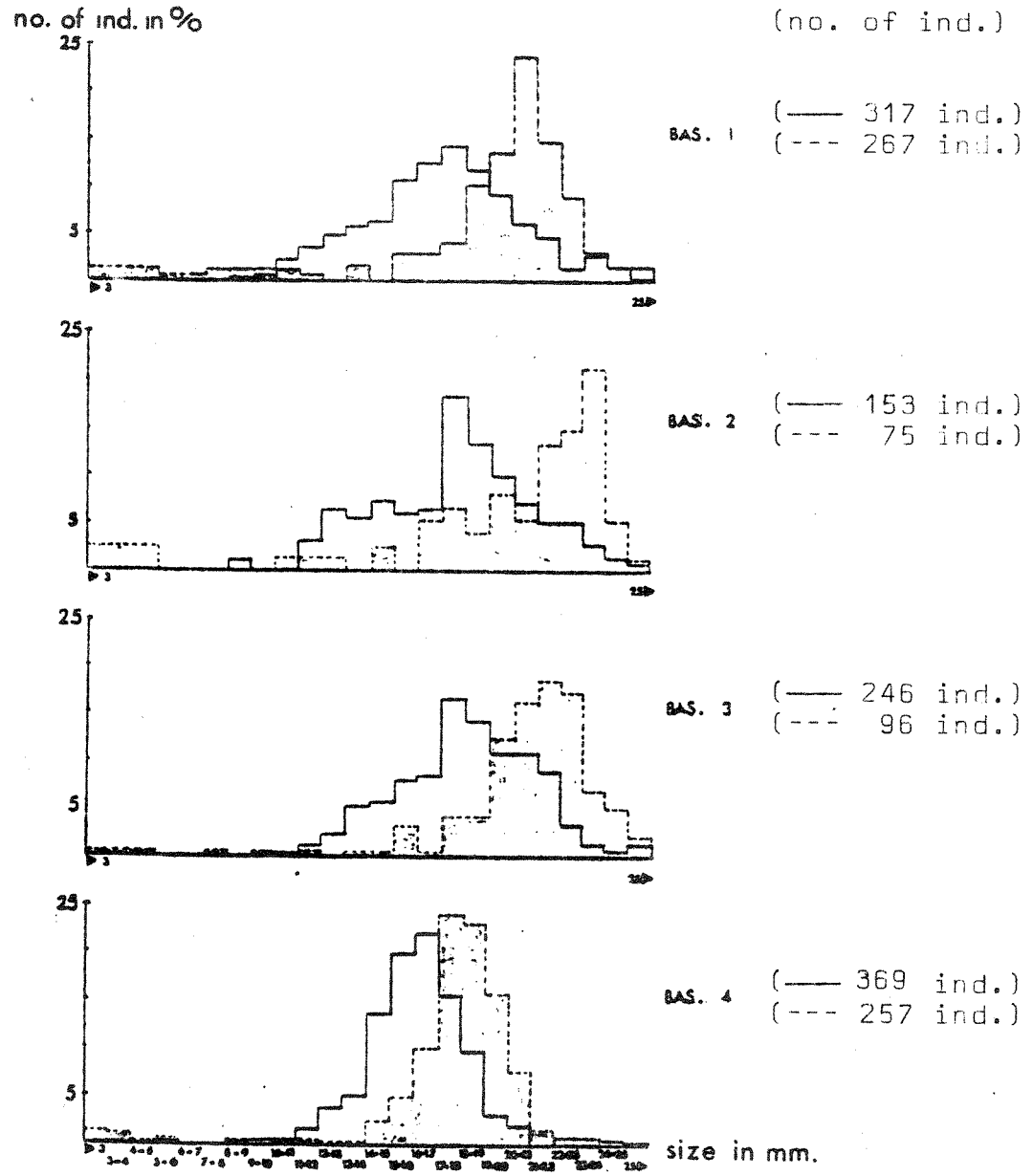

Fig. 1a. Size distribution of L.l. in each basin based on length. Full drawn lines give the values found 10-20/7-1982, dotted lines give the values found 19/3-1983.

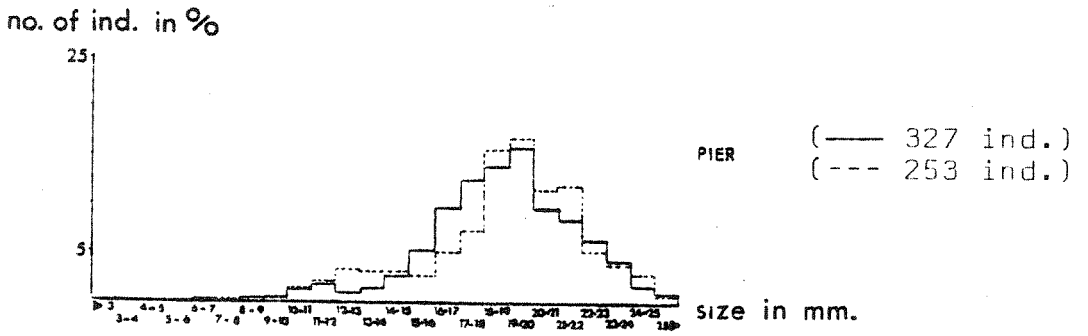

Fig. 1b. Size distribution of L.l. from the pier based on length. Full drawn lines give the values found 19/7-1982, dotted lines give the values found 19/3-1983.

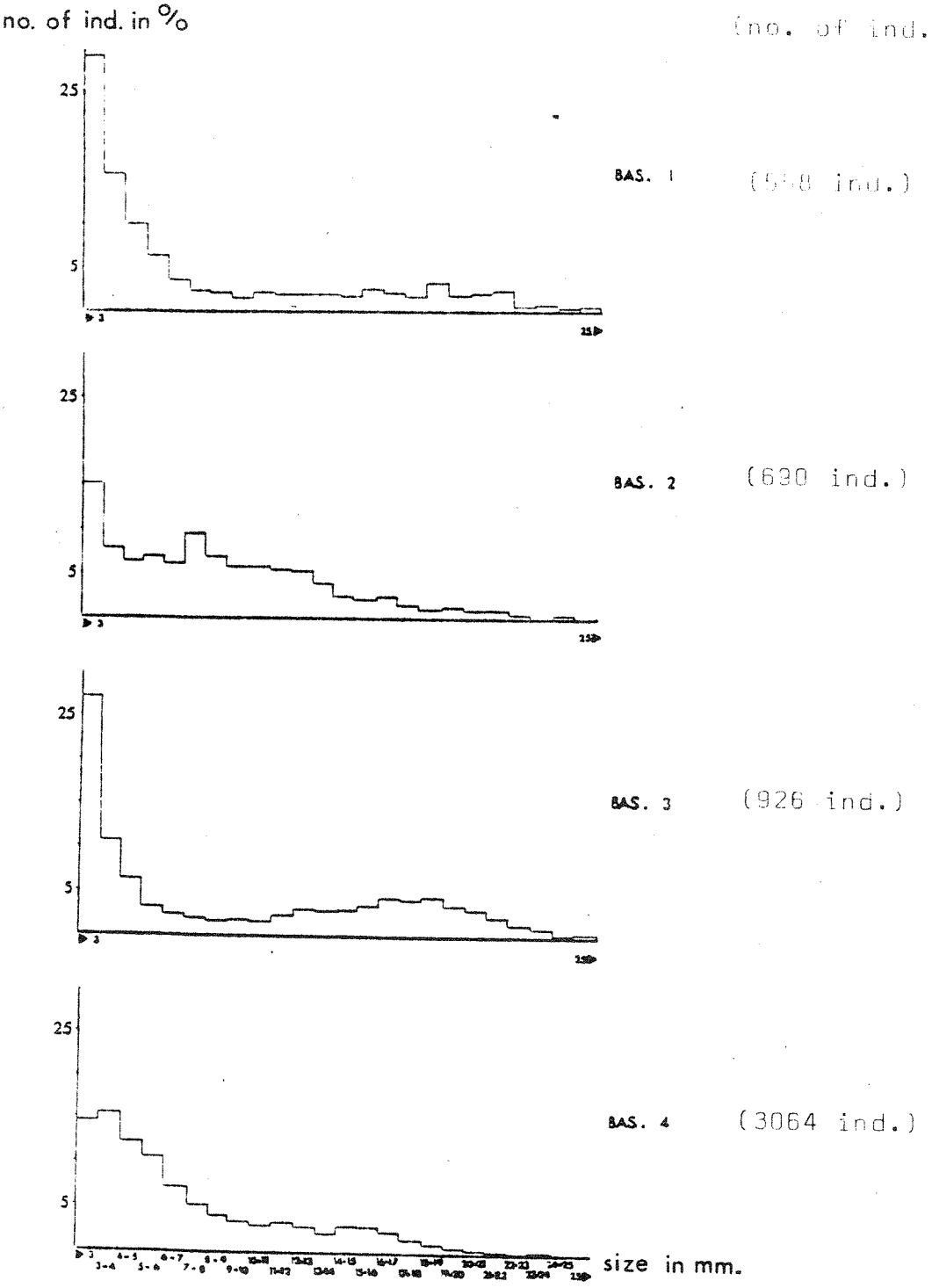

Fig. 2. Size distribution of dead L.l. in each basin based on length. The columns give the values found in sediment samples taken in each basin 1/2-1983.

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12. *Littorina littorea*, ENERGY BALANCE Torgeir Bakke

Participants: Torgeir Bakke
Kai Sørensen
Haakon Juelsen
Else Øyvor Sahlqvist

Also in cooperation with the research group of Dr. B.L. Bayne, IMER, Plymouth.

Aim and purpose:

- to investigate the energy uptake and loss status of the four basin populations of L. littorea at six points in time during at least one annual cycle beginning in February 1983;

- to investigate if oil has a long term effect on any of the main processes 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 hydrocarbons.

Work done

During May 10 marked individuals from each basin and 7 from the pier have been used to estimate respiration rates (VO_2), ammonia excretion rates (VNH_4), feeding rates (feed), faecal production for assimilation efficiency (ass.), shell size (L.) and body weight (W.). The measurements performed are given in Table 1.

All measurements went satisfactorily, but improvements in the

technique to estimate feeding rates will be sought.

Table 1. Physiology measurements in May 1983

Population \ Parameter	VO_2	VNH_4	Feed.	Ass.	L.	W.
B 1	+	+	+	+	+	+
B 2	+	+	+	+	+	+
B 3 (2 groups)	+	+	+	+	+	+
B 4 (2 groups)	+	+	+	+	+	+
Wild (pier)	+	-	-	-	+	+

Attempt to estimate aerial exposure has not been made.

RESULTS

Most of the Feed., Ass. and W. material is frozen for drying etc when the equipment arrives at SES (present by 1 Aug). The VO_2 measurements show:

1) B1 and B3 animals appear to have somewhat lower length specific respiration rates but not significant. Range of all groups: 85 - 120 µg O_2/h · individual

2) Respiration is positively correlated with size (L.).

The VNH_3 measurements show slight enhancement of excretion in B3, not in B1. Size dependence not yet determined.

Next measurement periods will be

Week 27 (8 - 9 July) and
Week 36 (4 - 10 September)

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13. CYTOLOGY OF *Mytilus edulis*

Title: Sublethal Biological Effects and Short-term Recovery of Mussels (Mytilus edulis) and Winkles (Littorina littorea) following chronic exposure to petroleum hydrocarbons.

Participants: J. Widdows, B.L. Bayne, M.N. Moore, D.M. Lowe.
Institute for Marine Environmental Research,
Prospect Place, The Hoe, Plymouth PL1 3DH.

Aim and Purpose: 1) To investigate the sublethal physiological and cellular responses of Mytilus edulis and Littorina littorea following chronic exposure to two concentrations of petroleum hydrocarbons.

2) To study the short-term recovery (20 days) of Mytilus and Littorina from the toxic effects of petroleum hydrocarbons.

Period: May-June 1983.

Description of work:

Physiological responses, such as rates of feeding, respiration, excretion and 'scope for growth' were determined for groups of mussels (Mytilus) and winkles (Littorina) collected from the 'high oil' (Basin 1), 'low oil' (Basin 3) and 'control' (Basin 4) experimental tanks at Solbergstrand. All groups of mussels were then transferred and maintained in 'clean water' in basin 4 and their physiological responses were measured after $2\frac{1}{2}$, 5 and 10 days of recovery.

Mytilus and Littorina from the 'high oil', 'low oil' and 'control' basins were sampled, fixed, sectioned and analysed for adverse cellular effects, including lysosomal latency, inhibition of gametogenesis and changes in digestive tubules. Specimens of both species were transferred from basins 1 and 3 to 'clean water' in basin 4, and their cellular condition was examined after 2.5, 5, 10 and 20 days of recovery.

Tissue samples were collected for hydrocarbon analysis by HPLC techniques.

Mussels were also sampled from the 'high oil', 'low oil' and 'control' experimental basins for measurement of gut retention time.

Progress

The work was carried out successfully and the results have been partially analysed.

Preliminary Results and Conclusions

A) Mytilus edulis

There was a marked effect of hydrocarbon exposure on the suspension-feeding (= clearance rate) of Mytilus. High and low oil exposed mussels had significantly reduced clearance rates, 0.537 and 0.824 1 h^{-1} respectively, compared with 1.79 1 h^{-1} for the control animals. As a result of the reduced ventilation/clearance rates the oil exposed mussels had lower rates of oxygen uptake. At present, the physiological data has not been corrected for slight differences in body size, but it is possible to state that the final calculation of 'scope for growth', an integration of the main physiological responses, will demonstrate a marked reduction in the growth potential of both oil exposed groups.

Preliminary analysis of the physiological data on the time-course of recovery indicates a rapid increase and recovery of the clearance rate of high and low oil exposed mussels within 2-5 days.

Mussels from the 'high oil' group had fewer developing and ripe gametes in the mantle and there was evidence of resorption. The digestive cells of the 'high oil' mussels were reduced in thickness and showed signs of vacuolation and atrophy. Lysosomal latency was also reduced in the 'high oil' group.

B) Littorina littorea

Winkles exposed to the 'high oil' condition had a significantly lower feeding rate, measured in terms of grazing on Ulva. Individuals from the 'low oil' basin had lower feeding rates but these were not significantly different from the controls. The rates of oxygen consumption by oil exposed winkles appear to be higher than the control animals, but these data still require weight correction.

Outline of future plans

1) There was considerable individual variability in the physiological responses of Mytilus to the higher oil concentration. It is proposed to identify, by measurement of clearance rate, those individuals that are most and least affected by oil exposure and then to carry out a genetic screening of the two groups of mussels.

2) To study the salinity tolerance of hydrocarbon exposed mussels, because there is preliminary evidence of hydrocarbons affecting water influx and regulation of water content at reduced salinities.

3) To examine the effect of hydrocarbons on the reproductive and nutritional storage cycles of Mytilus and Littorina.

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