

NORWEGIAN INSTITUTE FOR WATER RESEARCH

Oslo

0-82007-01

SOLBERGSTRAND EXPERIMENTAL STATION, DRØBAK

Long term effects of oil on marine benthic
communities in enclosures

LITTORAL ROCK COMMUNITY PROJECT

PROGRESS REPORT NO 2

March 1983

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Oslo, March 30 1983

F O R E W O R D

This document is the Second 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 December 1982 to 28 February 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, March 30 1983

*Torgeir Bakke
Programme Manager*

CONTENT:

	Page:
1. FOREWORD	1
2. PROJECT: Routine Monitoring of dosed hydrocarbons by fluorescence spectrometry	3
3. CHEMICAL ASPECTS - Activity report	7
4. COMMUNITY STRUCTURE	8
5. RECOLONIZATION AND POPULATION STRUCTURE	9
6. INDIVIDUAL ASPECTS - Growth of benthic algae	10
7. COMMUNITY METABOLISM	11
8. GENETICS SECTION Progress report for the population	13
9. <i>Balanus improvisus</i> Population aspects	14
10. <i>Mytilus edulis</i>	15
11. <i>Littorina littorea</i> Population structure & dynamics	17
12. <i>Littorina littorea</i> Energy balance	18
13. CYTOLOGY OF <i>Mytilus edulis</i>	19

SOLBERGSTRAND EXPERIMENTAL STATION

Progress report No 2

Period: December 1982 - March 1983

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

The concentration of the water accomodated fraction (WAF) has been monitored as described in the previous report. The analysis is simple and quick and generates concentrations of WAF in total diesel oil units in about 20 min after the samples have been taken. An outline of the concentrations at the outlet of the dosing unit is given in Figure 1. The concentration at the inlet to basins 1 and 3 has been somewhat smaller than this (Table 1).

Table 1. WAF concentration of B1 and B3 inlet in percent of WAF concentration in dosing unit outlet (mean percentages)

<u>Period</u>	<u>WAF temperature</u>	<u>B1</u>	<u>B3</u>
December	6-7 ⁰ C	99	106
January	12-15 ⁰ C	88	80
February	5-7 ⁰ C	90	96
Total:		92	94

Calculation of nominal hydrocarbon concentration of the water fed to the basins based on the WAF concentration and rates of feeding gave fluctuations which are too high to be satisfactory (Figure 2). There are three obvious reasons for the fluctuations:

- Changes in mixing propeller effect due to microbial growth in the dosing unit
- Changes in mixing efficiency with temperature of the water in the mixing chamber. A preheater was installed during week 52 to increase

FIG. 1. Hydrocarbon concentrations in the WAF at outlet of the mixing unit (Fluorescence analyses).

x: WAF concentration mg/l

·: Temperature in mixing unit

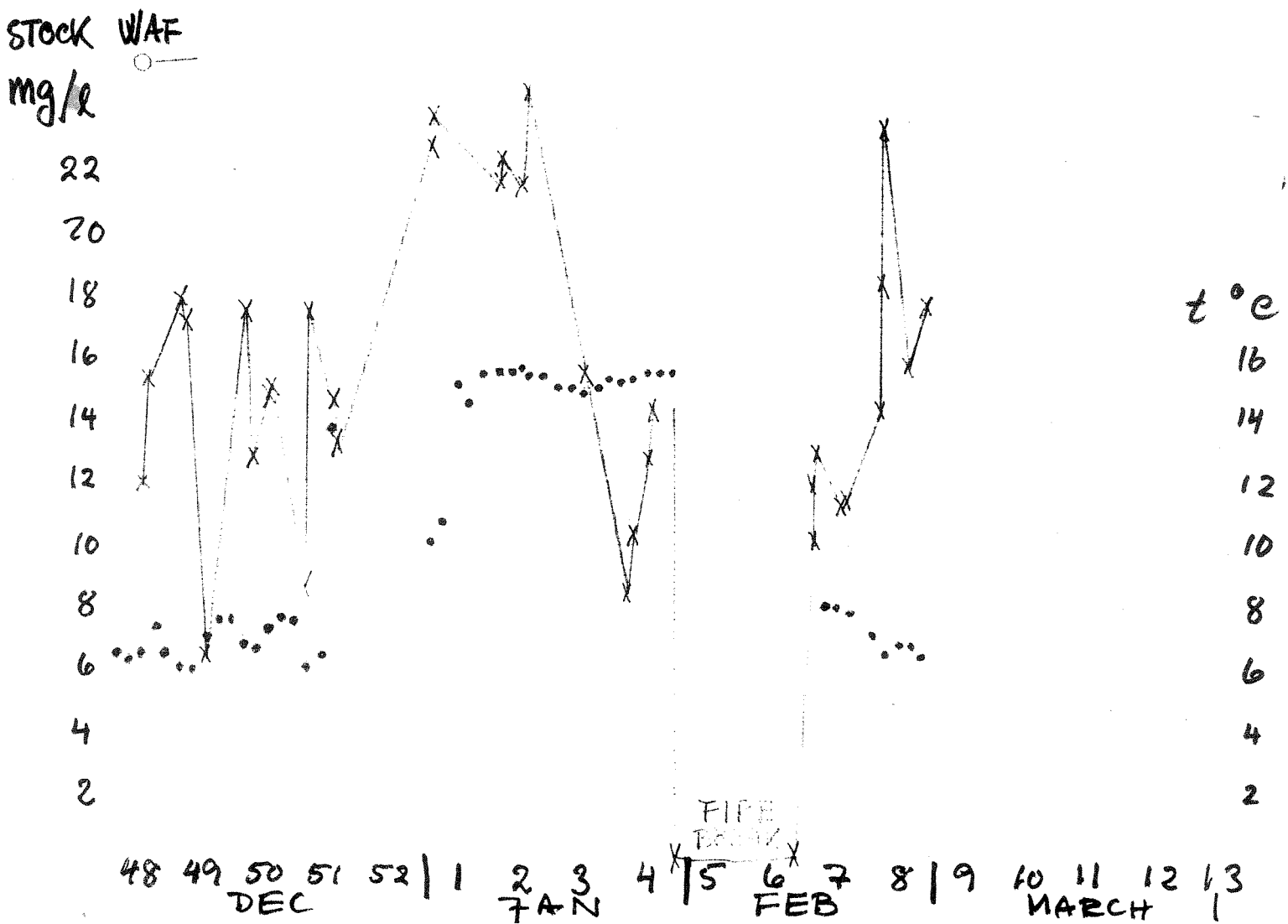


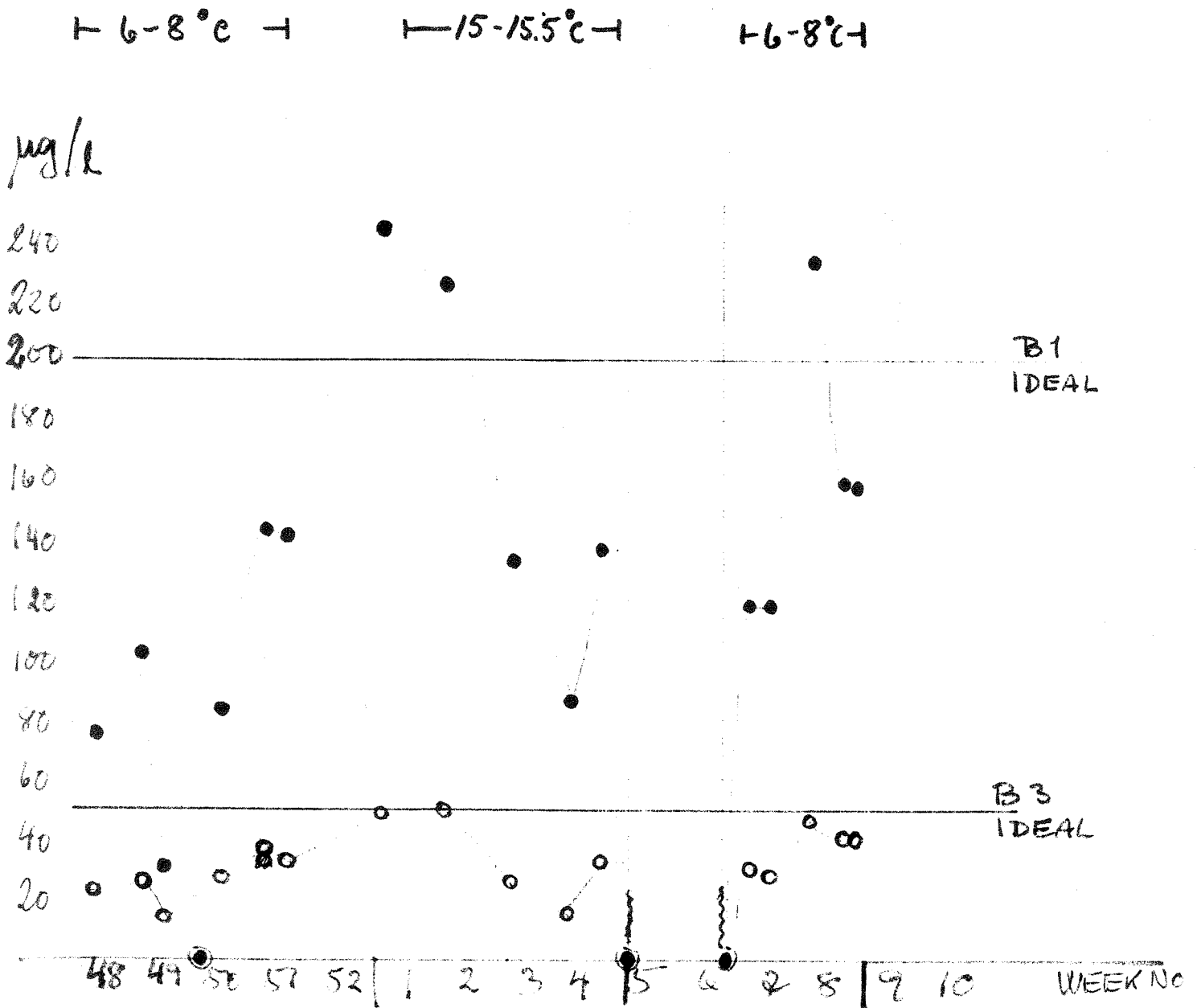
FIG. 2. Daily mean nominal concentration of hydrocarbons in the basins 1 and 3 calculated from:

Mean WAF concentration at inlet

Dozed WAF volume

1 m water input rate

● Basin 1 ○ Basin 3



the temperature from 6-7°C to 15°C.

- Faults and breakdowns of the dosing pumps due to heavy strain, especially as the temperature increase gave an increase in microbial growth in the pipelines.

A fire accident destroyed the dosing system on January 30. A new system became operative on February 12. With the building of this system we have tried to make improvements to reduce the fluctuations:

- Larger pipelines and pressure buffers to reduce pump strain.
- Two propellers at different depths in the mixing unit
- Improved preheating system
- A new dosing pump with higher capacity to enable us to compensate for low WAF concentrations.

We also intensified the cleaning routine for microbial growth of the system.

Torgeir Bakke
Project leader

SOLBERGSTRAND EXPERIMENTAL STATION

Progress Report No 2

Period: December 1982 - March 1983

3. CHEMICAL ASPECTS - Activity report

Sigve Sporstøl

In the period December 1982 - March 1983 7 water samples have been analysed with respect to their total amounts of hydrocarbons (THC).

RESULTS

SAMPLE	DATE OF SAMPLING	HYDROCARBONS (µg/l)	COMMENTS
Outlet water- Basin 1	6.12.82	130	Gas chromatogram indicates the presence of petrogenic hydrocarbons - likely as oil droplets.
Inlet water- Basin 1	6.12.82	84	Gas chromatogram indicates the presence of petrogenic hydrocarbons - likely as oil droplets.
Surface water- Basin 1	6.12.82	310	Gas chromatogram indicates the presence of petrogenic hydrocarbons - likely as oil droplets/surface film.
Inlet water- Basin 2	6.12.82	50	Gas chromatogram gives no indication of petrogenic hydrocarbons.
Inlet water- Basin 3	6.12.82	65	Gas chromatogram gives no indication of petrogenic hydrocarbons.
Water accomo- dated frac- tion	6.12.82	37000	Petrogenic hydrocarbons - likely as oil droplets.
Water accomo- dated frac- tion	19.1.83	6500	Petrogenic hydrocarbons - likely as oil droplets.

SI, 14. March 1983

Sigve Sporstøl

Sigve Sporstøl
Cand.real.

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Progress Report No 2

Period: December 1982 - March 1983.

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

Description of the work (since Progress Report No 1):

The monitoring since December 1982 is performed during 20 January - 17 February and the second sampling was started 21 March this year. Preliminary results have shown some normal annual changes and some significant reduction of furoid 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.

SOLBERGSTRAND EXPERIMENTAL STATION

Progress report No 2.

Period: December 1982 - March 1983

5. RECOLONIZATION AND POPULATION STRUCTURE

Participant: T. Bokn and Are Pedersen (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:

Since 16 December a polypropylene plate was mounted on the racks in basins 1 and 2 (B1 and B2). The first harvesting of chips was performed 18 January and since then chips have been changed 17 February and 17 March. These samples belong to the short time study. The chips in the long time study have been harvested 17 March. The degree of covering has been estimated, but the organisms have not yet been identified. They are conserved in formalin for later studies.

Dry weight and ash content are measured. Preliminary results do not show the difference between B1 and B2 corresponding to the results during 1982. Chips on the concrete steps are heavily grazed compared to the chips on the racks like the 1982-results show. Samples for petroleum hydrocarbon analysis of tissues are taken.

(For further information concerning this project - see Are Pedersens report).

SOLBERGSTRAND EXPERIMENTAL STATION

Progress report No. 2.

Period: December 1982 - March 1983

6. INDIVIDUAL ASPECTS - GROWTH OF BENTHIC ALGAE

Participant: T. Bokn

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 February and 22 March. 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. New tips will be tagged during May using another technique. Preliminary data do not show any differences between the oiled and control basins. At the start of March 25 individuals of *Laminaria digitata* and *L. saccharina* were tagged in each of B1, B2 and B3.

In the course of 19 - 21 days the mean length growth near the lamina basis, varied from 5 - 12 cm. The control *L. saccharina* showed a significantly higher growth than those of B1 and B3 ($p < 0.1$, Paired t-test).

Eleven samples of *Ascophyllum nodosum* (knobbed wrack), *Fucus vesiculosus* (bladder wrack) and *F. serratus* (serrated wrack) were harvested 20 December 1982 and 22 February this year. Analysis of hydrocarbons are not performed and the samples are still kept in the freezer.

SOLBERGSTRAND EXPERIMENTAL STATION

PROGRESS REPORT No 2

PERIOD: DEC 1st. 82 - MAR 1st. 83.

7. COMMUNITY METABOLISM

Are Pedersen (NIVA)

7.1 SAMPLING

The following survey program has been followed:

Survey no : date	Parameters	Long term chips	Short term chips	Chl.a	ID	CN	P	Counting	Meta-bolism
1. : 83.01.22			X	X	X	X	X	X	
2. : 83.02.01			(X)		X		X	X	
3. : 83.02.15			(X)		X		X	X	
4. : 83.02.17-18			(X)	X	X	X	X	X	(X)

- An excess of samples was taken on Survey no 1 to test the planned procedures and analysis of the material sampled.
- The oil dosing was interrupted after 14 days due to the fire. The value of the data from survey no. 4 would therefore be affected. Some chips were also then taken after 14 days of oil exposure (Survey no. 2). These chips were replaced by new ones which were subsequently sampled after 14 days without any oil dosing. (Survey no. 3).
- All samples are taken as described in Progress Report no. 1.

7.2 COMMUNITY METABOLISM

Due to the rather late delivery of the respirometer (Progress Report no. 1) it was necessary to build a simple oxygen chamber for metabolism studies during a winter-spring situation. After having some problems in solving the cooling system of the media, the chamber is now working satisfactory.

Unfortunately it is not possible to obtain parallel measurements.

7.3 RESULTS

Minor results from the sampling program have been obtained. It is, however, too early to predict any trends between the chips in the oiled basin and the controls. The metabolism results are being examined.

SOLBERGSTRAND EXPERIMENTAL STATION

Progress report No 2

Period: December 1982 - March 1983

8. GENETICS SECTION

Progress report for the population

Littorina littorea and *Mytilus edulis* collected in August and November, 1982, continue to be run with the horizontal starch gel electrophoresis technique. At this time (mid-May), 288 *Littorina* and 312 *Mytilus* (72 and 78 respectively, from each of the four basins) 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*. From these enzymes approximately 40 loci for *Littorina* and 20 loci for *Mytilus* are being scored.

In the summer new specimens of both species will be collected from each of the four basins in order to test any potential selection among the newly settled animals.

The data will be analyzed by means of a population genetics computer program devised by Swofford and Selander (1981).

Swofford, D.L. & R.B. Selander 1981. BIOSYS-I: a Fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72:281-283.

S.E. Fevolden

S.P. Garner

SOLBERGSTRAND EXPERIMENTAL STATION

Progress Report No. 2

Period: December 1982 - March 1983

9. *Balanus improvisus*
POPULATION ASPECTS

The measurements made in January and March went as planned. Growth and mortality was small during the winter season. No significant difference between the basins was observed with respect to these pop. factors.

Results from the sea station are more difficult to interpret. The mortality here appears to be larger, but some of this could be due to the fact that the plates with barnacles and the "stockings" with mussels were rubbing against one another. After the January measurements I attached the plates to an aluminium frame in order to reduce this problem. It helped, but the mortality still appears to be greater at the sea station. Growth, however, now seems equal at both locations. Measurements in the future will be as planned.

I intend to look for lamella starting mid-May in order to get larvae for the next barnacle generation.

Odd A. Frydenberg

Odd A. Frydenberg

SOLBERGSTRAND EXPERIMENTAL STATION

Progress report No 2.

Period: December 1982 - March 1983

10. *Mytilus edulis*.

Mats Walday

Pål Thome

Since the last progress report, we have been sampling in January and March, both went according to our plans. We have noticed (without doing statistical calculation) that the least growth is among the animals in basin1 (oil 200µg/l).

Generally in basin1, only a few of the shells were on the outside of the nets and they showed very little byssus-growth, and there was also high mortality, especially in the upper parts of the nets ie. close to the water surface.

In January we also found white bacterial-slime on the nets in basin1.

On Jan. 19th we established two new populations in basin3. One with animals from the 15-20mm interval and one from the 20-25mm interval. We are interested in seeing how the shells manage a smaller pollution like that in basin3 (50µg/l).

In December we had some rather heavy storms here and unfortunately our control-station was partly destroyed during one of these storms. The result was that we lost a lot of nets. I took a dive and found 17 of the 23 nets. Many of the shells were dead due to predation by starfish. This means that data on mortality for the control-station are not good enough for many of the intervals.

We are now discussing establishment of new populations in the fjord in order to get a better statistical background for our measurements.

The control-station has now been repaired and moved to a less exposed place in the same area.

But we still have some problems because we keep losing nets from the station.

The sampling in March showed that the shells in basin1 were in a bad condition. There are so few shells still alive that we might get problems finding enough animals for the CHN-analysis.

The newly established populations in basin3 were in a better condition. But they did not as good byssus-growth as the animals in the reference basin (no.4).

The mortality was lower in basin4 than in the polluted ones.

Out on the control-station the shells were in very good condition, but unfortunately we can't say anything about the mortality because of the accident we had.

We had algeal growth on all the nets (benthic diatoms) especially on those in the non-polluted stations.

There are now four more sampling periods left, and so far we have decided on weeks 18,23,30 and 37.

As already mentioned we haven't had the time yet to do statistical calculations on all the data so far received. So all information in this report is based on observations by Pål and me.

Yours sincerely Mats Walday

SOLBERGSTRAND EXPERIMENTAL STATION

Progress Report No 2

Period: December 1982 - March 1983

11. *Littorina littorea*

POPULATION STRUCTURE & DYNAMICS

Since last report in December recapture of animals have occurred twice, according to schedule. There will be no recapture in April due to a two week-project at the University of Oslo. The next recapture period will be in May although somewhat earlier than planned.

Recapture in all four basins proceeded as planned for this period. At the pier though, no marking of unmarked animals took place in January due to a 45% part of marked animals at recapture. In March, however, 872 unmarked individuals were marked to keep the high level of marked individuals also in later recaptures.

As for the steadily growing mass of data no analysis have yet been done, so no tables or figures can be brought forward at this point.

Kjell Moe and Einar Lystad

SOLBERGSTRAND EXPERIMENTAL STATION

Progress report No 2.

Period: December 1982 - March 1983

12. *Littorina littorea*, ENERGY BALANCE

Torgeir Bakke.

Due to other tasks especially the extra effort put on the persons involved in connection with the fire accident of the dosing system, the planned measurement period in February had to be postponed.

The following has been achieved:

Respiration measurements:

Aquatic: Various chamber sizes have been tested and the equipment is now operative.

Aerial: No tests performed.

Feeding:

Incubation of glass slides in basin water and artificial light gave too small growth of epiphytes to be of use in feeding experiments. System for outdoor incubation will be taken into use in March 1983.

Pilot study on consumption of *Ulva lactuca* showed insignificant grazing in 4 days.

Ammonia excretion:

Equipment operative. No pilot studies done to date.

Faeces production:

Pilot tests showed that a sample of 50 individuals incubated in 1 l of basin water for 2 hrs produces sufficient faecal material for dry weight and ash content determination in order to estimate assimilation efficiency.

SOLBERGSTRAND EXPERIMENTAL STATION

Progress Report No 2

Period: December 1982 - March 1983

13. CYTOLOGY OF *Mytilus edulis*

Participants: D.M. Lowe, M. Valday and P. Thome

The samples were of three sizes classes from each of three conditions:

Basin 1	(200 ug/l oil)
Basin 4	reference
Control 0	Fjord

It was only possible to obtain sections of the digestive gland from the smallest size class of animals as the digestive tissues were broken up during dissection in the two other size classes. Mantle tissues were only available in sufficient quantities for stereological analysis from the largest size class of animals.

Results

The difference in the digestive tubule epithelial cells between the three sites was very marked. Mussels from the oiled basin (1) exhibited large numbers of thin walled tubules with little evidence of digestive activity. In contrast the samples from the reference basin (4) exhibited a range of tubule types which appeared "normal" for a confined animal. Fjord mussels were a further improvement with well structured, well defined tubule epithelial cells.

A feature which contrasted sharply between the fjord sample and those of basins 1 and 4 was the presence of blood cells in the connective tissues. There was evidence of extensive granulocyte blood cell infiltration of the mantle and digestive gland connective tissues but no evidence of either granulocytomas or blood cell neoplasms.

Stereological analysis of mantle tissues from all conditions showed no significant difference between levels of either ADG or VCT cells. Similarly, the amounts of both developing and ripe gametes did not vary between conditions. However, the fjord mussels had significantly less ($P < 0.05$) atretic gametes than either of the two other conditions. Whilst the stereological analysis indicates no differences in terms of G V Fractions the sample size was small (i.e. 5 animals) and must therefore be viewed with caution. The appearance of the eggs, and indeed the spermatocytes, in mussels from basin 1 and 4 was "fragile" with evidence of egg membrane disruption.

The general condition of animals from both basin sites, taking into account the digestive tubule structure, granulocyte infiltration and gamete atresion suggests that these animals are undergoing gonadal regression.

The fact that our original request for 15 animals per condition was broken down into 3 size classes means that each sample is small (i e 5 animals) and does not give us the opportunity to establish a firm data base per size class or per condition. I think it would be more beneficial in the long term if we restricted the samples to, at best, one size class and at most two. If it is generally thought that two size classes are preferable then we should dispense with the medium group and have not less than 10 of each of the other two size classes. Personally I would favour 15 animals of the large size class only; this would reduce dissections at the Oslo end and, hopefully result in less tissue damage at dissection.

D M LOWE