

NIVA - REPORT

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Abstract:

The report describes the various research elements of the rocky shore community project at Solbergstrand, and their status until 1st December 1982.

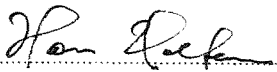
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Oslofjorden

Fremdriftsrapport nr 1

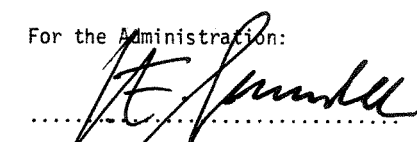
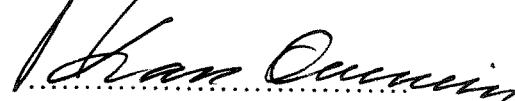
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Progress Report No 1


Project leader


Division leader:

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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 1

December 1982

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Oslo, February 24 1983

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F O R E W O R D

In spring 1982 a research cooperation was established between BP Petroleum Development Ltd., Norway u/a (BP, Norway), The Norwegian Institute for Water Research (NIVA) and The University of Oslo (UiO), with the aim to investigate long term effects of chronic hydrocarbon pollution on benthic ecosystems kept in large concrete basins. The research is sponsored by BP Norway and comprises two projects, one dealing with rocky shore communities and one with subtidal soft bottom communities. The experiments are conducted at NIVAs experimental station SOLBERGSTRAND by the Oslofjord, just south of Drøbak (referred to as SES).

The present progress report is the first in a series of quarterly reports from the rocky shore community project. It covers the period from the start of the project in spring 1982 to 1 December 1982.

The report compiles contributions from all participating scientists. Since the report period covers the initiation of most of the sub-projects, both project descriptions and progress reports for the actual period are given.

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, February 24 1982

Torgeir Bakke
Programme Manager

2. ROUTINE MONITORING OF HYDROCARBON EXPOSURE BY FLUORESCENCE SPECTROMETRY

Project participants: T. Bakke
K. Sørensen
U. Efraimsen
H. Juelsen

Purpose:

- to perform frequent estimates of the concentration of oil hydrocarbons in the water/oil emulsions (water accommodated 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.

2.1 Project description

With the present procedure duplicate WAF samples are taken at three points in the dosing system for the basins: the mixing/separation chamber outlet, the inlet to the header tank of basin 1 (200 ppb level) and of basin 3 (50 ppb level). The samples (50 ml) are extracted immediately with n-hexane (5 ml, p.a. grade) and the UV fluorescence intensity of subsamples of the extract measured at set excitation/emission level (256 nm and 302 nm respectively) by use of a Perkin-Elmer spectrofluorometer.

The fluorescence intensity is converted to mg total oil per litre WAF by automatic calibration of the instrument against a standard made up of the diesel oil used.

Sampling frequency:

The analysis is performed two days (Tuesday and Thursday) every week, with sampling at 9am and 2pm each day.

Laboratory facilities needed:

2 m length of bench space, one ventilation (air suction) cabinet, spectrofluorometer; all present at Solbergstrand at the moment.

Background parameters:

Records of temperature and salinity of the sea water supply to the mixing chamber is needed for data interpretation. At present the temperature of this water is maintained at 15-16 °C. Also oil and water flow rates to the mixing chamber have to be recorded preferably daily, as well as any irregularities in the mechanical mixing.

SOLBERGSTRAND EXPERIMENTAL STATION

2.2 PROGRESS REPORT

PERIOD: JANUARY - NOVEMBER 1981

Project: Routine monitoring of dozed hydrocarbons by fluorescence spectrometry

Preparative work: A Perkin-Elmer Spectrofluorometer, Model LS 5 with photomultiplier for the 200 to 800 nm range and an all-glass distillation unit for redistillation of solvents and extraction liquids was purchased in April. Most necessary glassware have been purchased and the instrument was operative at the end of August. Procedure for routine monitoring of hydrocarbons has been developed and was operative in mid September, when the oil exposure started. Also a Horiba continuous oil monitor, based on IR detection has been transferred from BP research center in Sunbury, UK to Solbergstrand, to enable continuous monitoring of the water accommodated fraction (WAF) from the dosing unit for periods of time. This will give an impression of short term variations in the concentration of the WAF over time. The instrument is presently being serviced at the NIVA instrument shop.

Results: The concentration of hydrocarbons in the WAF has varied between rather wide limits from 6 to 43 mg/l. Figure 1 shows the results throughout the first 4½ months of dozing for the concentration at the outlet of the mixing/separation chamber. The frequent extreme changes can mostly be attributed to mechanical faults in the beginning of the dozing, such as propeller break-down, changes in water level causing insufficient mixing, built up of oil-mucus-microbe layers in the chamber, interruptions in oil supply etc. In spite of this there was a tendency of increased WAF concentration throughout September and October from about 20 mg/l to about 40 mg/l. The flow rates to the test basins were adjusted accordingly. When a new mixing/separation chamber

cont. progress report Period January - November 1981

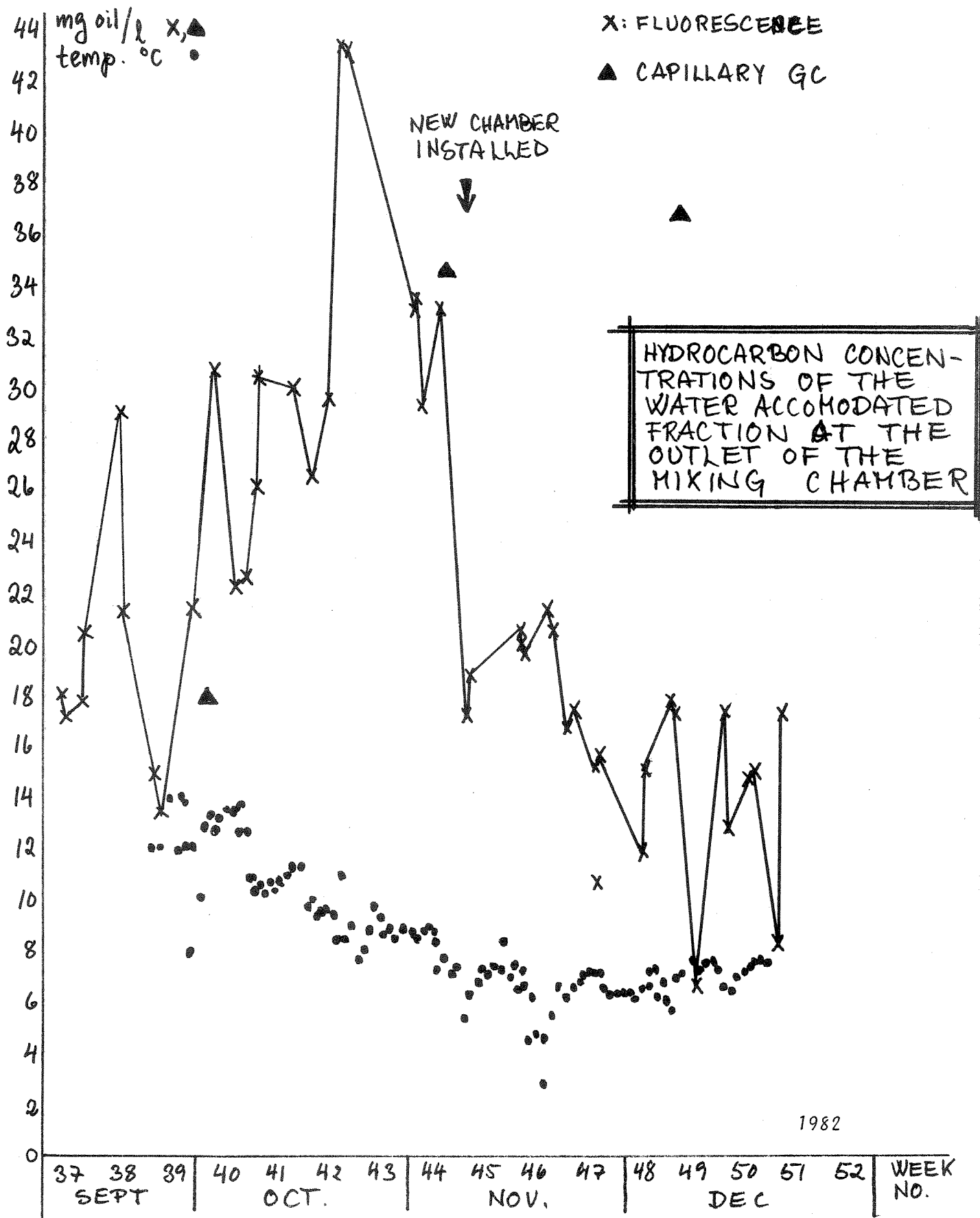
was taken into use in the beginning of November the concentration again dropped to about 20 mg/l with a subsequent tendency of decrease to about 13 mg/l in December.

As no temperature regulation of the water inlet to the chamber was installed until early January, there was a gradual decrease in temperature from 12°C in September to 6-7°C in Mid-November (Figure 1). After that the temperature was stable for the rest of the year. Hence temperature decrease seems not to explain the decrease in WAF concentration.

Microbial growth has been small after installment of the new chamber due to frequent mechanical cleaning.

The WAF concentration at the inlets to the leadertanks of basin 1 and 3 followed the trend shown in Figure 1. There has been a tendency of slightly lower values at the inlet to basin 3, but the significance of this has not been tested. Also a tendency of slightly higher values at 2 pm than at 9 am has been indicated. The correlation with daily temperature fluctuation or periods of mechanical rinsing will be tested.

Figure 1.





3. HIGH RESOLUTION CHEMICAL ANALYSIS OF OIL HYDROCARBONS

3.1 SHORT DESCRIPTION - CHEMICAL ANALYSIS

The high resolution chemical analysis on the ROCK LITTORAL PROJECT will be performed at the Central Institute for Industrial Research (SI) under project number 82 09 06; "Long term effects of oil on marine benthic communities in enclosures - chemical aspects".

The aim of the project is to support the biological investigations by monitoring the hydrocarbon levels in the water basins, organisms, sediments and water particles and thereby give information on the flow of hydrocarbons through-out the ecosystem.

Two different analytical methods will be applied.

1. Total Hydrocarbon Concentration (THC).

This analysis, performed by high resolution gas chromatography, gives total content of hydrocarbons in the samples.

2. Selected Aromatic Components.

The analysis, performed by computerized GC/MS, gives the concentration of selected aromatic hydrocarbons, i.e. naphthalenes, phenanthrenes, anthracenes, dibenzothiophenes, pyrenes/fluoranthenes plus the n-C₁₇ alkane/pristane, n-C₁₈ alkane/phytane and pristane/phytane ratios.

A sampling/analysis program proposal is presented in Table 1. It does not contain dates of sampling though these must be coordinated with the biological research programme. The proposal, with a budget of 675 400 NOK 82, overstep the analytical part of the hard bottom budget with approximately 25%. We feel, however, that the magnitude of the programme is kept as low as possible. With further reductions



it will be difficult to fulfil the aims of the project. We therefore leave it up to the Steering Committee to decide wheather the additional money can be transferred from the soft bottom project in the event of additional financial support.

The analytical project might be strenghtened by engaging a dr.scient student. This possibility has been partly evaluated, but at present no final decision has been made. We are, however, working with this task and will keep the Program Manager informed.

SI, 25. November 1982

R. G. Lichtenthaler.
Rainer G. Lichtenthaler
Dr.rer.nat.

Sigve Sporstøl
Sigve Sporstøl
Cand.real.



TABLE 1. Sampling/analysis proposal.

The budget is worked out for a project period of 2 years (1.10.82 - 1.10.84)
Budget in 1000 NOK 1982.

Parameter	Amount	Frequency	Analysis	Samples	Sum
Water ^{a)}	4 l	1/month/basin (mixed samples of 1 liter/week)	GC + 8 GC/MS	96	83.6
Particles	20 l; 0,45 µm GFC	4/year/3 basins	GC/MS	24	52.8
Algae	~ 1/8-1/4 m ² (basin walls)	4/year/basin	GC/MS	32	70.4
Sediments	20 - 50 g wet weight	2/year/3 basins	GC/MS	12	40.8
Littorina	1 individe	6/basin no.1 and 3; 2/basin no.2	GC/MS	14	47.6
Littorina	10 individs	4/year/basin	GC/MS	32	108.8
Mytilus	1 individe	6/basin no.1 and 3; 2/basin no. 2	GC/MS	14	47.6
Mytilus	10 individes	4/year/basin	GC/MS	32	108.8
Water ^{a)}	1 l	Additional analysis; "start up" phase	GC	20	15.0
Equipments/chemicals/administration/report writings/ discussions/travels					100.0
Total					675.4

a) The solvent extraction will be performed at Solbergstrand.



3.2 Activity report

October 1982 - December 1982

LONG TERM EFFECTS OF OIL ON MARINE BENTHIC COMMUNITIES
IN ENCLOSURE - CHEMICAL ASPECTS



In the period September 1982 - December 1982 chemical analysis have been carried out with special respect to

- monitor the levels of hydrocarbons in the water accomodated fraction and in the four water basis.
- distribution of diesel oil in the basins.

A total of 11 water samples and 3 surface film samples (taken with teflon plates) have been analysed. The analysis have been carried out using high resolution gas chromatography.

WATER ACCOMODATED FRACTION

Two water samples have been analysed. The results correlate very well with results from fluorescence spectrophotometric measurements on parallel samples, and range from 18.2 ppm (5.10.82) to 35.5 ppm (4.11.82).

WATER BASINS

A total of nine water samples and three surface film samples (teflon plates) have been analysed. Analysis of inlet water indicates that the hydrocarbons enter basin 1 and 3 as droplets (oil in water emulsion). Concentrations of 200 (basin 1) and 70 ppb (basin 3) are measured.

Analysis of the outlet water gave no evidence for the presence of oil droplets in any of the four basins. Hydrocarbon level in outlet water of basin 1 and 3 were not significantly higher than in outlet water of control basin 2 and 4. Small amounts of petrogenic aromatics (i.e. naphthalenes) was however identified in the outlet water of basin 1 and 3.

Although quantitative estimations are difficult, the analysis of surface water clearly indicate that the hydrocarbons are enriched on the surface as an oil film. This film contains relatively less of the most volatile compounds compared to the parent diesel-oil.



The gas chromatograms of the surface film samples further indicate that other organic compounds (from the seawater or from atmospheric fallout) also are enriched on the surface film.

Conclusively the analysis indicate that the hydrocarbons enter basin 1 and 3 as droplets. In the basins the droplets move to the surface. From the surface film the most volatile compounds are partly evaporated while the most water soluble of the remaining compounds (i.e. naphthalenes) are dissolved in the water column.

SI, 25. November 1982

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4. COMMUNITY STRUCTURE

Progress Report No 1 December 1982 by Tor Bokn

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: The composition of the littoral communities is characterized by monitoring percent algal cover and sessile animals and number of motile animals. A special frame adjusted to the dimension of the basin steps is used for this. The monitoring will be performed five times a year, which gives a picture of the structure changes at set areas in the four basins. The same areas are also photographed to document possible successions.

The work in 1982 was performed during the three periods 4 June - 6 July, 10 August - 14 September and 1 - 18 November. For the two last periods the community structure was also documented by photos.

Necessary equipment most of the year is a SCUBA-diving gear with a full face mask connected to a radio and a tape recorder via a water-proof cable. For documentation a normal camera and an underwater camera are needed.

Periods of sampling:

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

During these periods tape recording facilities etc. will be needed.

Background parameters: To have the opportunity to control some of the possible effects of the community structure it is necessary to have temperature and salinity data from the four basins available. Likewise the diesel oil concentration in the basin water is supposed to be needed.

5. RECOLONIZATION AND POPULATION STRUCTURE OF ALGAE

Participants: T. Bokn, A. Pedersen and R.L. Vadas (University of Maine)

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: Recolonization of algae will be studied by using clean granite chips (10 cm x 10 cm). Preliminary work started 8 June 1982 by having 8 clean chips in each of basin 1 and 2 (B1 and B2) at the levels corresponding to step 2 and 3 in the basins, where the same number of chips was arranged. The former arrangement was done to avoid grazing from periwinkles. 12 July the 2 x 8 hanging chips were mounted on two chips-supports (racks) constructed to keep grazers away from the chips. 15 September two chips from each level in B1 and B2 were harvested. They are all conserved in formalin for later studies.

16 September diesel-oil was mixed into B1 (and B3). 16 chips were boiled and mounted in the empty spaces on the racks and concrete steps. Every month since then chips are replaced for identifying organisms, and measurements, degree of covering of different organisms, dry weight and ash content. During the period September to October the development of algae cover was different from the racks of B1 (oiled) to B2 (control). The dominating alga on those chips from B1 was schizonema-phases of a diatom, while corresponding chips from B2 was dominated by the green algae *Enteromorpha* sp. Chips on the concrete steps are heavily grazed compared to the chips on the racks.

From the 16 December we will concentrate on one level in the two basins, which will correspond to concrete step two. This choice is based on the fact that this level is situated in the middle of the littoral zone, and will thus be exposed to air half of the time. In this zone we will find most of the common wracks. In each basin there will be mounted a polypropylene plate on the rack to give room for 44 chips. All together we will use about 170 chips on the racks and on the concrete steps in B1 and B2.

This project will be separated into a short time (1), a long time (2) and a recovery study (3).

(1) Every month (during May, June and July every fortnight) 20 chips will be harvested and changed with clean, boiled chips. In addition to the above mentioned parameters the organisms on the chips will undergo studies on metabolism " C^{14} -production", analyses on petroleumhydrocarbons, carbon-, nitrogen- and phosphorous content.

(2) Harvesting will start in the middle of March (after 3 months) and then continue every second month during two years. 12 chips will be harvested each time. The organism communities which are growing two months older every sampling time will be analysed for the same parameters as for the short time chips.

(3) To spot a possible recovery 72 chips will be put into the basins during January 1984. About one year afterwards and every third months until Summer 1986 12 chips will be harvested each time to be analysed as described for (1) and (2).

Periods of sampling: Two days every month about the 16.

Need of facilities: Compound and dissecting microscopes. Running seawater and aquaria. Flow-meter to measure primary production etc.

Background parameters: As for the community structure project we will need temperature and salinity data. The analyses of organisms demand nutrient data. Sedimentation on the chips demands measuring

of sedimentation rates. The data of diesel-oil content in basin water is needed.

Due to budget restrictions some parts of the project will be tried funded by NIVAs internal budget.

6. INDIVIDUAL ASPECTS - GROWTH OF BENTHIC ALGAE

Participants: 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: During June tips of *Ascophyllum nodosum* - knobbed wrack - were tagged in all four basins (25 tips in B1-B3 and 23 tips in B4). 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. In that way it will be possible to compare length growth with increase in weight. Tips are measured during June, July, September, October and December. The mortality has been too large. About 50 per cent of the specimens has been lost in different ways. The tagging system is now under revision.

Due to an extremely hot Summer the loss of laminariacean was large. Due to this *Laminaria digitata* and *L. saccharina* have not been tagged. Our intention is now to wait for the Winter season and then figure out the strategy.

During the Spring and early Summer 1983 the proportion of fertile to sterile tips of the branches of *Fucus vesiculosus* - bladder wrack - will be studied.

Periods of sampling: Every month.

Background parameters: Temperature, salinity, diesel-oil in the
water.

7. COMMUNITY METABOLISM

Project Report January 1983 by Are Pedersen (NIVA)

Participant: A. Pedersen

Purpose of the work: The project will be a study concentrating on the variation and changes in metabolism i.e. production and respiration in 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. This project will be closely related to the project: "Recolonization and population structure". (Re.: Progress Report No. 1 by Tor Bokn.)

7.1 Description of the work: The same granite chips used in the "Recolonization and population structure-project" will be used for analyzing community metabolism. As described in Progress Report No. 1, December 1982 (pp 2 and 3) by Tor Bokn, these chips will be exposed in basin B1 (oiled) and B2 (control), for different periods of time. The chips will be placed in a respirometer, designed by Dr. James Porter at the University of Georgia. It will measure in situ metabolism of sessile and some motile organisms.

The instrument (Fig. 1) measures oxygen concentrations and light intensity on three Yellow Springs Instruments model 57, Oxygen-Meters (accuracy to ± 0.01 ppm), and on a Licor Model LI 185A, Quantum Photometer (accuracy to $\pm 1 \mu E m^{-2} sek.^{-1}$), with attached LY 193 S, Spherical Quantum Sensor, respectively.

Data is recorded every 60 seconds from one light sensor, and three oxygenprobes on a data logger for max. three days (Aanderaa 12 channel DL 2, data logger).

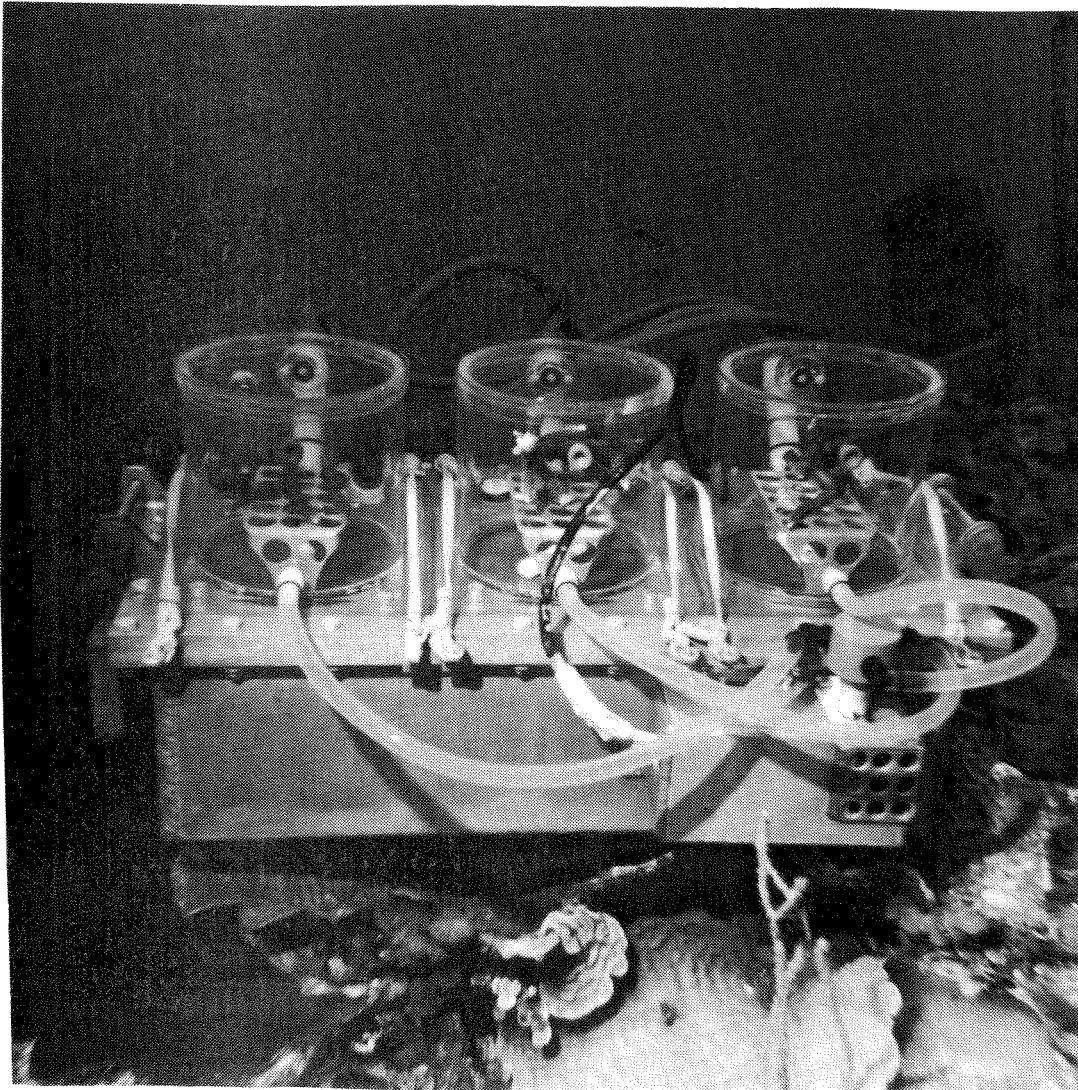


Fig. 1 The respirometer placed on corals at 20 m depth, in Virgin Island, St. Croix. It measures oxygen production from the zooxanthellz of the corals. (Photo: Dr. J. Porter)

The granite chips with fouling community attached, are placed on pedestals above continuously rotating magnetic stir bars in the three quartztopped chambers. These chambers are periodically flushed at any interval from 0.5 to 4 hours by a Deep Sea Impellar pump. The pump stays on for 2 minutes during each flushing operation and ensures that oxygen concentration in the experimental chambers never increases or decreases more than 20 % from ambient

concentrations (Porter 1980). The chambers are O-ring sealed at the bottom of each port. They also contain a vital serum stopper for introduction or extraction of radioactive tracers, C^{14} , DCMU, vital stains, water samples, etc.

Samples will also be taken from the chips in order to determine:

1. Chlorophyll a (chl a) - quantitatively measurement of biomass.
2. Dry weight (DW).
3. Total carbon, nitrogen, and phosphorous (C N P).
4. Identification (ID) of diatoms and other minor algae.
5. Numbers of diatoms per area, quantitatively countings.

Experiments: Three replicate granite chips will be placed in each part and the metabolism of the community on each chip will be measured during a 24 hour period. During daytime the algae will produce oxygen in excess of consumption (i.e. respiration) of the community. This net production will be recorded on and correlated to light intensities recorded simultaneously. Similarly the oxygen-consumption (respiration) during night hours will be recorded. These data will give a production v.s. light intensity curve for the community on the granite chips (Fig. 2) (P max curve if light intensity exceeds the maximum utilization point of the algae). This information will be used to estimate the P/R ratio v.s. time (Fig. 3).

Analysis: The process for handling the samples are:

1. Chl a will be sampled from a specific area, filtered on a GFC-filter, ground in acetone and measured on a fluorometer.
2. CN-samples will be dried in drying facilities at 105 °C for 4 hours and then weighted, analyzed on a Carlo Erba No.1106 Elemental Analyzer.
3. The samples for identification will be treated either with HCl or U.V. + H₂O₂ for cleaning and then embedded in Hyrax.
4. The counting-samples will be preserved in formalin.

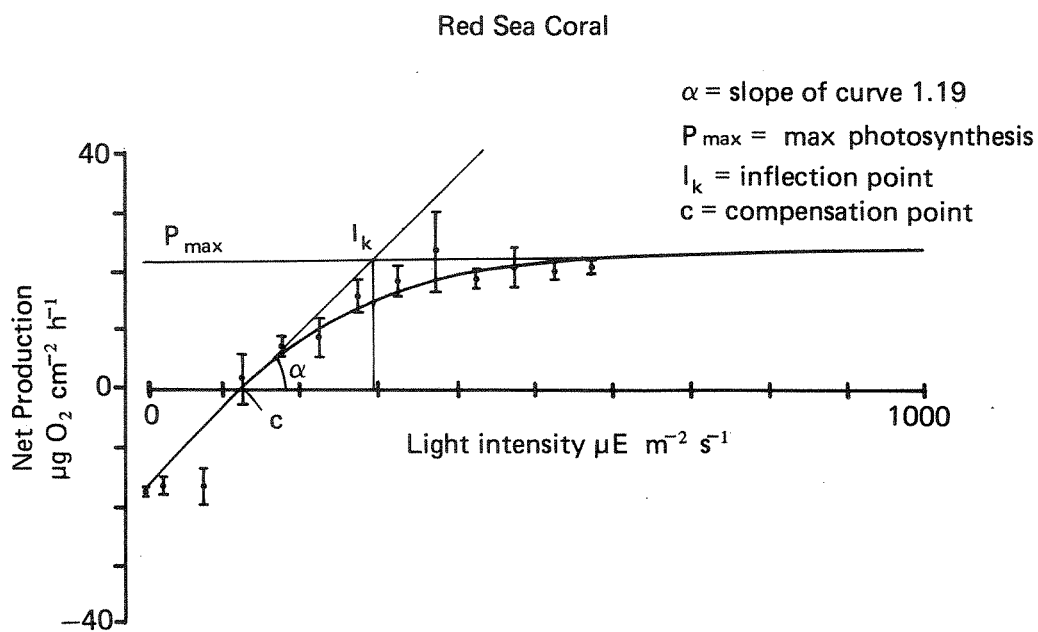


Fig. 2. P-max curve for a Red Sea Coral.

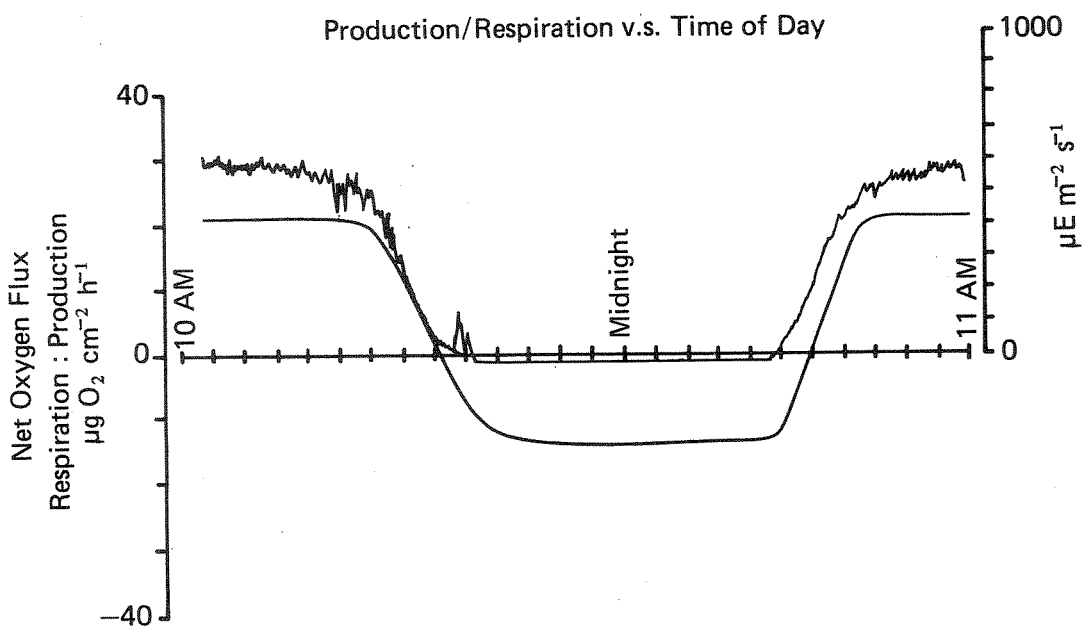


Fig. 3. Production and respiration of the Red Sea Coral during 25 hours. The curve for Oxygen Flux is based on the P-max curve on Fig. 2 (Porter pers. comm.).

The samples for chl a, CNP, DW, ID, and countings will be taken from the chips. Because patchiness and timelag can cause problems for correlating P/R measurements to the other samples, it is necessary to sample immediately after the P/R measurements are taken. This is just necessary for chl a and counting samples.

Labelling of chips: There are no labels on chips. They are mapped according to their location and orientation in the basins. For example, the chips marked with a circle around the E2 on Fig. 4, are in basin one referred to as:

B1RE2 (Basin 1, Rack 2, East No. 2).

The chips are also subdivided into two halves according to their orientation e.g. North/South or East/West.

Need of biological substrates: Each P/R experiment will be carried out with 6 chips from the oiled (B1) basin and 6 from the control (B2). Three of the six will be taken from the rack (R) and the other three from the steps (tribune, T). Three replicates will be used in order to satisfy statistical requirements for analysis of the results of the experiments. The chips used will be randomly chosen for each experiment.

The P/R measurements will for each site take about 36-48 hours. It will therefore be necessary to start these experiments one week before the sampling for the recolonization studies. The chips will not be disturbed while taking the P/R measurements.

The sampling of chl a, DW, CNP, ID and countings will be performed according to Progress Report No. 1 by Tor Bokn (p. 3). However, the samples for chl a and countings have to be sampled immediately after the P/R measurement.

Sufficient sampling requires the use of 4 chips, some of which can also be used in the recolonization project. The chips are placed either on a rack (R) or at the steps of the basin (tribune, T). Fig. 4 shows an example of the sampling in February 1983.

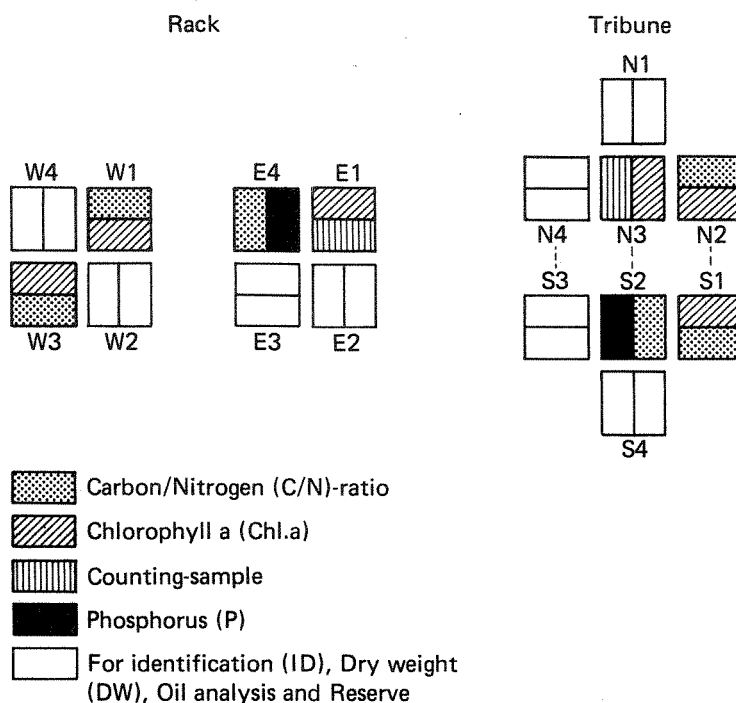


Fig. 4. Example of a sampling in Feb. 1983.

Needs for facilities: The respirometer has been ordered from the University of Georgia and will be delivered in April. Meanwhile the P/R measurements will be done in laboratory, using an oxygen jar. It will be necessary to have a dissecting and compound microscope with photo facilities. The compound microscope should be equipped with lenses and converter for normal light and for phase contrast. Other things wanted are:

1. Running seawater
2. Aquaria
3. Oxygen meter
4. Licor Quantum light meter with an intergrator
5. HP 85 Micro computer
6. Aanderaa printer
7. Access to NIVAs Nord 100
8. Literature on taxonomy of diatoms and micro algae.

Background Parameters

Salinity and temperatur in the basin must be measured. A light integrator stationed at SES would be beneficial. Other important parameters are:

1. Nutrients
2. Particle countings
3. Sedimentation rates
4. Diesel-oil content in basin water

7.2 Progress and travelling report

Background

As Lars Kirkerud unfortunately had to withdraw from this project as a result of increased work load, I was asked to continue his proposed work on community metabolism on rocky substrates.

At the meeting with advisory board for SES on 20th Sept. 1982, my plans for further work on the aspects of community metabolism were discussed and accepted. The advisory board suggested that I should seek out different scientists or institutions dealing with similar aspects. I therefore contacted some recommended persons, either by visiting or writing them.

Purpose of the trip

The purpose of the travel was to collect know-how and ideas from scientists working with different aspects concerning metabolism on different marine substrates and fouling communities.

USA

The first stop of my trip was the Univ. of Georgia, in Athens, Georgia, USA. Here Dr. James and Dr. Karen Porter were my hosts. The aim of this visit was to discuss ways of metabolism measurements. Dr. J. Porter has built a respirometer which after long discussions and also testing showed to be perfect for the kind of measurements I was interested in doing on the chips. We went through the design and description of the instrument. As the measurements by the respirometer are recorded at a data logger we also discussed the different ways of handling data on a computer. Dr. Porter who was most interested in our project, was willing to build a similar respirometer in the short time of three to four months. We also discussed this with the other technical parties involved.

At the University of Georgia I also had the pleasure to discuss the project with Dr. Pomeroy. In addition, I discussed the project with:

1. Dr. John Patton concerning oil pollution, chemical and microbiological aspects.
2. Phd. student Ester Fleishmann. She had helpful comments on UV-lights possible influence on production of benthic diatoms in very clear and shallow waters.
3. Phd. student Jason Smith who had done all the computer programs for the respirometer. I could obtain all programs without charge.
4. Phd. student Joe Neigel concerning genetics of marine organisms.
5. Phd. student K.R. Tenor concerning food chains and infauna.

St. Croix

On my way from Athens to West Indies laboratory, St. Croix, Virgin Island, I also contacted Dr. Brand at the University of Miami. He was, however, working with oil pollution in more pelagic oriented systems. I therefore continued to the West Indies lab. which is affiliated with Fairleigh Dickinson University, to visit Dr. Caroline Rogers and Dr. Robert Carpenter. Dr. Rogers has been working with coral reefs net production using an up-stream down-stream principle. This is also described by Dr. Odum and Dr. Pomeroy in salt marshes. It was a very interesting way of measuring production and could be a supplement to our experiment at SES.

Dr. Robert Carpenter had been using the Dr. Porter's respirometer at the University of Georgia and our discussions were of prime interest to my work at SES. He was looking at recolonization, grazing and metabolism on dead coral heads using an "artificial" substrate coral chips 8 square cm. cut out of large Acropora cervicornis. I was a bit concerned about the detection of the increase and decrease in O_2 -concentration in the rather large bell jars. Dr. Carpenter, however, could assure me that the O_2 -production on newly settled chips was so large that he had to handflush the chambers

on very bright days. I also had the opportunity of discussing the project plan with Dr. Rich. Rochester and Dr. Ogden. Dr. Ballard from Woods Hole Oceanographic Institute held a lecture on the rocky formation in the Atlantic. Dr. Ballard and Dr. F. Grassle were doing some diving with the submersible ALVIN in the West Indies. I was invited aboard the ALVIN and it certainly was small inside.

England

In England I visited Dr. R. Fletcher at Marine Laboratory Portsmouth Polytechnic. After touring the lab. facilities we discussed fouling communities. He provided many good ideas on how to sample from "artificial" substrates. Finally I spent one day in London visiting Dr. Girton at British Petroleum. He was most interested in our project and after my description of the plans of the SES project, we had a productive discussion. Dr. Girton had some remarks on our timing of sampling. A ten weeks "exposure" of artificial substrates in the sea will give a diversity up to 90 % of the normal communities. Such "normal/established" communities are, however, partly covered in our long term experiment. For my experiment with diatom communities in the initial phase of fouling, it is likely better to sample frequently.

Finally, I would like to summarize by saying that the trip was most interesting and beneficial to my further work at SES.

8. GENETICS SECTION

Progress Report for the Genetics section

Jan-Dec. 1982

The population genetics section of the BP project is now well under way. The spring was spent ordering supplies, equipment and chemicals, and the summer was spent setting up the lab. At this time two species of molluscs have been sampled to be run with the horizontal starch gel electrophoresis technique to test for enzyme polymorphism.

Littorina littorea was collected at Solbergstrand in 16 Aug. 1982. A minimum of 100 animals was taken from each of the four 1.5 m deep basins (numbered for this study as I-IV, with I designated as the most seaward basin). An additional hundred animals were collected from the rocks at the edge of the fjord at Solbergstrand. On 15 Nov. 1982, an additional one hundred Mytilus edulis were collected from each of the four basins. The Littorina were sampled before two of the basins (I+III) were exposed to oil hydrocarbons, beginning the middle of September; the Mytilus, however, were sampled after the hydrocarbon dosing had begun. During transport back to the University, the animals were kept alive in seawater and upon arrival, promptly deep-frozen whole at -80°C.

To determine which buffer systems worked best with the various enzymes, 72 of the Littorina from the fjord were tested for 27 enzymes on 6 different buffer systems. The resulting procedure established for Littorina is to run 24 of these enzymes on 5 of the buffer systems, and for Mytilus (tested later with basin animals) 15 (possibly 17) enzymes will be run on 5 (possibly 6) of the buffer systems. Some of these enzymes could eventually be dropped from analysis if they continue to exhibit weak and difficult to read bands.

Since early November, animals from the four basins have been run. As of the end of December 1982, the following samples have been collected:

<u>Littorina littorea</u> :	Basins I, II - 42 animals
	III, IV - 18 animals
<u>Mytilus edulis</u> :	Basins I, II - 30 animals
	III, IV - 6 animals

At this time most of the enzymes analyzed appear to be monomorphic. In Littorina, approximately thirty eight loci have been scored, and in Mytilus, approximately 25 loci. Of these, only a few exhibit fairly definite polymorphism (Five in Littorina, five in Mytilus) and an additional six and seven loci respectively show possible allelic variation. The five polymorphic enzymes in Littorina are as follows: Leucine amino peptidase (LAP), 6-Phosphogluconate dehydrogenase (6-PGDH), Phosphoglucose isomerase (PGI), Phosphoglucomutase (PGM), and Sorbitol dehydrogenase (SDH). All of these enzymes have previously been reported in the literatures as polymorphic but some authors have found polymorphism in a few additional enzymes that we have not observed as yet, e.g. Malate dehydrogenase (MDH) (Berger, 1977) and esterases (EST) (Vuilleumier & Matteo, 1972; Berger 1973, 1977; Beardmore & Morris, 1978).

In Mytilus, the five fairly definite polymorphic enzymes are the following: Isocitrate dehydrogenase (DH), LAP, Mannose phosphate isomerase (MPI), PGI and PGM. All of these except MPI have been previously reported in the literature as polymorphic, and, as with Littorina, some authors have found additional

polymorphic loci, again e.g. MDH (Koehn et al., 1976) and esterases (Levinton & Suchanek, 1978).

By spring, most of the hundred animals of each species will have been run and new specimens will be collected in April or May to sample the recently spawned juveniles. Hopefully, some of the questionable loci can be clarified as we continue to run animals, and maybe we will observe some allelic variation in those additional two enzymes as reported in the literature. Genetic variation will be measured by different methods, for example by frequency of polymorphic loci and by average heterozygosity.

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9. Balanus improvisus, POPULATION ASPECTS

Title of project

The effect of oil pollution on populations of Balanus improvisus at Solbergstrand Experimental Station.

Participants

Odd Alfred Frydenberg. I am a student at University of Oslo Dep. Marine Biology, Inst. of zoology.

Aim and purpose of the study.

I shall try to find eventually changes of growth and mortality between the B. improvisus who are polluted of oil and they who live in natural environment. From May, June next year I have a new generation of B. improvisus. The parents of the new generation are they who have lived in the polluted basins, control basin and the sea station. The new generation would be distributed back to all the basins and the sea station. I shall also try to find eventually changes of growth and mortality between the B. improvisus who are polluted and they who live in their natural environment of these generation, and I shall try to find eventually changes between the two generations. I will tell more about the new generation in later reports.

Description of the work:.....

The B. improvisus has settled on 15 plexiglass-plates. the plates are 20x20cm. 10 of the plates are from Holmestrand (742 animals), the other 5 plates from Frognerkilen (681 animals). The B. improvisus from Holmestrand are from the middle of July. They from Frognerkilen from 7/8-12/8-1982. Each plate are marked with nr. from 1-15. At first measurement I took a picture of every plate. On every picture I marked every animal from 1-x.

Estimate of biological substrate.....

The plexiglass-plates with the B.improvisus are distributed out to 3 basins and to the sea station.

Holmestrand=H, Frognerkilen=F.

Basin nr. 1 (200µg/l) it is 381 animals, H:225, F:156.

Basin nr. 3 (50µg/l) it is 376 animals, H:233, F:143.

Basin nr. 4 control it is 369 animals, H:119, F:250.

Sea station it is 294 animals, H:164, F:130.

The plates who I put out to the sea station has fallen down to the bottom where all was killed by predators. So from 24/11-82 I have put 2 plexiglass-plates from the control basin out to the sea station. From 24/11-82:

Basin 4 it is 183 animals H:57, F:126.

Sea station it is 186 animals, H:62, F: 124.

I measures all B. improvisus every time.

Assumed dates/periods of sampling.

And,

Assumed dates/period of in situ measurements

Measurement 13/9-15/9-82

" 23/11-30/11-82

" 15/1-20/1-83

" 15/3-20/3-83

" 20/4-25/4-83

" 20/5-25/5-83

" 20/6-25/6-83

" 20/7-25/7-83

The end August.

It is possible that it might be some changes in these dates.

The measurements of the new generation, see later reports.

Need of laboratory facilities/.....

I need to seat in the laboratory for measuring. In May, June next year when I shall work with the cyprids larvae it would be need of laboratory facilities. This work will go on at Blindern together with Henry Hovde. Today I do not have all the information of these project, but I would describe these in later report.

Specific demands of background parameters.....

The most important background parameters in my project are the temperature and salinity. Particle counts, nutrient and sedimentation are not so important.

Odd A. Frydenberg
Odd Alfred Frydenberg.

10. *Mytilus edulis*, POPULATION STRUCTURE AND DYNAMICS

Title of project

Long term effects from low concentration of oil on Mytilus edulis.

Participants

Pål Thome and Mats Walday, both hovedfag students at UiO.

Aim and purpose of the study

To increase our knowledge about growth, mortality and other possible effects of oil pollution on Mytilus edulis.

Description of the work

In July 1982 about 10 000 Mytilus were sampled from the basins and the fjord outside.

We measured max. length and sorted them after size in 5mm intervals: 10-15mm, 15-20mm and so on up to 40mm. The mussels were transferred to nets of polypropylen fibre and placed either in basin 1 (polluted with oil ; 200µg/l), basin 4 (reference) or outside in the fjord (control station).

About once a week we removed algeal growth from the nets. In the beginning of September we started sampling again. From our populations we took out 200 mussels from each size interval at each station.

We measured: Max. length

Volume (allometric growth)

Before volume measurement each mussel were rinsed from growth of algae and *Balanus*.

After this treatment they were placed in new nets and put out again. The rest of the mussels were stressed in a similar way: Carefully teared apart breaking up the byssus net, placed in new nets and back to their respective stations.

This work was finished just before the oil pollution commenced at the 16th of september.

In the periods 16/11 - 26/11 we followed the same procedure as above. In addition sampling for quality analysis (a total of 450 individuals from all three stations) and for CHN- analysis (total of 90 animals, 30 from each station), were sampled in the beginning of December.

Estimate of biological substrate or water material needed

We have estimated to measure 200 animals from each interval every sampling period, i.e. a total of approx. 3600 animals from the three stations.

During the winter, we have a sampling period every second month. When spring comes and activity in the sea increases we will sample more frequently, probably once every month.

Assumed dates/periods of sampling

Our first sampling period occurred in the middle of september, the second in the middle of november. The next will be in the middle of january.

In situ measurements will be made at the same time. A sampling period takes approx. 14 days. Quality and CHN- measurements will be done every second sampling period.

Need of laboratory facilities

Seawater, digital slide caliper, binocular magnifying glass, precision weight, freeze-dryer, CHN- analyser is needed during the sampling periods.

Background parameters

Temperature, salinity and particle counts must be measured.

PÅL THOME

MATS WALDAY



11. *M. edulis*, CONTENT OF MUTAGENIC COMPOUNDS

EXAMINATION OF MUTAGENIC COMPOUNDS IN MYTILUS EDULIS AFTER EXPOSURE TO DIESEL OIL

Inger Hagen and Jan Hongslo

Central Institute for Industrial Research, Oslo, Norway

Mutagenic compounds have been demonstrated in extracts of clams and shrimps from the marine environment of Kuwait, and it is suggested that the mutagenicity is due to crude oil pollution (Mut. Res. 104 43-48, 1982). The aim of the present study is to examine whether mutagenic compounds will appear in *Mytilus edulis* after exposure to diesel oil.

Samples of *Mytilus edulis* (~ 50 individuals) are collected from a control basin and from the basin containing 200 µg/l of diesel oil, before starting of the oil dosing and at 3 months intervals until June 1983 (i.e. September and December 1982, March and June 1983) and from then on twice a year. When possible our sampling will be performed at the same time as the group in Plymouth that are studying neoplasms in hemocytes, and it is the intention that our results will be correlated at the end of the study. Also, the results will be correlated with the analytical data on the hydrocarbon content in *Mytilus*.

12. *Littorina littorea*, POPULATION STRUCTURE AND DYNAMICS

Description of the project

Title

Population dynamics of *Littorina littorea* in a natural and an oil contaminated environment at Solbergstrand experimental station.

Participants

Kjell Moe and Einar Lystad. Both students at University of Oslo , Dep. Marine Biology , Inst. of zoology.

Aim and pupose

Register eventual changes in population dynamics in the polluted versus the natural environment. Try to find in what way *L.littorea* responds to this kind of stress (pollution by hydrocarbons) both at an individual and population level. Further on correlate this response to that of *L.littorea* in an open population in the Oslofjord not stressed by oil contamination.

Description of the work.

Sampling of *Littorina* is done by hand on the stairs in all four basins. The area is divided into squares 42,5 X 42,5 cm. on the horizontal part. Each square is labeled with a letter (vertically) and a number (horizontally). This means that every square has both a horizontal and vertical part.

All *Littorina*'s are then sampled in the labeled area , marked with a number and colour which are encrusted with cyanoacrylat (a quick sticky glue) as protection. Every snail is then put back to its own area. after beeing measured with a digital calipher. Recapture is done in 6 squares at each level, that means 30 in each basin. In the open population at the pier we recapture in all 15 squares. The marked individuals are then

measured and let back in the water in its own square.

Estimate of biological ...

Sampling , marking and recapture have taken place in all four basins and at the pier.

Number of individuals.:	Basin 1	1973
marked	" 2	705
	" 3	368
	" 4	245
	The pier	1100

The low number of individuals in basin 4 is due to partly sampling. The open population at the pier demands a different kind strategy so all unmarked individuals are marked at recapture. Therefor there will be a rising number of individuals at this site. We also take some individuals (20) for examination of gamete material and nutrition status. Plans for genetical studies seem to be out in the blue as far as Littorina is concerned because of difficulties with proteins and homogeneity. Recapture is to take place every second month with increasing frequency as production and growth increases.

Assumed dates/periods of sampling

Capture and marking	6/7-21/7
Recapture	" 6/9-12/9
"	15/11-5/12
"	10/1-16/1
"	7/3-13/3
"	18/4-24/4
"	20/5-26/5
"	26/6-2/7
Recapture/Slaughter	August

There might be some changes in these dates if necessary.

Assumed dates/period of in situ ...

These dates will be the same as sampling dates.

Need of laboratory facilities

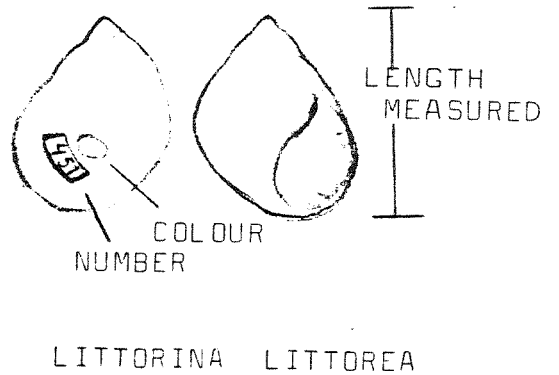
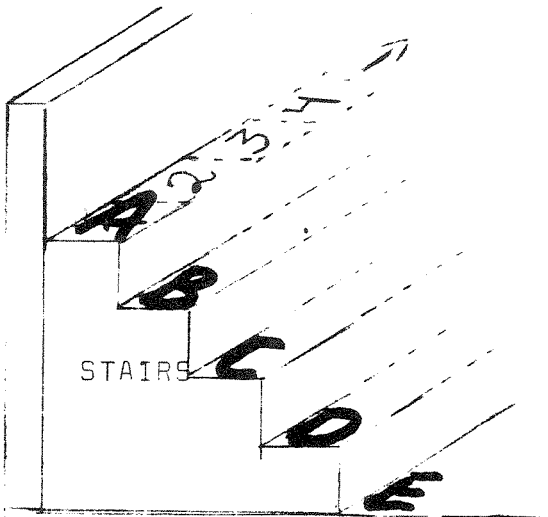
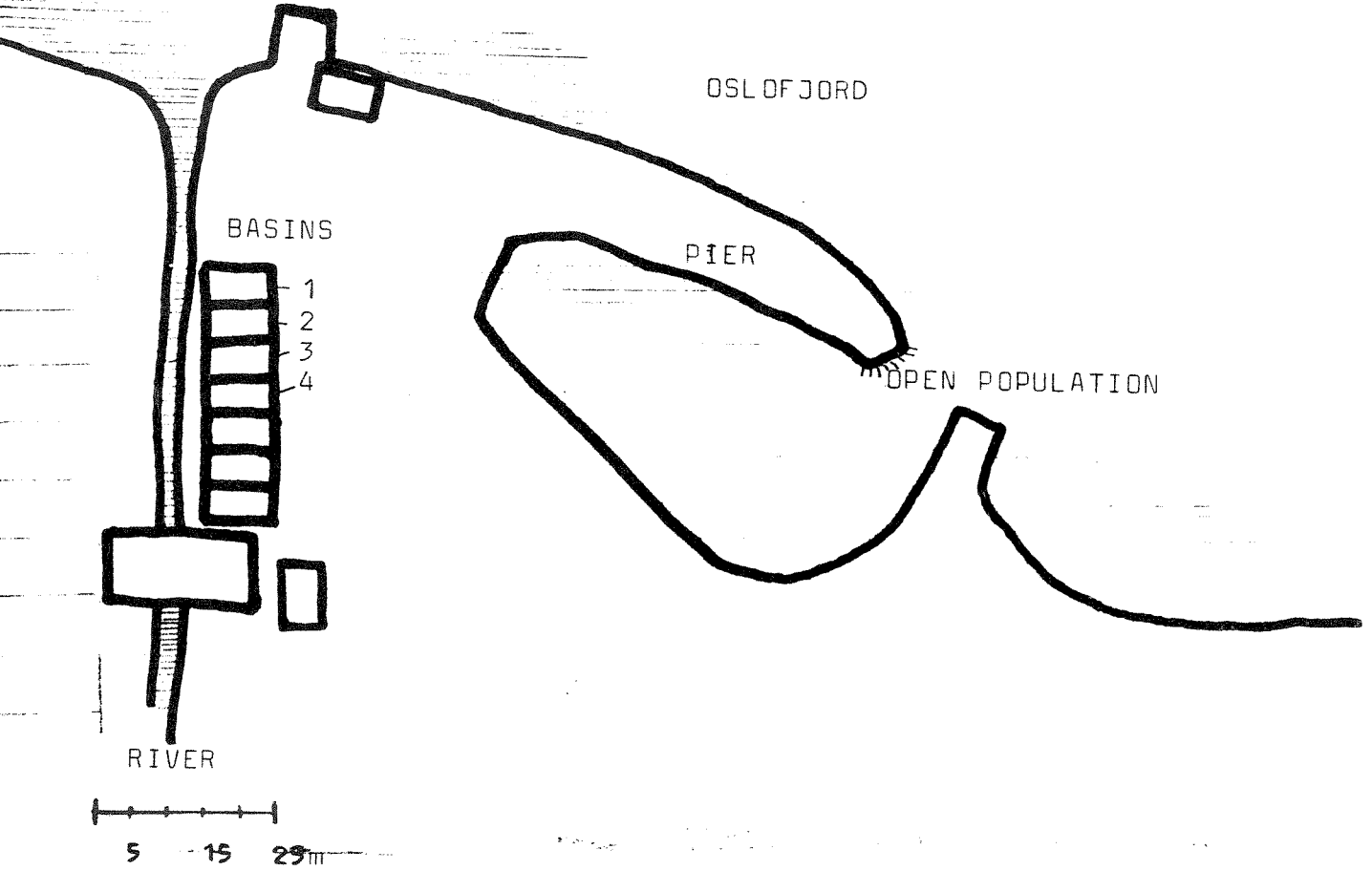
We need to seats in the laboratory for measuring and marking Littorinas. Some space in an aquarium for the snails during sampling period is also needed.

Specific demands ...

We are interested in sedimentation and eventual development of anoxic conditions in the basins. Also an investigation on microalgae film epilithic or epiphytic.

Kjell Moe and Einar Lystad

SOLBERGSTRAND EXPERIMENTAL STATION



LABELING OF SAMPLING AREA
IN BASINS

13. *L. littorea*, ENERGY BALANCE Description of the project

Project title:

"Energy balance in Littorina littorea".

Participants:

T. Bakke
K. Sørensen
H. Juelsen

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.

Methods:

The investigation will be based on measurements on samples of 10 individuals of average size from each population taken six times during one annual cycle. Each individual will be subjected to immediate laboratory estimates of the rates of the following processes:

Food uptake and grazing intensity.

The snails will be allowed to graze singly on glass substrates which have been pre-incubated in the corresponding basin (summer) or in an algal culture (winter) to develop a microalgal layer. After grazing has been allowed for a fixed period of time, the biomass reduction on the substrates will be estimated by comparison with non-grazed reference substrates. As biomass parameter will be used ash-free dry weight and/or carbon and nitrogen content of the algal layer. (Differences in grazing behaviour may be studied from the pattern of radula strokes on the substrates.)

Grazing rate will be estimated at least once for each snail of the sample in a flow through chamber in the laboratory with water supply from the corresponding basin. Laboratory in stead of in situ incubation will allow frequent observation of snail activity which in this respect is crucial.

Most of this procedure has only been tested out in small pilot scale and several logistic problems may arise. In the worst case one will have to rely on population estimates of grazing intensity at different times of the year based on the differences in microalgal growth on grazed and nongrazed

stone chips in the basins (Bokn & Pedersen) and population densities and rates of individual movement (Lystad & Moe) for the energy budget considerations.

Oxygen consumption.

Aquatic oxygen consumption will be estimated on individual snails in closed respiration chambers. Changes in the oxygen concentration of the chambers will be measured by use of YSI mod 5331 oxygen probes. The chamber size will be adjusted to give reliable reading of oxygen decrease within an hour, and the shape will allow a microalgal substrate to be inserted for estimates of oxygen consumption during grazing. These measurements will be performed in the laboratory for the same reasons as the grazing.

Aerial oxygen consumption will be sought performed by use of a Warburg type respirometer. This is not available within the project, and hence a cooperation with the Institute for zoophysiology at the University of Oslo is a prerequisite.

Absorption efficiency.

This will be estimated according to the method of Conover (1966). Faeces will be collected from a large number of snails (about 50) transferred directly from each basin to the laboratory. Each group will be incubated in an aquarium with frequent partial replenishment of filtered basin water. The faeces collected from the bottom of the aquarium will be analysed for dry weight and percent ash content. Similar analysis of weight and ash content will be performed on the microalgal layers on which the snails are normally grazing.

Nitrogen excretion.

This will be done by transplanting snails to individual open chambers with Millipore filtered sea water. The increase in the concentration of ammonium with time in the water of the chambers will be measured either by probes or photometrically at the chemistry laboratory at NIVA.

After the measurements described above the snails will be sacrificed for measurement of shell size and tissue wet weight, and the tissues from at least 5 individuals pooled for hydrocarbon analysis.

Based on the individual rates of food uptake, respiration and excretion adjusted to a standard size individual, and the population estimate of absorption efficiency, we will calculate the energy available for growth and gonadal production (scope for growth), the ratio of this energy to total energy intake (growth efficiency) and the ratio of oxygen consumption to nitrogen excretion. These aspects of the individual energy budget may then again be linked to the other sub-projects mentioned earlier.

Material needed:

10 individs of Littorina littorea from each basin (preferably marked) six times during one year will be sacrificed. In addition 50 individuals from each basin will be used each time for faecal collection and replaced afterwards. Also scrape-off samples from the basin walls will be needed to estimate the organic content of the food of the snails.

Periods of sampling/analysis:

The estimates will be performed six times annually. We aim to do the first estimates in February and after that every second month. A full series of estimates/measurements is expected to take one week. If time is limiting the effort will be reduced to three basins leaving out one of the controls. Other reductions of the program will have to be the number of measurement periods during one year.

Need for facilities:

During each one-week period the following will be needed:

- about 3 meter bench space with shelves above. (The aquaria room at SES has the adequate equipment.)
- an YSI oxymeter with accessories (presently at SES).
- ammonium analyser (to be borrowed from NIVA)
- aerial respirometer (in cooperation with UiO)
- drying and ashing facilities (at NIVA)
- microbalance (at NIVA)
- algal culture during winter (at NIVA)
- dissecting microscope (borrowed from NIVA)

Background parameters:

No specific needs outside what is presently monitored or included in other sub-projects.

14. BIOCHEMISTRY AND CYTOLOGY OF *M. edulis* and *L. littorea*

Joint NIVA/University of Oslo project on oil pollution: BP sponsored

Notes for discussion on IMER's involvement

After discussions in Oslo and in Plymouth, the following programme has been drawn up as representing the first stage of research by IMER personnel.

We envisage research on two species, *Mytilus edulis* and *Littorina littorea*. The research involves cytological and physiological approaches to assessing the responses of these animals to low levels of hydrocarbon pollution, and to measuring the processes of recovery (both in the short and long term) from pollution stress.

1. *Mytilus edulis*

a: Cytological measurements (i.e. cell structure, the distribution of cell types within tissues, measurements of lysosomal function) made on cells of the digestive gland and mantle.

In particular, we wish to establish tidal and seasonal aspects of the presence/absence of storage tissue (adipo-granular cells and vesicular connective tissue), the sequence of events comprising the annual storage and gametogenic cycles, quantitative estimates of gametogenesis, and the detailed processes of storage and digestion in the cells of the digestive gland, including the role of lysosomal mechanisms.

These measurements to be made on mussels experiencing pollution, control mussels and polluted mussels during short term (i.e. 3 weeks) and longer-term (2-12 months) recovery from pollution.

b: Physiological measurements, made on whole animals, will include feeding rate, aspects of gut passage and digestion, respiration and excretion.

These measurements are designed to establish the scope for growth for individuals in each condition, details of the adaptive link between energy loss and gain (particularly when assessed in terms of gut passage time and net food absorption), and to allow the calculation of reproductive effort and reproductive value for individuals in the population.

These experiments will also include assessments made on the time-course of recovery from pollution stress.

The physiological and cytological components of this project are mutually interdependent, particularly in aspects of the reproductive cycle, events in the digestive gland and in providing "whole animal" interpretations of cytological (and cytochemical) effects of pollution.

2. *Littorina littorea*

In the first instance we envisage only cytological measurements made on this species (but see Section 3). These measurements will include structural (histological) surveys of the digestive and reproductive tissue, coupled with cytochemical assessment of lysosomal function in the digestive cells and the oocytes. Elements of the mixed-function oxygenase system, particularly the enzyme neotetrazolium reductase, will be screened by quantitative cytochemistry as a measure of response to hydrocarbon pollution.

Experiments with this species will include, as with Mytilus, a study of short- and long-term recovery from pollution.

3. General comments

This programme is envisaged as being reasonably self-contained, but we are very conscious of the overall team effort involved in the NIVA project as a whole.

Results of the IMER research will provide a quantitative assessment of pollution effects in the two species over time and including aspects of recovery. In addition, some elements are designed to allow the extrapolation from effects on individual animals to effects at the population level; this refers particularly to measurements of reproductive effort and reproductive value.

On the other hand, we see the need for close collaboration with others on two aspects in particular:

a: In order to put our work on Mytilus into a general context, we will need to refer to life-table measurements on this species, in particular to estimates of size-related mortality and to population growth.

b: In order to relate cytological observations in Littorina to effects on the whole organism, we will need to refer to physiological measurements (i.e. energy budget calculations) made on this species. If necessary, we can undertake some such measurements ourselves.

4. Logistics

A study of seasonal aspects of energy storage cycles and gametogenesis requires regular monthly samples. We propose that monthly collections of mussels (10 individuals from each condition) be made, the flesh dissected from the shell, the pallial muscle cut away (this facilitates subsequent histological treatment), the tissue fixed in Bakers Formol fixative and sent to Plymouth for further study.

Remaining aspects of the study can best be carried out by four scientists from IMER (B.L. Bayne, J. Widdows, M.N. Moore and D.M. Lowe) visiting Oslo for periods of about three weeks twice each year for 2 or 3 years. A preliminary assessment suggests that March and August/September, 1983, would represent optimal dates.

However, two problems remain. Our first visit should precede natural spawning in the mussel population by 2-3 weeks; what is known locally of the spawning cycle of Mytilus? Secondly, what arrangements have been made (or should we make) concerning analyses of hydrocarbon body burdens in animal tissues?

B. L. Bayne

24 August 1982