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SOLBERGSTRAND EXPERIMENTAL STATION, DRØBAK

LONG TERM EFFECTS OF OIL ON MARINE BENTHIC COMMUNITIES IN ENCLOSURES

RESEARCH PROGRAMME

Oslo, May 1982

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Front page : Photography of the rock littoral basins with the laboratory building.

NIVAs hustrykkeri

NIVA - REPORT

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Abstract:

The report outlines the scope of work of the research programme to be performed at Solbergstrand Experimental Station under the sponsorship of British Petroleum. The aim of the programme is to study long term effects of low levels of oil hydrocarbons on littoral rock communities and sublittoral sediment communities kept in large basins.

4 keywords, Norwegian
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2. Oljeforurensning
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4. Solbergstrand Drøbak Oslofjord

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PREFACE

The present document is a revison of the Solbergstrand Experimental Station Research Proposal, G 304, Notat, June 1981. The programme has been reduced to cover those projects which can be accomplished under the sponsorship of British Petroleum with a funding of 12 mill. N kr (1981) adjusted annually for inflation. Efforts will continue to obtain additional funding to include important aspects of the programme which have had to be reduced or excluded under the present economic framework. The strategy adopted following the revised budget is a high investment in construction, equipment and manpower during the first years at the expense of the activities in later years.

The scope of work under each project has been adjusted to recommendations given by the Advisory Board of the programme during a seminar held in Oslo March 29-30 1982. Elements of work supported by internal funds and elements to be dealt with by research students are also described.

Oslo, May 1982

Torgeir Bakke

PREFACE TO 1st EDITION

The present document has been worked out in order to:

- Outline the research philosophy behind the plans for experimental research activities within the field of marine pollution biology (ch. 1 and annexes)

- Give a short presentation of Solbergstrand Experimental Station (ch. 1)

- Describe ongoing and planned projects in main features (ch. 2) and as research proposals (annexes)

- List some examples of other projects of relevance to marine pollution problems in Norway, which may be treated experimentally at Solbergstrand (ch. 2)

The wide scope of experimental marine biology requires cooperation between several categories of experts. Consequently, the present account should be regarded as an invitation to participation in future as well as ongoing activities. Both the projects and the plans for the experimental station would benefit from the contributions of other specialists; adding their ideas, methods and observations to the endeavours of those already engaged.

Oslo, June 1981

Jon Knutzen

C O N T E N T S:

1. INTRODUCTION

1.1 Perspectives

The present situation within aquatic pollution biology is to a large extent characterized by descriptive work on specific recipients. Further, many short-term experiments are performed using bioassays with one or a few species. Due to the complexity of the recipient environment, and test conditions deviating from the situations met with in nature, results from these types of studies are difficult to interpret or have uncertain relevancy. Little new knowledge of cause/effect relations is gained.

The prime purpose of research activities at Solbergstrand is to perform cause/effect studies with systems and procedures which permits a more direct application of results to recipient situations.

Even if aiming at specific pollution problems, the experimental methods may in principle be applied to different kinds of polluting substances and environmental disturbances. Thus it should be in the mutual interest of authorities and industry to have at their disposal a tool for relevant pollution studies. Most of the work today is either too closely tied to the recipient, with inadequate consideration of the overwhelming number of factors which interact, or too far from the actual situation, and thus giving results warranting the comment "so what ?". Both extremes - the field ecology approach alone and short-term tests under strictly defined conditions merely existing in the laboratory - tends more to keep us aware of basic questions than allowing us to answer them.

The platform for the experimental research at Solbergstrand can be given as follows:

- Model ecosystems (hard and soft bottom communities of approximately natural composition in simulated environments)
- Simulated loading or stress (i.e. the same type of stress as in existing receiving waters) under controlled conditions
- Performance tests in long-term and life cycle studies with key species of different communities

- Low doses/long-term effects
- Bioassay with sensitive life stages (based on introductory life cycle tests)
- Flowthrough systems
- Combination of stress factors
- Uptake/excretion/biomagnification
- Evaluation of physiological/biochemical methods for field observation.

Several of the above approaches demand considerable resources in terms of manhours and expertise. Thus it is necessary to concentrate work on a limited number of projects and to seek cooperation with other research institutions. Further, it is hoped to bring a variety of experts into the programme in time, as the main experiment will run for more than a year.

1.2 The experimental station

The field station is situated south of Drøbak on the east side of the Oslofjord, about 50 km from Oslo by car (Fig. 1). The former trout hatchery borders to the shore and includes a laboratory building of 160 m^2 and 20 concrete basins (Fig. 2).

Seven of the basins are about 50 m^3 (8 x 5 x 1,25m) and thirteen larger basins are about 400 m^3 (20-30 x 6 x 2,5 m). The smaller basins are in use (see below) or they can be made operative on relatively short notice. The larger basins have sand bottom covered with vegetation and their walls are in need of repair.

In addition to two large laboratory rooms, the indoor facilities include a kitchenette, a climate room with cooling and freezing machinery and 4 smaller rooms which may be used for diverse purposes (Fig. 3). At present the laboratory building is under repair and furnishing.

The laboratory building is owned by the Norwegian Institute for Water Research, whereas the surrounding 15 decare property is leased. So far, total investment have been close to 2.5 mill. N.kr.

In addition the University of Oslo has extensive facilities (laboratories, equipment, aquaria with running sea-water, accomodation and eating facilities, 42' stern trawler and small boats) at the Biological Station in Drøbak.

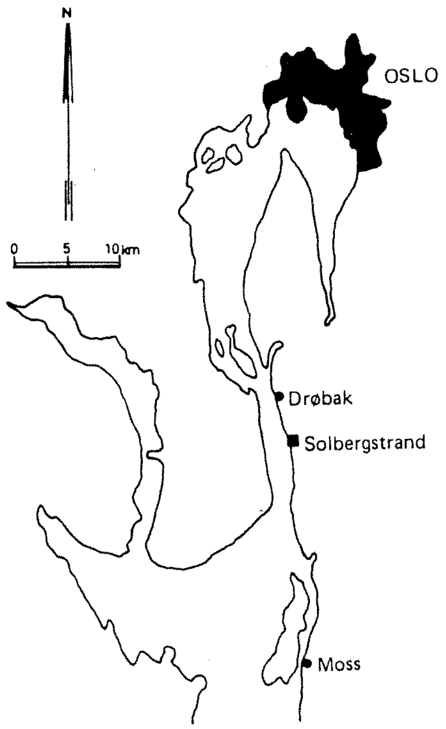


Fig. 1. Location of Solbergstrand Experimental Station.

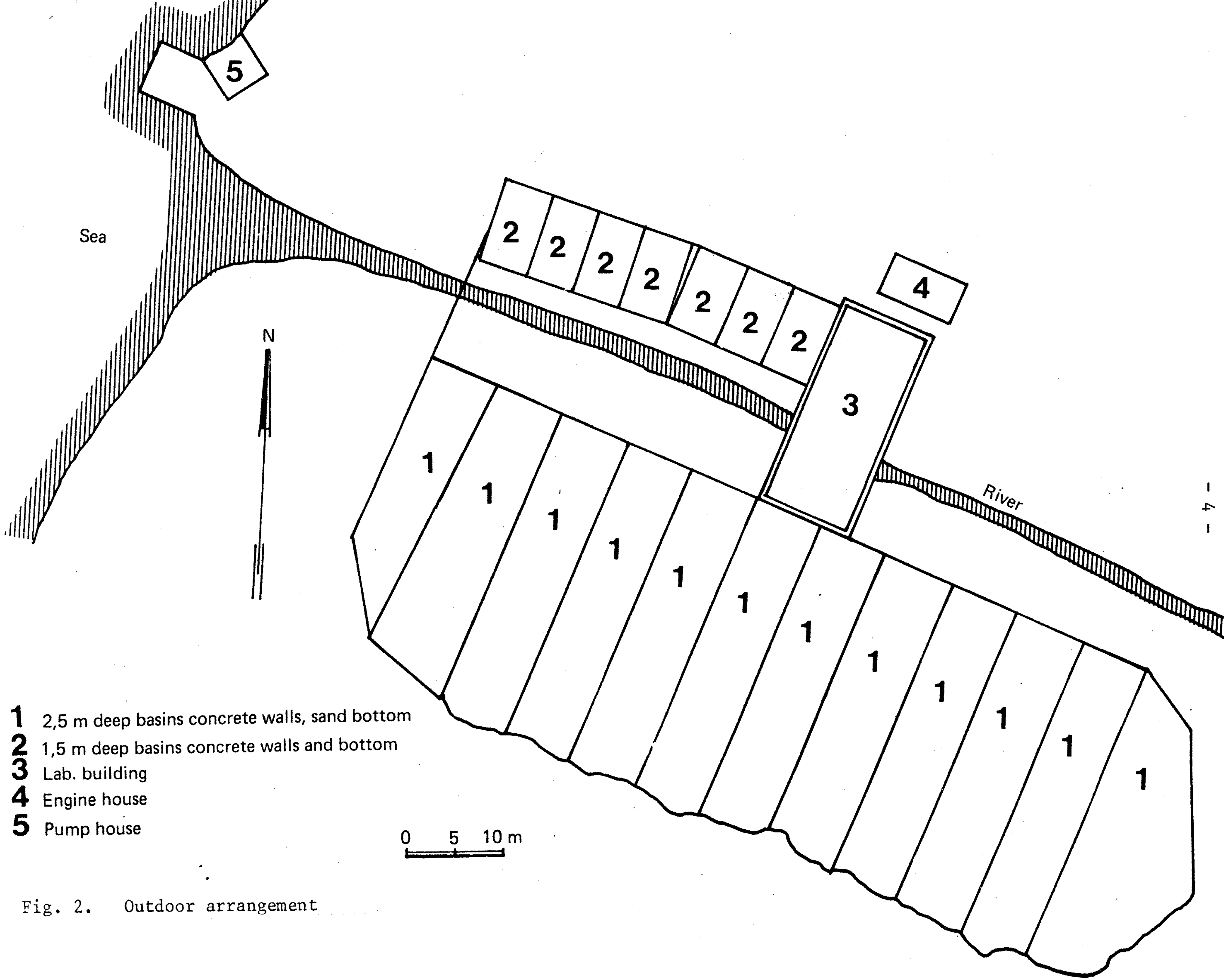


Fig. 2. Outdoor arrangement

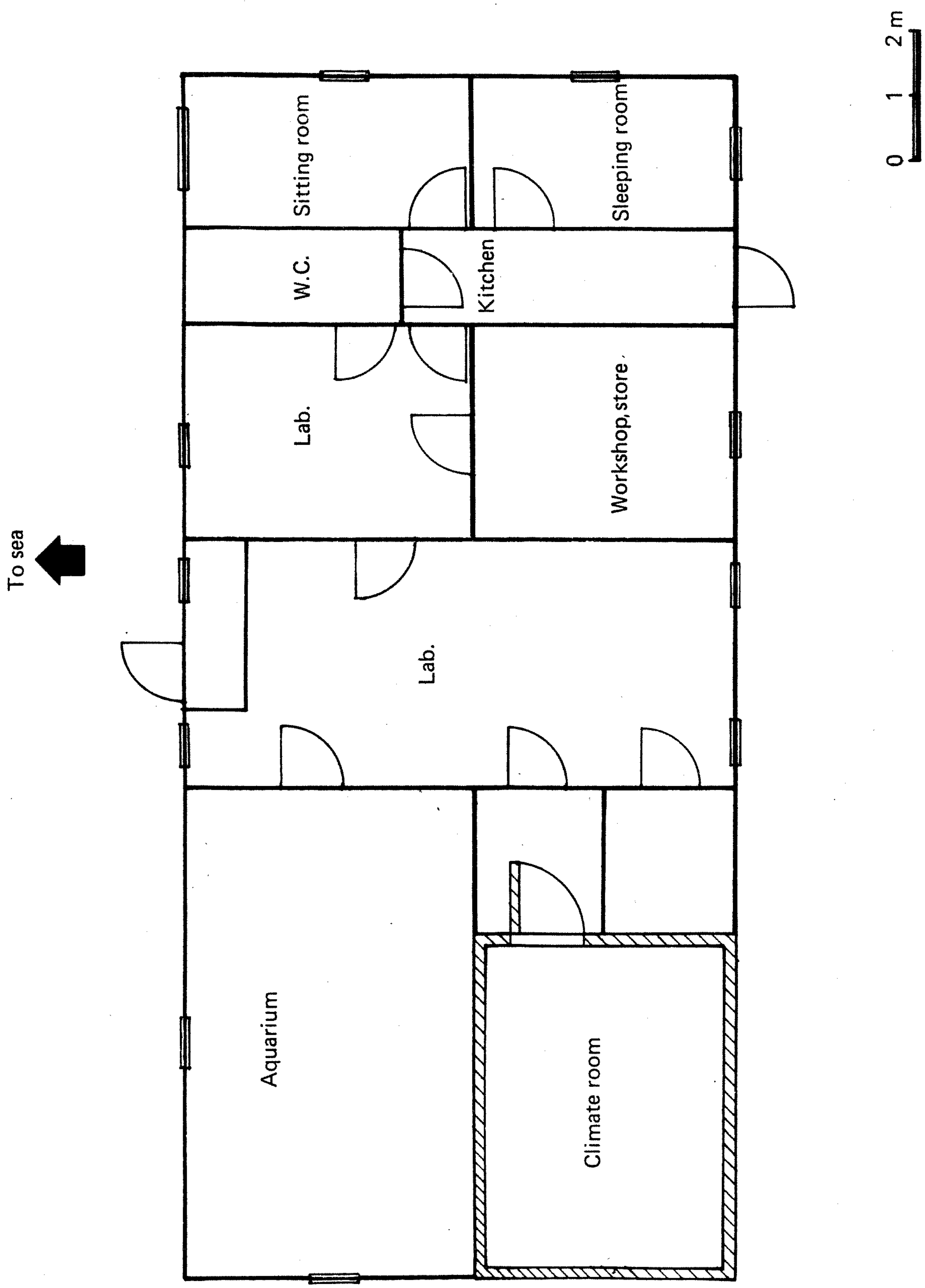


Fig. 3. Preliminary laboratory plan.

2. PROGRAMME ORGANIZATION

A programme of this magnitude, with several institutions involved, requires a firm coordination and administration. The administrative elements established are:

- a Steering Committee responsible for the fulfilment of the programme. This committee has representatives from BP, NIVA and the University of Oslo. Sponsors coming in at a later stage should also be represented. The members are appointed for two years. Present members are:

  Dir.Gen. L. Overrein, NIVA (chairman)
  Professor J. S. Gray, University of Oslo
  Dr. W.J. Syratt, British Petroleum, London.

- an Advisory Board with members selected on basis of their scientific expertise. The tasks of the Board are to recommend adjustments of the research activities (annually), suggest elements to be included and inform about potential co-working institutions or groups. Three of the present Board members are from outside Norway, two Norwegian members have been appointed by NAVF.

  The Advisory Board members are appointed for two years. Present members are:

  Dr. B.L. Bayne, Institute for Marine Environmental Research, Plymouth, UK
  Dr. J.F. Grassle, Woods Hole Oceanographic Institution, Massachusetts, USA
  Dosent E. Sakshaug, University of Trondheim
  Professor R.L. Vadas, University of Maine, USA

- a Programme Manager responsible for coordinating the research activities and reporting of these to the Steering Committee. The Programme Manager also functions as secretary for the Steering Committee and the Advisory Board. The present Programme Manager appointed for two years, is

  Forsker T. Bakke, NIVA.

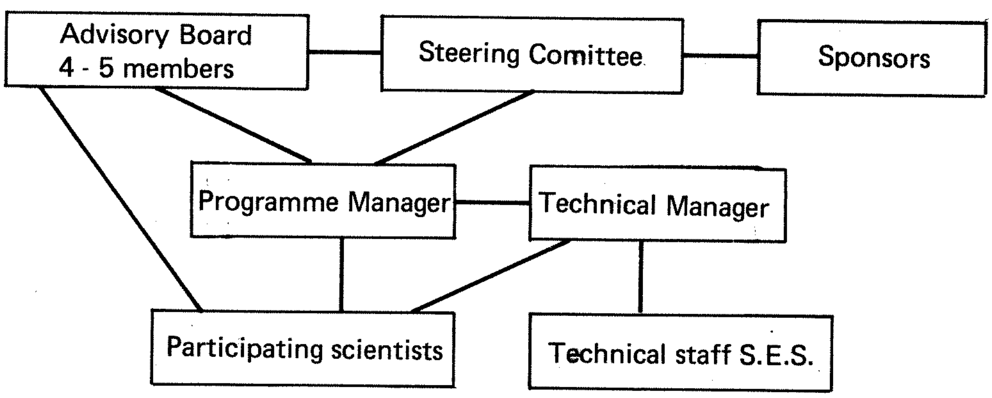
- a Technical Manager responsible for planning and running of the experimental facilities at Solbergstrand. The Technical Manager appointed for two years is:

  Forsker L. Kirkerud, NIVA.

The organization is given schematically below.

Solbergstrand Experimental Station

Administration



3. PROJECTS

3.1 Long-term effects from low concentration of oil on a simulated littoral rock community

Background

The intertidal zone is the most accessible part of the marine environment for studies; hence its ecological structure and dynamics have been more thoroughly described than other marine communities. This zone is also potentially the most vulnerable to pollution impact at the surface of the sea, such as oil spills. Several large spills (e.q. "Torrey Canyon", "Amoco Cadiz", "Florida") have caused great initial damage to flora and fauna, with subsequent recovery time of several years (Southward & Southward 1978, Sanders 1978). Thus we have a substantial empirical basis for judging effects of massive spills on various types of littoral communities, and for deciding which types of cleanup procedure is optimal for a particular spill on a particular type of shore (Siva 1979, Cowell & al. 1979 and others). But the littoral community is also exposed to chronic pollution stress, especially by oil hydrocarbons and degradation products thereoff, in the vicinity of harbours and other areas of heavy boat traffic and around petrochemical refineries and other coastal industrial plants.

Rocky and soft bottom intertidal communities, and their constituent populations of plant and animal species have been included in several biological and chemical monitoring programmes (Jones 1980, Bowman & Lewis 1977, Goldberg & al. 1978, Phelps & Galloway 1980 and others), and serve as targets in contingency plans at oil spills (Gray & al. 1978). Yet there have been conducted few experimental studies of the long term effects of chronic oil pollution on littoral communities, especially with relevance to East Atlantic boreal and arctic coasts. Within the FoH (Norwegian Marine Pollution Research and Monitoring Programme) experiments on oil pollution of rock-pools have been conducted recently (FoH Project 202) and it has been proposed (Gray & Brattegard 1979) to mobilize the ecologist teams established under the Norwegian Ecological Action Plan to perform experimental oil spill effects and recovery studies along the Norwegian coast. These have, however, not been initiated.

Community establishment

At Solbergstrand Experimental Station littoral communities have been established in 4 concrete basins, each measuring 8 x 5 x 1,5 m with about 25 m^3 of running sea water. The communities are intended for studies of the effects of low chronic oil hydrocarbon exposure for extended periods of time. The basins are equipped with wave generators and tidal simulation reflecting the tidal range and timing of the fjord outside (Fig. 1). Sea water is supplied by means of centrifugal pumps from 1 m depth outside the station and with a present basin turnover rate of 2 hrs. The basins lie in connection to the laboratory which, when fully established, has facilities for use of advanced instruments and for specific tests in aquaria. Establishment of the facilities has been funded in part by FoH for equipment and manpower, with a total of N kr 300 000,- for 1979 - 1981.

The littoral communities were established in October 1979 by transplanting stones from the outside shore together with their natural cover of algae and animals. The stones were positioned on steps along one side of each basin, with the same vertical distribution as on the shore. Later recruitment has occurred both internally and by dispersion stages brought in with the water. The communities experience the same seasonal and climatic fluctuations as the shore outside except damaging ice movements in winter.

The algae associations are dominated by the common fucoids of the Oslofjord: *Fucus vesiculosus, F. distichus* spp., *edentatus, F. serratus* and *Ascophyllum nodosum* together with *Laminaria digitata, L. saccharina* and *Ulva lactua*. Less conspicuous species of red and green algae vary during the year. The dominating invertebrates are *Littorina littorea, Mytilus edulis* and *Asterias rubens*. There are also substantial populations of *Littorina obtusata, Balanus balanoides*, gammaridean amphipids, isopods *(Idothea* sp.) and during the summer a strong growth of the hydroid *Dynamena pumila*. Several other species have been recorded in the basins such as *Carcinus maenas, Ciona intestinalis, Pleuronectes flesus* and various freeliving polychaetes.

A description of the Experimental Station and a preliminary investigation were presented at an IAWPR-Symposium in Munich 1981 (Bokn & Kirkerud 1981).

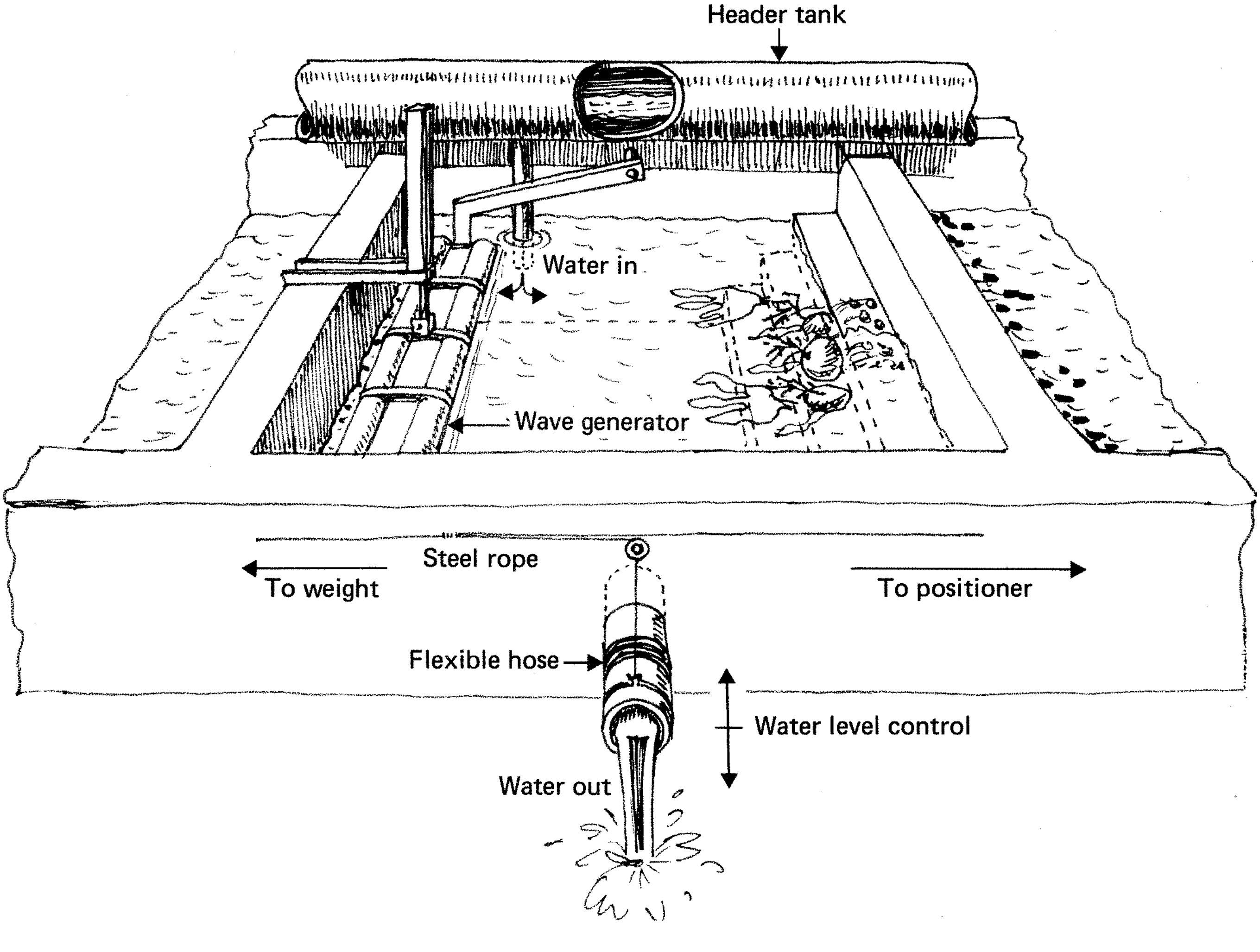


Fig. 1. Sketch of a basin.

Dosing of hydrocarbons

Exposure of the communities to pollutants will begin in late summer 1982. The water of the basins will be given a low continuous supply of oil hydrocarbons, simulating the pollution stress one can expect in the vicinity of harbours or industrial sites, for a period of at least one year, with subsequent recovery. It is aimed to apply a diesel type oil and an exposure level of about 200 µg/l total hydrocarbons in two basins. The others will act as controls. Most of the hydrocarbons will be present at microscopical droplets as it is not practically feasible to scale existing systems for production and dosing of a water soluble fraction to the large quantities of sea water used in the system. However, this type and magnitude of hydrocarbon exposure reflects a chronic oil pollution situation better than exposure to the water soluble fraction alone.

The dosing system is a replicate of a system developed at the Bermuda Biological Station and consists of a unit for vigorous mixing of oil and water, a separator for larger oil droplets and a dozing regulator coupled to a header tank. To facilitate cleaning and maintenance two parallel mixing and separation units will be constructed.

The oil/water proportion, mixing speed -type and -duration will be tried out during the summer 1982. With a turnover time in the basins of 3-4 hrs and a stock water accomodated fraction (WAF) containing at least 20 mg/l total hydrocarbons there will be an oil consumption of the system of less than 2 l/day.

Routine monitoring of the basins

This monitoring will run independently of the particular research activities. Temperature of air, basins and surface water outside the station and salinity is recorded continuously. Nutrients, particle content and size distribution, and phytoplankton biomass will be monitored 2-3 times a week. Hydrocarbons of the WAF will be analysed routineously by fluorescence spectrophotometry on the site (Gordon & Keizer, 1974), and by gas chromatography/mass spectrometry (GC/MS) for total content and specific compounds on selected samples. The water of the basins will be analysed in the same way. The GC/MS analysis will be performed at the Central Institute for Industrial Research (SI).

Biological effects studies

The effects of hydrocarbon pollution can be traced at all levels of biological organization, and ideally several levels should be included in such large scale experiments. The present scope of work comprises studies on community, population and individual/subindividual levels, but following the recommendations given by the Advisory Board emphasis will be placed on the population and individual levels. The basin communities are in principle closed, which gives the opportunity to trace the performance and fate of single marked individuals for extended time periods with and without oil stress.

Community structure

The composition of the littoral communities will be characterized by monitoring percent algal cover and number of motile animals. A special frame adjusted to the dimension of the basin steps will be 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 will also be photographed to document possible successions. This monitoring is very time consuming and can only be performed to the desired extent by participation of research students.

Community function

Based on recommendations from the Advisory Board the gross community metabolism aspects will be centered around the effects of oil on community establishment, i.e. effects on the microbenthic layer covering stone surfaces in the intertidal zone. This initial community is composed partly of bacteria, microalgae and microfauna, partly of the recruitment stages of macrobenthic plants and animals, which have been stimulated to settle by the microorganisms. The project will put emphasis on community respiration and production. Oil may interfere with these processes in both positive and negative directions (Baker 1971, Bakke & al. in press, Davies & al. 1980, Gordon & Prouse 1973 and others). Thus continuous exposure to oil may cause a shift in the balance between community photosynthesis and respiration and possibly a gradual change in the major energy pathway. It is aimed to estimate these processes in microbenthic communities on removable substrates positioned in the basins. Both roughly cut stone plates and plates covered with thin glass fibre gauze will be used. The former will be transferred to the laboratory for studies and returned to the basins. The latter will be sampled by use of a cork borer and the samples mounted in small respiratory chambers with continuous flow. The parameters dealt with will be

- community respiration
- community production
- light intensity
- nutrients
- chlorophyll
- tissue CNP
- taxonomic composition.

Measurements will be carried out at 0.5 to 2 months intervals, depending on season and visual examination of growth.

Production and respiration will be analyzed from changes in oxygen and total carbonate (measured by O_2 and pH probes).

Population genetics

Discovery of the main principles of molecular genetics - the structure and functioning of DNA and the so called central dogma describing the flow of hereditary information from the genetic code in DNA through RNA to aminoacid sequences of polypeptides - had widespread ramifications for all fields of biology, especially so for population genetics since variation in natural populations could now be studied at the protein level. Electrophoretic techniques for separation of proteins (mainly enzymes) in animals, followed by specific enzyme staining has today become the method of choice in assessing genetic diversity of natural populations.

When hypotheses of adaptive neutrality are ignored, one is left with the theory that genetic variation among populations is maintained by balancing selection which means that different genetic variants are favoured in different environmental niches. From this follows that the degree of genetic variation in a population should be correlated with the degree of heterogeneity of its environment (environmental heterogeneity hypothesis). From knowledge of the population structure of a species it is therefore possible to study the long - term evolutionary response of that species to environmental challenges such as oil-pollution.

The primary objectives will be to investigate at the population level any potential effect from oil-related pollution on electrophoretic variants at several gene-loci in different organisms from the Oslofjord.

The approach to be used will be studies of genetic variation over a series of gene-loci coding for enzymes and other unspecific proteins.

A variety of electrophoretic techniques have been employed to study genetic variation, or enzyme polymorphism, in natural populations. The most frequently used - and also the choice for this project - is horizontal starch-gel electrophoresis. The popularity of starch as a supporting medium lies in the ease with which replicate slices from a slab gel may be stained for several different enzymes.

Some of the advantages of the electrophoretic methods are: 1) Mass screening of hundreds of individuals for 30 or more genes is possible; 2) Variable as well as non-variable gene-enzyme systems are visualized so that one may estimate the proportion of genes that are polymorphic in a population; 3) The genetic similarily of populations may be quantified because the proportion of shared alleles at each locus is readily obtained. Therefore by comparing populations from oil exposed versus nonexposed basins, it is possible to evaluate whether there has been any selection for genetic variants due to environmental stress.

Different enzymes have been found to have quite different levels of variation within and between populations; thus the need to assay as many gene-enzyme systems as possible in such studies has been emphasized. We will sett up a battery of at least 25 enzyme-systems. Since isoenzymes are often encoded by two or more gene-loci, this enables us to investigate a fairly large samples of genes, and hopefully some diagnostic markers might be found.

Earlier studies attemped to explain (adapted) genetic differences among species and populations in total average heterozygosity evaluated over all gene-loci studied. This approach has been criticized due to the complexities of the animal-environment interaction and the functional diversity of gene products usually examined in electrophoretic studies. One common approach to use today is to separate enzymes according to their biological/physiological function, most often classifying them as central metabolic enzymes (group 1) or those enzymes acting upon externally derived substances (group II). More recent environmental-heterogeneity hypothesis assert that heterogeneity for group I enzymes is positively correlated with the environmental heterogeneity and that group II enzyme heterogeneity is positively correlated with phyletic diversity of trophic resources. This separation of enzyme systems will be followed in the proposed study to investigate on which group of enzymes oil in the environment will have the most (if any) severe effect.

Initially the work will be concentrated on populations of *Littorina littorea* and *Mytilus edulis*. These animals have established themselves in substantial numbers in the Solbergstrand basins. Additional studies will be performed on *Balanus balanoides* and gammaridean amphipods, also accessible in large numbers from the hard bottom basins. At a later stage selected organisms from the soft bottom basins will be subject to similar studies.

Electrophoretic analyses of these samples will provide data on the amount of genetic divergence among subpopulations of the animals selected for the study.

The electrophoretic data will be immediately evaluated for the precence of any diagnostic differences that might be used to classify individuals to their environmental "quality" - that is to oil exposed water versus "clean" water.

Gene frequency data will be subjected to gene diversity, an analysis designed to partition total genetic variance in a hierarchical fashion representing the various levels of population structure - that is to see how extensive have been the potential effect of pollution to the population structure.

Any variation between polluted and control basins will be tested by analysis of variance. Particular emphasis will be placed on comparing the genetic diversity of juveniles with the genetic diversity of the spawning adults in order to evaluate whether recruitment represents random sampling of adult reproductive output. Severe mortalities suffered by the pelagic larvae of marine organisms in general and in polluted areas in particular may contribute to very unequal reproductive success among spawning adults.

Population structure and function

Population densities of major plant and animal species on the basin steps will emerge from the transect approach described in the community chapter, with use of frames. One aim of the transect approach is to follow structural changes of populations with a methodology frequently applied in field effects monitoring to evaluate and improve this methodology under controlled conditions.

Being semiclosed, the basin systems also give opportunity to study population structure and dynamics of the dominating motile forms with high precision.

Aquisition of detailed information on the structure of populations is also an integral part of the interpretation of data on genetic variability. For both *Littorina littorea* and *Mytilus edulis* populations will be sampled, individuals marked and returned to the basins. From mark and recapture data it will be possible to estimate population size, growth rates of individuals, recuitment to the population and survival and mortality. It can be expected that any short-torm selection will alter some of these aspects of the population structure and thus interpretation of any genetic changes can be linked to these aspects.

Similar experiments will be done outside the basins thus giving a natural condition, control basin and oiled basin comparison. As this work is time-consuming it will be done by research students from the University of Oslo. (UiO).

Techniques are available at UiO for culture of cypris larvae of *Balanus balanoides* and *B. improvisus* year round. These larvae can be induced to settle on experimental plates in set densities and patterns and then placed in situ in natural conditions, the control basins and the experimental basins. Here detailed life-table studies on growth survival, mortality of distinct, genetically similar material will be studied. Genetic work on some of the individuals can be expected to give data not obtained elsewhere since the technique is unique to UiO.

Recolonisation of algae will be studied by using clean granite chips (10 cm x 10 cm). To see if oil has any effects on gametes, zygotes larvae and/or germlings, 32 chips in two basins (one oiled/one control) will be studied ten times a year during three years. In every basin 8 chips will be kept away from grazers to see if the possible oil effects are directly on primary producers or on their grazers separately. This aspect will be tied closely to the community metabolism study. Due to budget restrictions the part concerning recolonisation will be tried funded by NIVA's internal budget.

Individual aspects

To check if petroleum hydrocarbons have effect on overall growth of macroalgae, linear growth will be measured in three selected species. In *Fucus vesiculosus* (bladder wrack) the proportion of fertile to sterile tips of the branches will also be studied. About 300 tagged and numbered individual plants will be measured every month during three years.

Tissue content of carbon, nitrogen and phosphorus of algae from various levels of the basins will be analysed. The relative proportion of these compounds could indicate nutritional or other stress caused by the exposure to hydrocarbons. These analyses will be performed several times a year by an external researcher.

Effects of hydrocarbon exposure on energy conversion of the littoral fauna will be studied with emphasis on *Littorina littorea* and *Mytilus edulis* as these species also will be subjected to genetic and population dynamic studies. The aims of the studies are:

- Laboratory experiments with the presumably tough species *Mytilus edulis* have shown that several important physiological processes such as food uptake and assimilation, oxygen demand, and nitrogen excretion has been significantly altered by short-term (4 weeks) and long-term (5 months) exposure to low levels of hydrocarbons (about 50 µg/l total amount), resulting in a disadvantageous individual energy budget (Widdows & al., 1982). In an ecological context the individual is insignificant, but a general response of this type can lead to serious negative consequences for the growth and reproduction of a commercially and ecologically important species. It is thus important to test these results in natural populations of *M. edulis* to judge the vulnerability compared to the laboratory test animals. It will also be of great value to test the precision in estimating these responses in natural populations versus the precision in the laboratory. The advantages of using the experimental in stead of field populations are the defined long time exposure, the direct comparison of test and reference populations with the same origin and history and the near-by laboratory facilities.

- The referred laboratory investigations (Widdows & al., 1982) have also showed a significant negative correlation between body burden of aromatic hydrocarbons and scope for growth (e.g. energy available for somatic and reproductive growth at any time) in *M. edulis*. It is imperative to investigate if the same correlation exists in populations in natural communities defined as clean or to a varying degree hydrocarbon exposed, and during different seasons. This will provide an empirical basis for evaluating if body burdens of field populations of mussels (or similar species) approach a level at which negative biological effects can be expected to occur.

These aims are also the basis for the *L. littorea* studies planned. Methodology and stress response knowledge is, however, less well developed for this species. The main reason for including *L. littorea* is that it feeds as a substrate grazer, and thus covers another important route of hydrocarbon entry to the littoral than *M. edulis*. In this respect it also substitutes the even more appropriate species *Patella vulgata*, which is not present in this part of the Oslofjord. *L. littorea* has also a different reproductive strategy (copulation and production of egg capsules) than *M. edulis* (pelagic fertilization and totally pelagic larval life). The success of these two strategies under chronic hydrocarbon stress may therefore be compared.

The energy budget studies will be performed at 1 - 3 months intervals (i.e. 6-7 times during one year) each time with 10 individuals from each basin. The physiological parameters will be tied to measurements of body size and tissue weight, condition index (*M. edulis*) and hydrocarbon loading of tissues. A study of the relationship between physiological processes and body size will be conducted twice during one year. The *L. littorea* studies will be covered by the BP funds whereas the *M. edulis* work will be performed by research students in close cooperation with the *L. littorea* work.

The physiological methods which will be applied, have been developed and described by Bayne et al. (1977), Bayne & Widdows (1978) and Widdows et al. 1982) and will be adjusted to the present conditions. A cooperation with the research group of Dr. Bayne at Plymouth, UK, has been established and it was decided during a recent Steering Comittée meeting (April 15) that funds up to 75 000 N kr per year should be allocated from the contingency reserve to cover travel expenses etc. for the participation of the Plymouth group. The particular topics which this group will consentrate on, will be decided upon in a meeting with Dr. Bayne on May 25, but of special interest are the long term effects of oil on nutrient storage cycles, reproductive processes and reproductive efforts as well as cytochemical aspects especially of *L. littorea*.

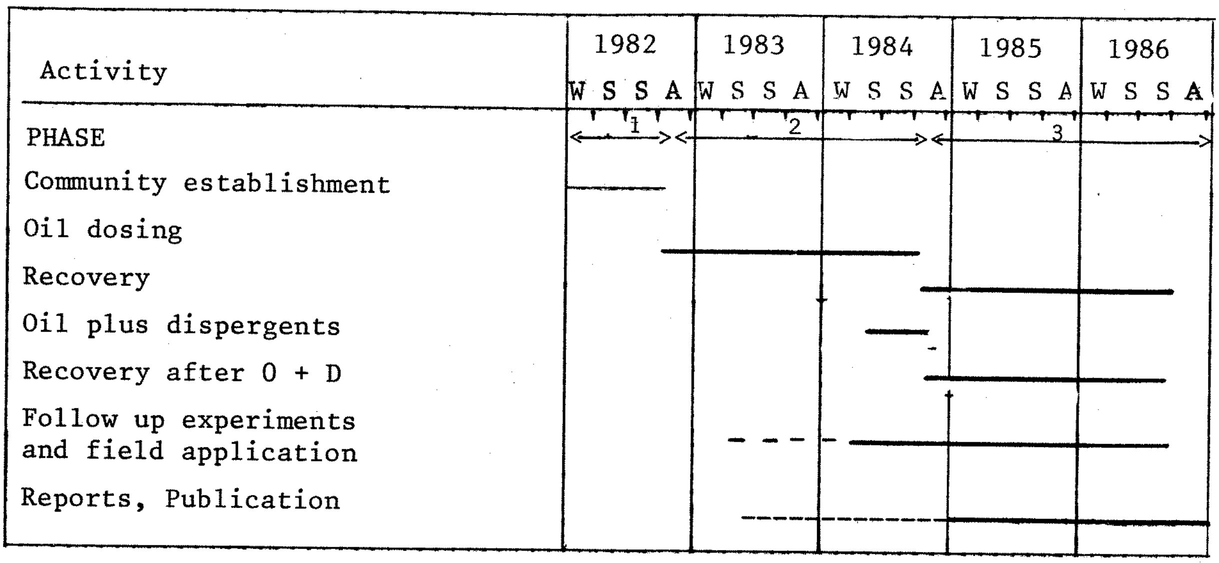
Staff and laboratories

The various aspects involved will be covered in the following way.

	Funded
Hydrocarbon dozing and analysis	
NIVA (T. Bakke)	BP
SI (R. Lichtenthaler)	BP
Community metabolism	
NIVA (L. Kirkerud)	BP
Community structure	
NIVA (T. Bokn)	BP
UiO (F. Moy)	BP
Population studies algae	
NIVA (T. Bokn)	BP
Recolonization algae	
NIVA (T. Bokn)	NIVA
Population dynamics invertebrates	
UiO (research students)	UiO
Population genetics	
UiO (S.E. Fevolden)	BP
Individual studies algae	
NIVA (T. Bokn)	BP
UiO (R.A. Kornfeldt)	UiO
Individual studies animals	
NIVA (T. Bakke)	BP
UiO (research students)	UiO
IMER Plymouth	BP/NERC
Routine environmental monitoring and maintenance	
NIVA (H. Juelsen, E. Johannessen)	BP

Time schedule

The following time schedule for the rock littoral experiment is suggested with dosing started in summer 1982



The present research proposal gives detailed plans for a 3 years period (1982-1984), but we find it necessary to be able to continue the research beyond this phase. The type of activity in the third phase depends very much on the results from the second phase, but it is expected that the magnitude of effort and thus of funding at least in 1985 will be similar to that of 1982-84. Among relevant aspects are: long time exposure to oil and detergents with subsequent recovery, other types of oil stress, "short" time laboratory and basis experiments to test hypothesis emerging from the 82-84 experiments, application of results and methods at field situations, and presentation of the results in scientific publication.

External activities

The Steering Committée seeks to stimulate cooperation with other relevant research bodies on the rocky shore hydrocarbon experiment and on the use of the experimental communities generally. An extended scope of the investigations ought to be beneficial for all the aspects involved. The following projects are already discussed:

Førsteamanuensis O. Grahl-Nielsen, University of Bergen, will cooperate on comparative chemometrical studies of total organic composition of tissues in *M. edulis* (and possibly other species) from test and reference communities.

Førstelektor F. Beyer and *hovedfagsstudent Follum, University of Oslo,* are conducting studies comparing recruitment and growth of algae and animals on artificial substrates between the basins and the fjord outside.
This project started in April 1981.

Professor R.L. Vadas, University of Maine will cooperate with T. Bokn on the recolonization studies of algae.

3.2 Long-term effects of oil on sublittoral soft bottom communities

Introduction

The effects of oil on the fauna of marine sediments have been predominantly studied following acute pollution resulting from oil spills (e.g. 'Torrey Canyon', Santa Barbara, 'Amoco Cadiz'). Of this type of study the West Falmouth spill, in the proximity of the Woods Hole Oceanographic Institute is by far the best documented and carefully studied (Sanders et al. 1980). Yet acute pollution is uncontrolled and biological changes that are followed are usually of recolonisation processes, the work stopping when the community has returned to a 'normal' level. In their review of oil pollution research the Norwegian Forskningsprogram om Havforurensninger FOH) expert group indicated that results of studies on acute pollution indicate that recovery times took 3-10 or more years depending on the degree of exposure and type of sediment in which the oil occurred. The group felt little was to be gained from pursuing similar research on artificial spills.

Study of chronic pollution over a long-term is extremely difficult in the marine environment. Attempts have been made to enclose bodies of water within plastic bags and examine effects of small amounts of oil on the whole plankton system. Work covering the whole ecosystem involves large teams of workers and yet the work is limited in time to a few weeks since nutrient limitation occurs and the communities rapidly diverge from natural community and in control bags. Yet the insights given into how planktonic systems are structured (the CEPEX experiment, Menzel (1977), Steele (1979), Gamble (1977), and the Kiel experiment, Bodungen (1976)) could not have been obtained by following traditional research lines.

The Norwegian bag experiments in Lindåspollene, Bergen, financed by FOH, has concentrated on effects of oil and has shown effects at very low oil concentrations and the finding of the potential competition for nutrients between oil degrading bacteria and phytoplankton is of great importance.

The most comprehensive benthic experiment is the University of Rhode

Island's Marine Environmental Research Laboratory(MERL) facility with research done in conjunction with several other US research institutions such as the Wood Hole Oceanographic Institution and the Skidaway Institute of Oceanography. Studies of both natural processes: benthic metabolism, metal flux, interactions plankton/benthos, particle resuspension etc. and effects of alien substances such as radioisotopes, trace metals, hydrocarbons and sewage on benthic structure (meio-, macrofauna) and processes (microbial interaction, benthic respiration, nutrient flux) have been conducted under the MERL frame-work. Here natural benthic communities were carefully transferred into tanks with a 5 m overlying body of water. Effects of 180 and 90 ppb of water accomodated oil were studied for up to a 2 year period on the whole system (Grassle, pers. comm.). Clear effects were observed on the benthos and on benthic/planktonic interactions. Thus although simplified and unnatural in many ways, the use of enclosed bodies of waters to study of whole systems has produced results which could not have been obtained in other ways.

With the recent acquistion and availability of large concrete tanks at Solbergstrand the potential for similar ecosystem studies on benthic communities in Norway is realised. Sediment communities are of particular interest in this context since sediments cover the largest part of the seabed. With the exception of MERL and the Kiel plastic bag, the benthic component of the ecosystem has been largely ignored.

Under the FOH "small"-scale field experiments involving long-term periodic hydrocarbon load on enclosed sublittoral sand communities (FOH Project 204, Bakke, NIVA); and effects of oil-contaminated sediment (FOH Project 203, Berge, UiO) and oil-contaminated bottom water (FOH Project 204) on benthic recruitment have been performed.

Use of experimental soft bottoms has several advantages over field studies, provided they are large enough for sampling without interference with the whole community. Sampling can be much more precise and controlled than from a research vessel and it should be possible to establish communities from below diveable depths and yet maintain their ambient light and water conditions, allowing physiological, biochemical and other shallow water experimental techniques to be used <u>in situ</u>.

Solbergstrand Experimental Station has available 13 concrete basins of size 20-30 x 6 x 2.5 m, i.e. a bottom area of about 150 m^2 each. When fully utilized they may offer benthic communities of different characters for large scale experimental studies. The size of the basins will permit extensive sampling without excessive disturbance of the communities, even after subdivision of the basins for special purposes.

A joint University-NIVA, (Drøbak/Solbergstrand) experimental facility is envisaged with large tanks and wet laboratory at Solbergstrand and dry lab, accomodation, small aquaria at Drøbak with use of boats when available. Small aquaria, dry lab facilities and accomodation at Drøbak will be used and CHNS analyser will be housed there. Nevertheess much routine work will be done in UiO where extra facilities are available including computing and microscope, library and office facilities, as well as at the sections of biological and chemical analysis at NIVA. Thus request is made for transportation between Oslo-Drøbak-Solbergstrand.

Work done on intertidal sediments in recent years has shown that exclusion of predators from sediments can lead to a claimed 20 fold increase in prey abundance (see Gray, 1981 and Petersen 1981 for reviews of this topic). If such is the case generally then effects of oil (or other pollutants) may be studied almost exclusively in relation to effects on predators rather infaunal deposit feeders. At UiO Berge (1980), Berge and Hesthagen (1981) and Berge and Valderhaug (in prep.) have shown conclusively that in subtidal sediments in Oslofjord and on the south coast of Norway predators have little effect save on recruitment of bivalves. Work is in progress examining recolonisation rates of oiled sediments placed in situ at various unpolluted localities.

In addition some 10 research students are currently engaged in benthic field studies and thus UiO has a considerable involvement with soft-bottom research.

At NIVA research on fate and effects of pollutants in marine sedimentary systems is central.

Rygg has conducted soft bottom surveys and monitoring tied to various types of pollution situations along the Norwegian coast for several years with emphasize on effects on macrofauna and on selection of sensitive indicator species (Rygg, 1981 and others).

Skei is doing comprehensive research on fates and environmental levels of PAH, PCB's and metals in coastal waters, especially on elementary flux between sediments and bottom water under oxidizing and reducing conditions (Skei 1979, 1981 and others).

Under FOH Bakke has conducted long term enclosure experiments with oil effects on various biological components of subtidal sediments, such as bacteria (biomass and activity), micro algae, meio- and macrofauna as well as on community metabolism and benthic recruitment (Bakke & Johnsen 1979, Bakke & al. in press). This work, involving several Norwegian research institutions, has indicated the main influence of low-level periodic hydrocarbon exposure to be on microbial structure and metabolic characteristics, and on gross community energy conversion, but also a selective negative influence on amphipod recruitment and on macrofauna tolerance towards oxygen deficiency.

Objectives

The primary objectives will be to study long term effects of chronic additions of oil hydrocarbons on benthic sediment communities. At a later stage detergents could be incorporated in the experiments.

During autumn 1982 four of the large basins will be made ready for use. The concrete walls will be repaired and concrete bottoms made. Also transverse walls of concrete with sluices will be constructed in two basins dividing them into 2 x 3 sub-basins each with a bottom area of 6 x 7 meters. The two subdivided basins will have booms and trolleys for positioning of sediments and sampling gear and a roof reducing light intensity to what is natural for the experimental community. The basins will be supplied with running sea water from the depth of the community. The turnover time will be kept at about 7 days. This allows the use of piston water pumps which should give the least harm to recruitment stages brought in with the water.

Criticism can be levelled at the fact that it may be difficult to establish natural communities within the basins. The same criticism was raised against the CEPEX and MERL experiments yet these proved acceptable. On the bottom of the basins a layer of at least 10 cm of sand may be placed to prevent establishment of a fauna typical of hard substrata.

Two methods will be used to establish communities. Firstly, in one series of experiments natural sediment will be taken with box corers and placed in trays (1 x 1 m) and then transplanted to the basins. These communities will be allowed to establish themselves through one year with natural recruitment. By the subdividing of the basins experimental and control systems will be established. The large size of the basins allows a favourable species/area ratio to be established.

Bottom sediments will be taken from Bjørnhodebukta, Håøya, an area of silt-clay at 30-35 m depth with a rich community comprising some 83 species. This community is used in another benthic project at UiO Gray and Valderhaug's, NAVF project) and thus considerable background information is available on the fauna and environmental factors. Transplantation of the sediments will occur in March-April 1983 to minimize temperature shock of the organisms transplanted.

The second method will be to utilize the current UiO stystem, where boxes of frozen sediment are placed in situ at various sites and then after various periods of time these boxes can be brought into the basins for exposure. The communities that are established are closely similar to the natural ones taking into account species/area relationships, after a year of recruitment.

Current flow in the basins and organic input to the communities will be assessed in pilot experiments. Also a pilot experiment on the method of transplantation will be done in summer/autumn 1982. Box corer samples will be taken from Bjørnhodebukta and placed in experimental trays. One tray will be removed to the SES facilities and one left in situ. By sampling with corers of the two trays, population changes over time can be compared. This information will be incorporated in the design of the final experiment.

Methods

Under the phase of establishment of the natural community in the basin, micro-, meio- and macrofauna and monthly settlement, will be routineously monitored together with nutrients in water and sediment, primary production, settling organic matter, CNP, ATP and total bacteria and redox potential in the sediment.

Once a reasonable community is established (probably about one year), low concentrations of a water accomodated oil fraction will be led into three sub-basins continuously, the other three acting as references. Type of dozing will be based on the experience gained from the rock littoral project. The variables listed above will be followed over a 3 year period.

Of special interest will be shifts in important meio- and macrofaunal species' numerical rank (dominance), shifts in trophic structure, and changes in community indices (total biomass, diversity, distribution of individuals on species etc.). The purpose will be linked to practical monitoring, by testing under semi-controlled conditions the sensitivity of procedures used in soft bottom monitoring.

Changes in community metabolism and in the balance between autotrophic and heterotrophic processes will be followed by estimates of gross respiration and production.

Shifts in bacteria biomass and metabolic characteristics towards greater proportion of oil degraders have been demonstrated during short time (about 14 days) low-level exposure to hydrocarbons (FOH Project 204, and FOH Lindåspoll-project). During the long-term exposure of the basin communities, one may be able to pursue the dynamics of this change and to study the type of long-term steady state that developes (if at all), and whether this has consequences for the mineralization capacity for natural organic substrates. The role and responses of microorganisms should therefore be an important aspect of the studies. However, based on recommendations from the Advisory Board, the expences allocated to microbiologist salary in the earlier revised budget to BP should preferable be used to cover crucial technical assistance for the performance of the other aspects, as technical assistance have been reduced to a minimum. Hence, efforts will be made to establish funding for the microbiological aspects elsewhere.

During 1983 a variety of sediments varying from coarse sand to mud will be taken from a number of sites around the coast frozen and placed *in situ* to allow natural recolonisation. After 1 year these will be taken to Solbergstrand and treated as control and experiment using the same amounts of oil as in the first experiment. The exact location and number of sites will be specified after consideration of a variety of factors including sediment type, diver access etc. This part of the work will give information on likely effects on other types of fauna than those adjacent to Solbergstrand.

It is expected that the experiments will give important insights into: 1) how mortality affects benthic fauna, (are some species affected first? and are these species naturally rare? or is mortality merely in proportion to abundance? are amphipods and meiofaunal copepods really more susceptible than polychaetes and nematodes?) 2) effects on fecundity of benthic species. 3) how oil affects the transformation of organic matter through the system (does oil inhibit oxygen percolating into sediments? does oil disrupt bacterial degradation of particulate organic matter? will oil change the balance between autotrophy and heterotrophy? are predator-prey relations upset? 4) whether competition exists between oil degrading bacteria and natural bacteria for available nutrients. Undoubtedly other unexpected findings will be made.

As a second phase of the basin experiment, studies will be made of autecological responses of some of the species which seem most affected by the long-term exposure to reveal why these are sensitive. In situ estimation of physiological, biochemical and other functional responses with the aim of later use in the field will be done in cooperation with Dr. Bayne's research group at the Institute for Marine Environmental Research, Plymouth, England.

Genetical aspects

In ecology much research is in progress on identification of local populations using enzyme polymorphisms. Yet marine genetics is a neglected area of research particularly in Norway. The technique most widely used is that of electrophoresis of certain enzyme systems to establish degrees of heterozygosity. The enzyme system is referred to as a genetic marker and one seeks to correlate variability in that enzyme to an environmental gradient. The use of the term marker is because one cannot be certain that the enzyme studied is linked to one locus or whether the same locus may be coupled to another enzyme. Thus the functioning of the particular enzyme system is not usually related to say a gradient of pollution since the enzyme is only a marker.

Using biochemical knowledge on Glycera alba (Blackstock and Filion - Myklebust, 1982), we have however come a step further than is usual. A group of enzymes in the glycolysis cycle has been found to increase in quantity under slight pollution since the activity of the animals increases. Now one can use the same enzymes (LDH, MDH, PFK etc.) to study genetic changes along known pollution gradients (Oslofjord and in the Solbergstrand experiments), for enzymes known to play a functional role, thus linking ecology to biochemistry to genetics. It is hoped that additions finance to support the biochemist (C. Filion - Myklebust) will be raised.

The research in this part of the programme will concentrate on population genetics of G. alba and also investigate population genetics of amphipod crustaceans since this group appears to be highly sensitive to effects of pollution. Following organic enrichment and oil pollution, this group was one of the first to be displaced. Thus we feel the coupling of ecology to biochemistry to genetics is a highly exciting and profitable research field.

Chemical aspects

Studies at MERL and elsewhere have indicated a selective transport of hydrocarbons to the sediments with greatest accumulation of alkanes and least of aromatics. This is to be expected as the processes involved in transporting hydrocarbons to the bottom, and trapping them in the sediments act selectively on the various components. It would be imperative to study the rates of build-up of the types of hydrocarbons during really long-term well defined exposure both in conjunction with selective accumulation in benthic organisms, and selective bacterial mineralization of hydrocarbons. This requires involvement of a marine chemist on the project.

This study will be tied to a subproject on the influence of animal activity (bioturbation) on hydrocarbon accumulation in sediments, and on hydrocarbon flux between sediments and water.

Routine fluorescense analysis of hydrocarbons in dosed water will be done at Solbergstrand and GC/MS analyses of selected compounds in basin waters, sediments and organisms by the Central Institute for Industrial Research (SI).

Additional funding will be necessary to perform a high resolution analytical program of desired magnitude and to hire an analytical chemist to cover these research aspects.

Staff and laboratories

The aspects involved will be covered in the following way:

	Funded by
Basin construction and equipment	
NIVA (L. Kirkerud, H. Øvrebø, H. Juelsen, E. Johannessen)	BP
Community establishment	
NIVA (T. Bakke, L. Kirkerud)	BP
UiO (J.S. Gray, research student (?))	BP/UiO
Hydrocarbon dozing and analysis	
NIVA (T. Bakke)	BP
SI (R. Lichtenthaler)	BP/External
Community metabolism	
NIVA (L. Kirkerud)	BP
Meio-, macrofauna structure	
NIVA (B. Rygg)	BP
UiO (J.A. Berge)	BP
Population genetics	
UiO (S.E. Fevolden)	BP
Individual performance	
NIVA (T. Bakke)	BP
IMER Plymouth (?)	BP
Sediment chemistry and contaminant fluxes	
NIVA (J.M. Skei)	BP
UiO (?)	Uncovered
Microbiology	
UiO (?)	Uncovered

J.A. Berge is envisaged as a day-to-day leader of the UiO side of the project. Berge is at present 100% financed by FoH and in order to complete his project there, a gradual transfer to BP funding over a two year period is planned.

T. Bakke will coordinate the NIVA side of the project.

4 BUDGET

The present budget is in accordance with the revised budget agreed upon by the Steering Comittée in December 1981. It is based on 1981 prices for equipment, manpower and analysis and must be adjusted for inflation annually. It is assumed that the money is transferable from one year to another. The contingency reserve will be used to strengthen aspects of the projects worth pursuing in more detail, to cover travel expenses for scientists from UK, and to cover expenses connected to the activities of the Steering Comittée and Advisory Board.

There are certain important elements of the programme which are not covered by the BP grant (i.e. microbiology and HC chemistry) and others which are only covered satisfactorily during the first 2-3 years (technical manpower and hydrocarbon analysis). Efforts will be made to obtain funding from other sponsors for these elements.

Revised budget in 1000 N kr 1981

	1982	1983	1984	1985	1986	Total
General Repairs	100					100
Rock littoral project	1276	1141	703	476	404	4000
Subtidal soft bottom project						
Repair of 4 basins	1000					1000
Community establishment	50	150				200
Running costs Univ. of Oslo	364	565	624	442	442	2437
Capital costs [1] Univ. of Oslo	288					288
Running costs NIVA	528	794	881	289	280	2775
Capital costs NIVA	50	50	50			150
	2280	1562	1555	731	722	6850
Total annual costs	3656	2703	2258	1207	1126	10950
Contingency reserve						1050
Total cost of BP programme						12000

Budget breakdowns

Rock littoral project	1982	1983	1984	1985	1986	Total
Time researcher	425	425	300	225	225	1600
Time assistant[2)]	304	304	304	152	80	1144
Equipment	330	45	45	45	45	510
Analyses	150	300	-[1)]	-	-	450
Travels[3)]	67	67	54	54	54	295
Annual Costs	1276	1141	703	476	404	4000

Subtidal soft bottom project

Running costs University of Oslo	1982	1983	1984	1985	1986	Total
J.A. Berge		75	150	150	150	525
S.E. Fevolden	125	150	150	150	150	725
Technical ass.[2)]	75	100	100			275
Equipment, travels etc.	80	110	80	40	40	350
Total	280	435	480	340	340	1875
30% University of Oslo	84	130	144	102	102	562
Annual costs	369	565	624	442	442	2437

Running costs NIVA	1982	1983	1984	1985	1986	Total
Time researcher	196	240	224	168	168	996
Time assistant[2)]	243	318	318	84	84	1047
Equipment	47	47	47	-	-	141
Analyses [1)]	50	200	300	-	-	550
Travels[3)]	42	42	42	37	28	191
Annuals costs	578	847	931	289	280	2925

1) The budget covers analytical service up to a total of 1 mill N kr. This leaves 3 mill N kr uncovered of the original analytical costs.

2) Technical assistance is at an absolute minimal level especially in the budgets of 1985 and 1986.

3) Includes local travels from NIVA/UiO to Solbergstrand.

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